Associations between microbes and macroalgae: Host, epiphyte and environmental effects

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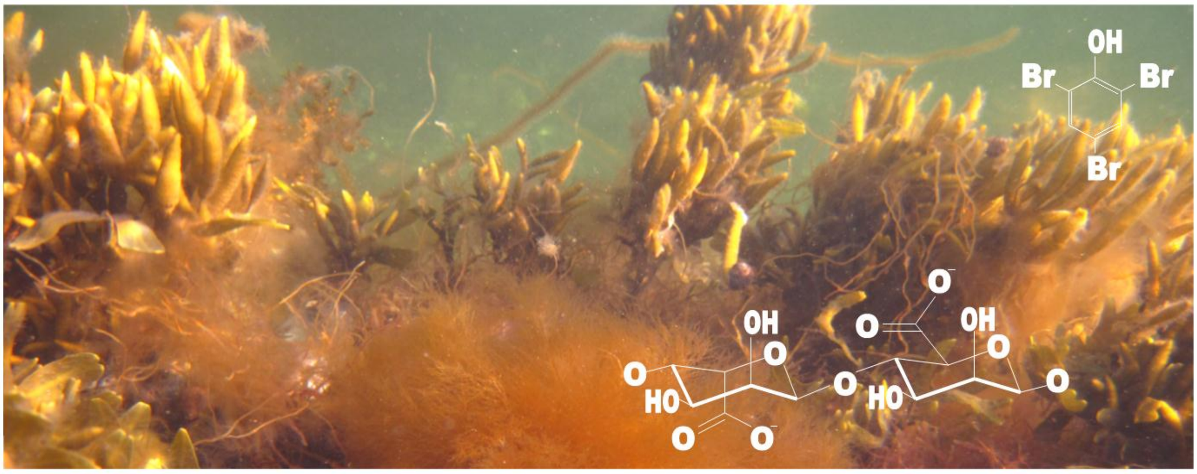

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Associations between microbes and macroalgae: Host, epibiota and environmental factors

M.Sc. Franz Goecke Saavedra

Mathematisch-Naturwissenschaftliche Fakultät
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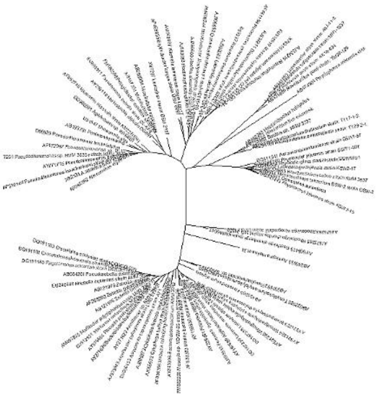

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Para mi abuelo Helmuth…

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Paper III

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Paper V

Goecke, F., Núñez A., Labes A., Wiese J., Neuhauser S., & Imhoff, J.F. Gall formation in the bull-kelp *Durvillaea antarctica* (Chamisso) Hariot in the central coast of Chile is associated with two different endophytes. (under preparation)

Short note

Goecke, F., Labes A., Wiese J., Schmaljohann R., & Imhoff, J.F. Observation of bacteria over the surface of released oogonia from *Fucus vesiculosus* L (Phaeophyceae). Gayana Botánica submitted 31 October 2011

TABLE OF CONTENTS

List of the Supporting Information provided in the present investigation.

List of Figures

General

Chapter I

Chapter II

Chapter III

Chapter IV

Chapter V

List of Tables

Chapter I

Chapter II

Chapter IV

Chapter V

1 Zusammenfassung

Während der letzten drei Jahre stand die Assoziation zwischen Makroalgen und Bakterien im Fokus vieler Untersuchungen. Die Arbeit wurde vorgelegt, um zu diesem Verständnis beizutragen. Verschiedene Makroalgen im selben Habitat können die gleiche wichtige ökologische Rolle als primäre Produzente spielen, aber im Begriff eines Zusammenwirkens kann jede einzelne Art eine eigene Mikro-Umwelt repräsentieren, die von eigenen mikrobiellen Gemeinschaften besetzt ist. In dieser Arbeit wurden die potenziellen Effekte des Wirtes, der Jahreszeit und der Epibionten auf die Zusammensetzung dieser Gemeinschaften untersucht. Zusätzlich wurden die Möglichkeit spezifischer Assoziationen und krankheitserregender Beziehungen bearbeitet.

Mit molekularen Methoden wurde kürzlich dargestellt, dass unterschiedliche Arten von Makroalgen im gleichen Habitat mit verschiedenen mikrobiellen Gemeinschaften verbunden sind. Wir bestätigten dies durch Kultivierungsexperimente an zwei Makroalgen, die in der Ostsee zusammen vorkommen. Diese besonderen Mikro-Umwelten können durch die Form des Thallus, die Oberfläche und einmalige metabolische Eigenschaften (z.B. sekundäre Metaboliten und Zellwand) produziert werden. Für all diese Faktoren wurden Einflüsse auf die Assoziationen gezeigt. Außerdem bestätigte diese Arbeit durch eine phylogenetische Studie von neubeschriebenen Bakterien, dass Algen eine reiche Quelle für die Isolierung von Bakterien sind.

Die Assoziation von Algen und Bakterien können einen ökologischen Vorteil gegenüber mikrobiellen Epibionten darstellen. So ist die Bildung von antimikrobiellen Metaboliten weit verbreitet unter Algen-assoziierten Bakterien, eine Eigenschaft, die von der Makroalge genutzt werden kann. Eine mutualistische Beziehung könnte darin bestehen, dass die bakterielle Gemeinschaft den Wirt vor Aufwuchs (biofouling) schützt, während die Oberfläche des Wirtes Nahrung und Schutz für die Bakterien anbietet. Die Rolle als Verteidiger des Wirtes durch diese Bakterien gegen Krankenerreger ist fast unerforscht, da Krankheiten in Algen wenig studiert worden sind. Daher haben wir einen Krankheitserreger von einer chilenischen Alge beschrieben.

Algen sind Schlüsselkomponenten der aquatischen Umwelt, nicht nur als Habitat für viele Makroorganismen, sondern auch für Millionen von noch unentdeckten Mikroben. Chemische Substanzen regulieren die Beziehung zwischen Makroalgen und Bakterien. Damit sind Wirt und Epibionten eine potenziell reichen Quelle von neuen sekundären Metaboliten. Sowohl Makroalgen als Mikroben sind wichtige Organismen im Meer, dennoch sind wir nur am Anfang, die Komplexität des Verhältnisses zwischen Algen und Bakterien in der Natur zu verstehen.

2 ABSTRACT

During the last 3 years, there was a special enhancement of research focused on association between macroalgae and bacteria. The present work was carried out in order to contribute towards our understanding of those alga-bacteria relationships. Different macroalgae in the same habitat may all exert the same important ecological role as primary producers, but in terms of interactions every single species represents a completely different microenvironment occupied by rich and diverse microbial communities. The present study covered potential effects from the host, season and epibiota in regulating the associated microbial communities. It also covered pathogenic relationships and possible specific associations.

Recently it has been demonstrated by molecular methods that different species of marine macroalgae in the same habitat are associated with different microbial communities. We confirmed these molecular data by means of a cultivation-based study on two co-occurring macroalgae from the Baltic Sea. Those very unique microenvironments may be formed by the thallus morphology, by microtopography of the surface and by unique metabolic properties (e.g. secondary metabolites and cell wall structures). All these factors were shown to influence the associations with organisms of their immediate surroundings. The present study also confirms algae as a distinct source of bacteria by a phylogenetic study of all newly described bacteria from algal sources available in GenBank.

The association of algae with members of the microbial community may represent an ecological advantage against microbial epibiosis. The development of antimicrobial metabolites is widespread among alga-associated bacteria, a quality which the macroalgae may use for it´s own benefit. A mutualistic relationship can be postulated, in which the bacterial community protects the host from biofouling, while the host may provide nutrients and physical protection to the bacteria. The role of the associated bacteria as defense of the host against pathogens is almost completely unknown, starting with the fact that diseases of algae are mostly neglegted. In this sense, we described a microbial pathogen affecting a macroalga at the Chilean coast.

Algae are key components of the aquatic environments, not only habitats for many macroorganisms, but also for millions of microbes waiting to be discovered. Because chemical compounds seem to rule the macroalga-bacteria relationships, host and epibiota represent also a potential rich source of secondary metabolites. Both, macroalgae and microbes are key organisms in marine environments. Nevertheless, we are only starting to discover the complexity of relationships between algae and bacteria in nature.

3 INTRODUCTION

3.1. Marine microorganisms in their interaction with macroalga

Microorganisms are an essential component of earth's biosphere represented with an enormous number in aquatic environments (Whitman *et al*. 1998). Seawater contains a range of millions of viruses and bacteria, thousands of fungi and microalgae, and hundreds of microscopic larvae and spores per mL (Harder 2009). Many of those microorganisms have biofouling potencial or interact with marine macroorganisms, in pathogenic or non-pathogenic ways. The aquatic environment favors the formation of biofilms on surfaces (Weinberger 2007). Macroalgae are especially susceptible to epibiosis because they live in an environment with strong competition for space amongst benthic organisms (Hellio *et al*. 2001, Lam *et al*. 2008). Therefore, algae are continuously challenged by microorganisms as well as by grazers (Weinberger *et al*. 1997, Bouarab *et al*. 2001, Fig. 1). Indeed, many of the organisms that feed directly or indirectly on macroalgae live in close association with them (Thomas 2002). Seawater as environment has usually relatively low nutrients concentrations, whereas living surfaces such as algae exude large amounts of organic carbon and nutrients (Kong & Chan 1979, Armstrong *et al.* 2001, Lane & Kubanek 2008). Bacteria in the seawater must also protect themselves from damaging ultraviolet radiation and predation, whereas microbial communities on living surfaces form biofilm structures shielding them from environment stress (Burke *et al.* 2011). Algal mats and turf algae, for example, can act as microbial reservoirs through adverse environmental conditions. Those mats may provide a favourable environment in which certain bacteria are able to persist by reducing or eliminating many of those common stresses bacteria faced in the water column, as well as by providing a more stable habitat in which to exist (Beleneva & Zhukova 2006, Englebert *et al.* 2008).

The algae and their associated communities form complex and highly dynamic ecosystems, consisting of a diverse range of organisms (Holmström *et al*. 2002, Fig. 2). Bacteria are an inherent part of the physical environment of micro- and macroalgae (Hold *et al.* 2001) and dominant among the primary colonizers of algal surfaces, followed by diatoms and fungi (Lam *et al*. 2008). While some macroalgae are heavily colonized, other algal species in the same habitat remain almost free of epibionts. This indicates the presence of an established antifouling defense in only some macroalgal species (Steinberg & de Nys 2002, Nylund & Pavia 2005) and, on the other hand, species-specific contact mechanisms between algae and the microorganisms (Goecke *et al*. 2010).

Figure 1. Marine macroalgae from their very beginning dealt with positive and negative interactions with a great variety of microorganisms. In the picture, the red alga *Polysiphonia* sp. grows on a rock and has the frond submerged into water in direct contact with the microorganisms around. In detail few examples of them (from left to right): epiphytic algae, diatoms and microalgae, microcrustacea, cyanobacteria, bacteria, invertebrate eggs, and oomycetes.

3.1.1. Algae as source of new bacterial taxa

Although the isolation source of a determined microorganism not necessary implies exclusivity and ecological associations, certain bacteria are consistently found as algal epiphytes suggesting that they can be highly specific and may express adaptations to utilize resources provided by algae (Wada *et al.* 2007). Worldwide, several new bacterial species, genera and orders have been described after isolation from algal material, indicating that algae represent a distinct source for new bacterial taxa (Goecke *et al.* 2010, Paper I). Even if the origin does not necessarily include specific association, the repeated isolation of species of a determined bacterial group from an algal taxon, or in general from algae, could be explained by evolutionary adaptations, i.e. metabolic pathways, niche specificity, or even established symbiotic relationships that allow those bacteria to grow associated with the algal hosts.

3.1.2. Algae-microbial interactions

3.1.2.1. Microbial communities associated with macroalgae: Bacteria

Descriptive studies of bacteria isolated from the surface of macroalgae were reported as early as 1875 (Johansen *et al*. 1999). The interest in bacterial populations living in association with macroalgae has increased during recent decades. Recently, 107 studies were evaluated that found bacterial communities associated with a total of 148 macroalgae (36 Chlorophyta, 46 Phaeophyceae, 55 Rhodophyta, 12 undetermined algae) within the last 40 years (Paper I in Supplementary Information: S01). And yet this represents only a tiny amount of species of algae. This increasing number of publications concerning bacteria associated to algae can be attributed to the combined use of improved methods in bacterial culture, microscopy and molecular biology (Fig. 1 of Paper I, S01). However, many questions concerning the occurrence, distribution, persistence and ecological function of the associated bacteria remain unresolved (Goecke *et al*. 2010). Although some of the bacterial–algal interactions have been discussed earlier, the ecological relevance of most naturally occurring bacterial communities on macroalgae remains unclear and in most cases the bacterial species involved have not yet been identified (Ivanova *et al*. 2002). Studies of macroalga–microbe interactions have lagged, mainly for methodological reasons (Largo *et al*. 1997). Suitable tools for the analysis of epiphytic bacterial communities including culture-independent approaches were not available until molecular techniques were introduced to this field of research (Goecke *et al.* 2010).

Marine macroorganisms can be expected to display the whole spectrum of host-bacterial associations on their surface, ranging from passive and random epibiosis to highly specific and obligate symbiosis (Bengtsson 2011). On one hand, algae are the major primary producers in aquatic ecosystems (Thomas 2002), and on the other hand, heterotrophic bacteria play a key role in regulating accumulation, export, remineralisation and transformation of the largest part of organic matter in those aquatic systems (Mudryk & Skórczewski 2006). By utilizing macroalga-derived substances, bacteria take an important place in the food web and make the alga primary production available to a number of organisms (Bengtsson 2011). Therefore, algae are key components of the aquatic environments, not only microhabitats for many invertebrates and fishes, but also for millions of microorganisms. Interactions between bacteria and algae are thought to be important in controlling the dynamics of both communities and yet there is presently little knowledge of the pathogenic and symbiotic nature of bacterial communities of most marine living surfaces (Jasti *et al*. 2005, Bengtsson 2011). Recently it was shown by molecular methods that macroalgae ‘harbor’ different associated microbial communities (Lachnit et al. 2009).

Different macroalgae may mediate bacterial surface colonization by compounds excreted from the thallus (Lachnit *et al*. 2010, Saha *et al*. 2011, Sneed & Pohnert 2011a).

3.1.2.2. Epibiosis

The growth of one organism on the surface of another organism is referred to as epibiosis (Bengtsson 2011). Despite some beneficial aspects of epibiosis for the affected macroalgae (see Wahl 1989), biofilm formation produces a permanent threat for the host (Steinberg & de Nys 2002, Wahl 2008). It is likely that the epibiosis leads to an increased hydrodynamic drag on the basibionts, reduces buoyancy and elasticity of the tissue, may also attract grazers, and thereby increases tissue loss of the host or even result in the death of the host. Beside these mechanic effects, bacteria may directly compete with the algae for nutrients (Berland *et al.* 1972). Biofilms also may inhibit gaseous exchange and reduce incident light, thereby decreasing photosynthetic activity (Wahl 1989, 2008, Steinberg *et al.* 1997). In addition, the bacterial biofilms enhance the attachment and growth of a range of other fouling organisms, such as diatoms, invertebrate larvae, and algal spores (Harder 2009). Finally, the host may be damaged due to the production of a variety of toxins, digestive enzymes, inhibitors and waste products of the bacterial community (Weinberger *et al.* 1997, Ivanova *et al.* 2002, Table 2 of Paper I, S01). Microorganisms that are common on the surface of macroalgae themselves might become detrimental if they are able to enter the algal tissue (see below 3.1.3.2). Physiologically, fouling organisms have negative effects on host growth and reproduction. Hence, evolutionary pressure on marine macroalgae has favoured the development of mechanisms to defend their surfaces against biofilms (Wahl 1989, Steinberg & de Nys 2002).

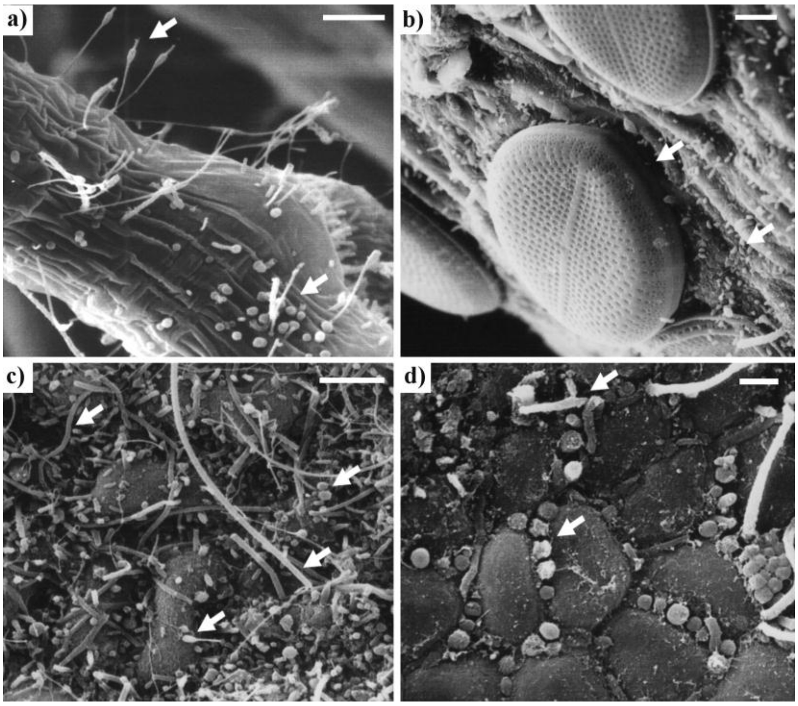

Figure 2. Different examples of microorganisms living associated with macroalgae surfaces. **a)** Detail of the thallus of unidentified filamentous red algae of the order Ceramiales in the Baltic Sea. **b)** Diatoms over the surface of *Delesseria sanguinea* also from the Baltic Sea. **c)** Dense biofouling over the brown alga *Fucus vesiculosus* in Germany (Baltic Sea). **d)** Poor biofouling over other brown macroalgae of the order Fucales, *Durvillaea antarctica* in Chile. SEM microphotographs, scale bar= a-c) 5 µm; and d) 2 µm.

3.1.2.3. Macroalgal diseases

As any other organisms, algae are susceptible to a wide range of pathogens (Andrews 1976). Especially in the aquatic environment, diseases, parasitism, epibiosis and biofouling are quite common phenomena that constantly threaten the health of algae (Harder 2009, Fig. 3). Historically, it has been considered that diseases among algae are quite rare, but the enhancement of global aquaculture activities has shown an increase of dramatic outbreaks with serious economic losses (Gachon *et al.* 2010). Macroalgal diseases and the respective bacterial pathogens are listed in Table 3 of Paper I (S01).

The recent development of new detection and identification technologies is supporting the recognition of the role of parasites and diseases in food web dynamics and ecology of the aquatic environments (Neuhauser *et al.* 2011a, b). Pathologies seem to be much more common and important in regulating algal populations as previously thought. Although algae do not have a cell-based immune system, evidence for induced defence reactions of algae upon pathogen recognition is emerging (see Goecke *et al.* 2010). Recent findings of bioactive secondary metabolites associated with the surface of the algae and their influence on microbiota (Saha *et al.* 2011, Sneed & Pohnert 2011) suggest evolutionary adaptation of the algae to overcome the threat by potential harmful organisms. On the other hand, in order to attack the frond tissues, a pathogen must penetrate the cuticle layers of the macroalga (Craigie *et al*. 1992). Algal cell walls and cuticles contain a great diversity of polysaccharides, which make them chemically and structurally more complex and heterogeneous compared to those of terrestrial plants (Polne-Fuller & Gibor 1987). Many of pathogenic bacterial strains produce extracellular enzymes that enable them utilize the polysaccharide as a carbon source (Bengtsson 2011). Bacteria capable to degrading the macroalgal cell wall are important factors for the damage of algal tissue and provide an entrance for pathogenic and opportunistic bacteria too (Buschmann *et al*. 1997, Ivanova *et al*. 2005). Not only bacteria but also algal endophytes are able to breach the cuticula and cell wall and facilitate secondary infections (Craigie & Correa 1996).

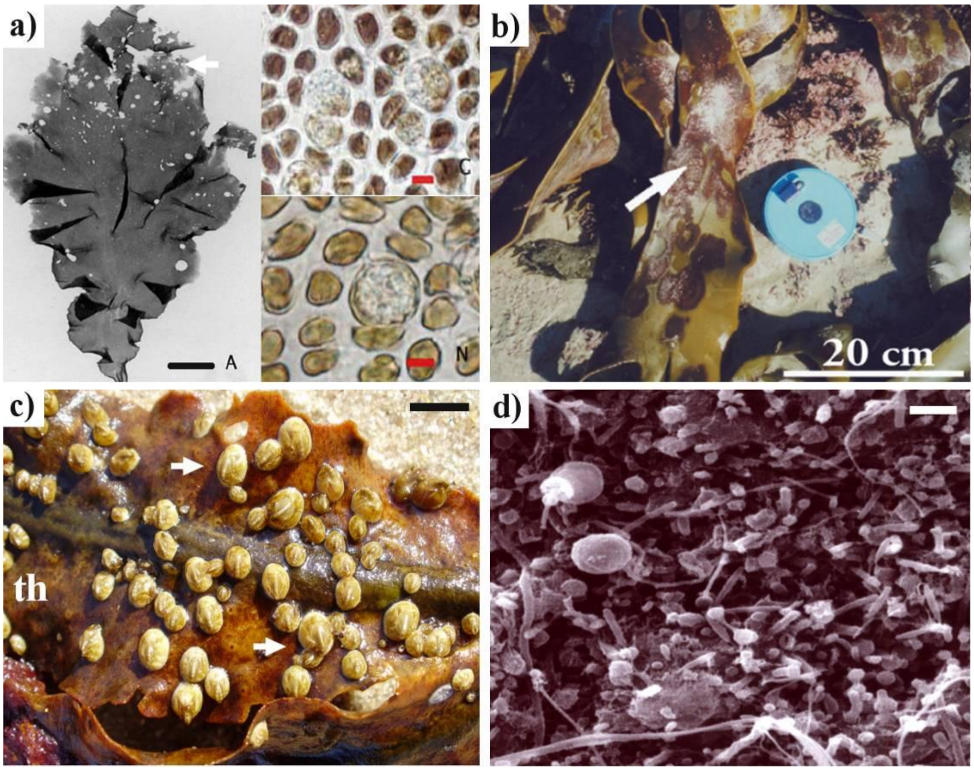

Figure 3. Diseases, parasitism, epibiosis and biofouling over algae. **a)** *Porphyra yezoensis* with ‘red rot disease’ and a chytrid infection in the detail of the chytrids (C, N) (modified from Ding & Ma 2005); **b)** The obligate algal parasite *Herpodiscus durvillaeae* over *Durvillaea antarctica* (from Heesch *et al*. 2008); **c)** Cirripedia growing over the thallus (th) of *Fucus serratus* in Kiel; **d)** Scanning electron microphotography of the biofilm growing over *Fucus vesiculosus* in Kiel. Scale bar: a) 1 cm, b) 20 cm, c) 0,5 cm, and d) 5 µm. Arrows indicate the epiphytic algae (in Figure 3b) and the epibiotic cirripedia (in Figure 3c).

3.1.3. Chemical defense

Given that algae lack cell-based immune responses and are continuously exposed to a broad array of potentially deleterious microorganisms, it is reasonable to hypothesize that the production of bioactive secondary metabolites acts as a fundamental mechanism of antimicrobial defense to deter microbial attack (Engel *et al.* 2002). Secondary metabolites mediate numerous biological interactions and play a particular important role in mediating host-microbe associations in the ocean (Lane *et al.* 2010). To inhibit settlement, growth and biofilm formation by bacteria, macroalgae are able to influence bacterial metabolism and quorum sensing, and produce antibiotic compounds (Goecke *et al.* 2010). Macroalgae may secrete antifouling compounds into the surrounding seawater and retain antigrazing compounds within the thallus structure (Armstrong *et al.* 2001).

Macroalgal chemistry is rich and diverse, spanning most natural product classes and including functional group characteristics found from no other source (Maschek & Baker 2008). The production of inhibitory substances from macroalgae was noted as early as in 1917 (Ara 2001) and since then antibacterial activity of different extracts of macroalgae from almost all groups has been described in many studies around the world (Sridhar & Vidyavathi 1991, Hellio *et al.* 2000, Freile-Pelegrin & Morales 2004, Engel *et al.* 2006, Puglisi *et al.* 2007, Dubber & Harder 2008, Lane *et al.* 2009; Table 5 of Paper I, S01). It has been recently suggested that macroalgae without their own chemical defense are able to rely on the secondary metabolites produced by their associated bacteria (Egan *et al.* 2000, Armstrong *et al.* 2001, Fig. 4).

3.1.4. Beneficial relationships

The role of epiphytic bacteria in maintaining the health of the host has received little attention. Though beneficial associations between bacteria and their host have been identified (Weinberger *et al*. 1997, Dobretsov & Qian 2002, Rao *et al*. 2006), the advantages for algae are less obvious.

Beneficial relationships may be based on the algal capacity to produce organic compounds and oxygen which are utilized by bacteria (Brock & Clyne 1984, Coveney & Wetzel 1989). In turn, bacteria mineralize organic substrate, supplying the algae with carbon dioxide and nitrogen, minerals and growth factors (Croft *et al.* 2006). In addition, microbes play a role in the protection of the macroalga against toxic compounds such as heavy metals or crude oil (Dimitrieva *et al.* 2006, Semenova *et al.* 2009). Positive macroalgal–bacterial interactions may include phytohormone production, spore germination and morphogenesis of macroalgae triggered by bacterial products; specific antibiotic activities affecting epibionts and elicitation of oxidative burst mechanisms (reviewed by Goecke *et al*. 2010, Paper I, S01). However, after more than 20 years of research on this topic, there is still no experimental evidence demonstrating if or how host organisms selectively attract and harbor such epibionts (Harder

2009). There is an enormous variety of different metabolites as possible mediators of interspecies interactions in the algal biosphere, including products of the algal host, pathogens, foulers, and symbionts. Although bacterial secondary metabolites are likely to participate in such interactions, little is known about the role of bacterial secondary metabolites in mediating these ecological interactions (Meusnier *et al*. 2001).

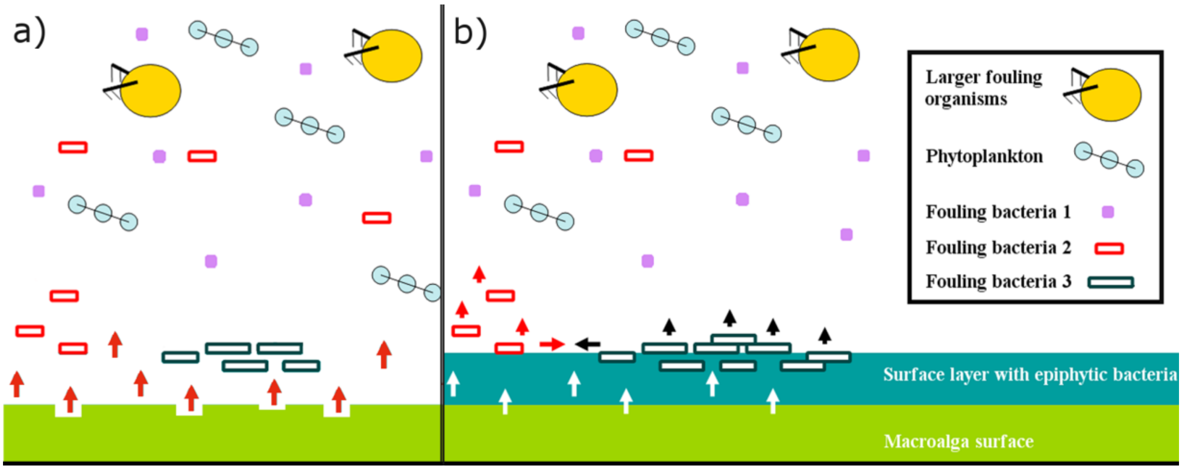

Figure 4. Schematic diagram shows the influence of secondary metabolites on macroalgal surfaces. Antifouling chemicals may be produced by either the algal cells or by epiphytic micro-organisms. **a)** Algae are able to produce compounds and release them at surface level that can inhibit the colonization and growth of planktonic bacteria. **b)** Symbiotic relationship between algae and epiphytic bacteria that may control biofilm formation and subsequent fouling on algal surfaces (based on Armstrong *et al.* 2001).

3.1.5. Goals of this investigation

The present investigation started with a review on the widespread relationships among bacteria and macroalgae presented in **Paper I** (Supplementary information S01). It can be addressed that chemical interactions regulate the bacteria-macroalga relationships, may cause specific associations, and that the production of biologically active compounds by the algal host might influence those relationships between the bacteria and macroalgae (Persson *et al*. 2011). Nevertheless, there are many gaps in knowledge to be closed to achieve a better understanding of the macroalgae-bacteria relationship.

It is clear that some macroalgae, especially from tropical environments, are able to produce potent antibiotic substances with a widespread pattern of antibiosis (Engel *et al*. 2006, Puglisi *et al*. 2007, Lane *et al*. 2010). The question arises whether those chemical interactions are common in the aquatic environment? Are common macroalgae of a temperate environment able to regulate the microbial population over their surface? In order to test that assumption, we decided to study the antibiotic activity of extracts of 16 macroalgae that co-occur in the

Kieler Fjord, Baltic Sea (Germany). Recently it was suggested that the nature of the microbial strains used in bioactivity tests has influence on antibacterial activities (Hughes & Fenical 2011) and that the selection of bacterial test strains may rush conclusions on the effect of macroalgal extracts and metabolites on bacteria. Therefore, we decided to check if a different response is produced by the selection of the microorganisms used in the tests. We used ecologically relevant bacteria (macroalgal surface-associated bacteria and pathogenic bacteria) and compared them with standard microorganisms (used normally in the bioactivity tests). The results will be published in **Paper IV**, building chapter IV of this work.

If macroalgae are able to chemically regulate their epibiotic microorganismic communities, it is reasonable due to the unique chemistry of every species to expect that species specific associations occur and that some bacterial species associated commonly with same hosts. To answer the question if algae are a distinct source of bacterial taxa, we conducted a phylogenetic study of all those bacteria that were described to be isolated from macro- and microalgae from all eukaryotic algal divisions. The phylogenetic study was based on 16S rRNA gene sequences of bacteria described as new species available in GenBank in September 2011. The results will be published in **Paper II**, building chapter I of this work.

If chemical interactions regulate the bacteria-macroalga relationships, there is a possible effect of its associated microbiota itself regulating those relationships. Previously it has been observed that antimicrobial activity is widespread among alga-associated bacteria (Wiese *et al*. 2009b). This production of chemical defenses by associated microorganisms may represent an added level of complexity to the mechanisms by which marine plants avoid deleterious microorganisms (Engel *et al*. 2002). In order to analyze the ability of the (macroalgal) associated microorganisms in shaping the microbial communities, we tested their ability to produce secondary metabolites of bacterial isolates obtained from two co-occurring macroalgae from the Baltic Sea. The experiments were conducted to answer the questions: Is there a common pattern among algae associated organisms even if they co-occur? Do these microbes present different antibiotic patterns among hosts, and are therefore responsible of different associations? In the present study, we investigated the epiphytic bacteria associated with two perennial macroalgae: The red macroalga *Delesseria sanguinea* and the brown macroalga *Fucus vesiculosus*. The surface of both algae were examined seasonally (summer and winter for two years) by scanning electron microscopy and processed in order to isolate associated bacteria, which were classified by phylogenetic analysis based on 16S rRNA gene sequences. The results will be published in **Paper III**, building chapter II of this work.

As mentioned, many of those microorganisms can display pathogenic relationships over macroalgae, affecting physiology and even morphology of the host (Apt *et al*. 1988). A small population of one macroalga in Chile has been observed to produce malformations of the thallus. Because algae are commercially valuable in many parts of the world, pathogenic microorganisms that compromise harvests have been a concern and a subject of research (Bengtsson 2011). Aim of this part of my study was to describe an outbreak occurring in the central coast of Chile, which produced lesions, galls and discolorations on the fronds of the brown macroalga *Durvillaea antarctica*, an important socioeconomic algal resource. Are those malformations produced by a bacterial attack? Or are there any other microorganisms involved? The results will be published in **Paper V**, building chapter V of this work.

3.2. SPECIES UNDER STUDY

3.2.1. *Fucus vesiculosus* Linnaeus 1753 (Fucales, Phaeophyceae) is an intertidal, common brown alga widespread in the Northern Hemisphere (Lüning 1990, Fig. 5a). The alga occurs most on rocky shores usually growing over stones and mussels. It often forms monospecific belts on mid-intertidal rock (Rueness 1977). *Fucus* species are perennial and may live up to 4 years (Lee 2008). Due to its widespread distribution and its structurally important role in intertidal communities, many aspects of the biology of this species have been studied in deep detail (Berger *et al*. 2001, Rindi & Guiry 2004).

The thallus of *F. vesiculosus* is intensely branched and has a variable form which grows until 75 cm height and 2 cm width (Rueness 1977). The branching is dichotomous, with each flattened segment having a prominent central midrib surrounded on both sides by a narrower wing (Fig. 5a), usually bearing scattered cryptoblasts (sterile conceptacles with large number of long hairs) (Lee 2008). Air bladders originate not far from the apex, characteristics of the species, but may depend on environmental conditions (Rueness 1977, Lee 2008, Fig. 5b). The thallus is supported by a short narrow stalk that is attached to a discoid holdfast (Fig. 5b). The internal anatomy of *Fucus vesiculosus* is composed by a mucilaginous cuticle covering the epidermal layers of cells. Inside this is the cortex with the medulla in the center. Hyphae are produced by the inner cortical cells (Lee 2008, Fig. 6b). More detailed structural information was given by Moss (1950) and McCully (1966, 1968).

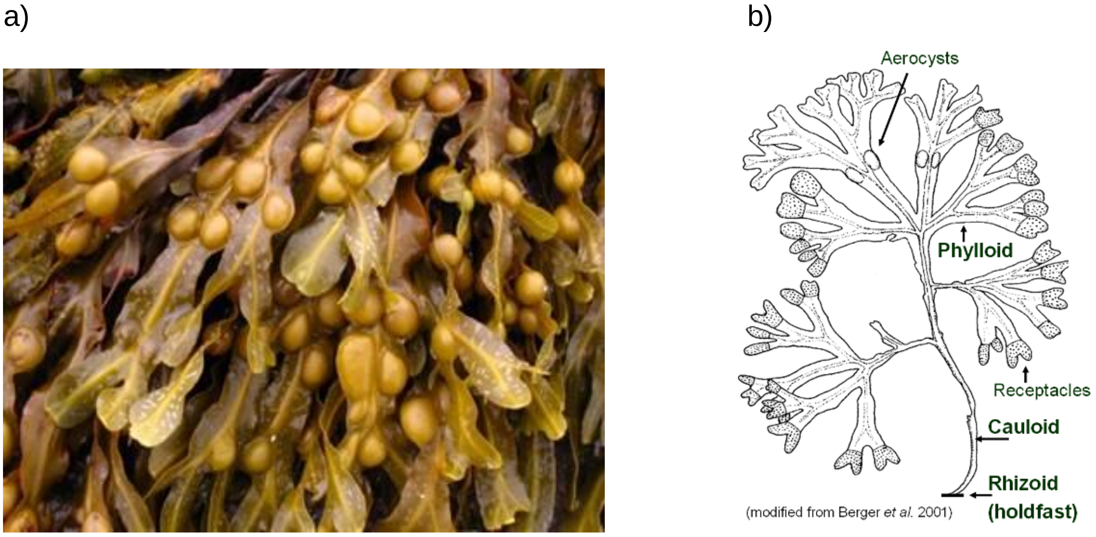

Figure 5. a) *Fucus vesiculosus* in the Baltic Sea. **b)** Schema of the habit of one alga showing the different parts of the frond (rhizoid, cauloid and phylloid), including the air bladders (aerocycsts) and receptacles (after Berger *et al*. 2001).

When reproductive, the tips (in the apical zone) of the thallus of *F. vesiculosus* are swollen into receptacles (full with muscilage), which contain the fertile conceptacles where the gametangia are produced (Fig. 6). The plants are either monoecious (antheridia and oogonia in the same conceptacle) or dioecious. The oogonia yield 8 haploid eggs, which are released to the water in a packet after a maturation phase (Lee 2008, Fig. 6d & 7). *F. vesiculosus* presents an isomorphic life cycle (Fig. S02 in Supplementary information), it is reproductive the whole year. However, in the Baltic Sea two peak periods of reproduction are observed. The main reproduction period is at late May-June (Rueness 1977, Lee 2008). The common names for *F. vesiculosus* are “bladder wrack rockweed” (USA), “lady wrack” (UK) or “black tang” (UK) and “Blasentang” (D) (Guiry & Guiry 2011).

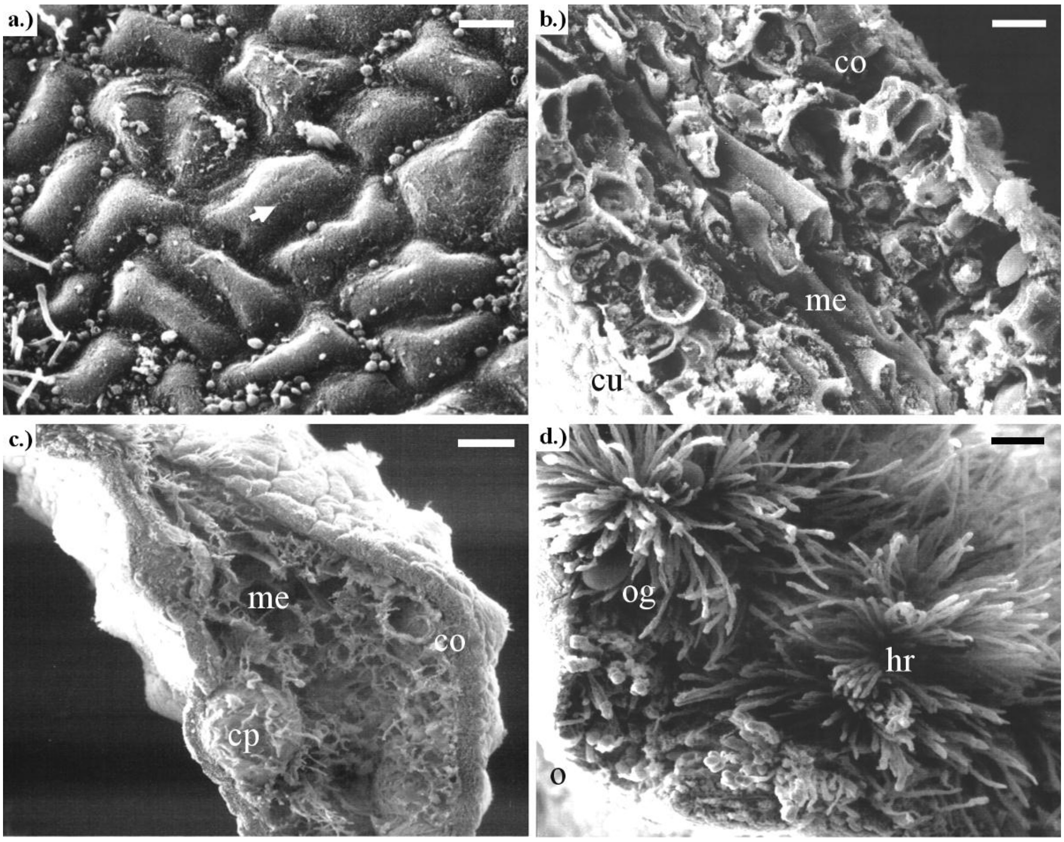

Figure 6. Scanning electron microscopic section through a vegetative (a-b) and a reproductive (c-d) *F. vesiculosus* plant. **a)** Cell (arrow) disposition of the surface of *Fucus*. **b)** Cross section of an immature frond where was possible to observe the internal cell dispositions. **c)** Cross section of a fertile frond with a large internal medullar space and conceptacles. Where cuticle (cu), cortex (co), and the medulla (me) are shown. **d)** In a cross section of a single conceptacle, ostiole (o), female oogonia (og) between a large number of hairs were observed. Scale bar = a) 5 µm; b) 20 µm; c) 500 µm; d) 100 µm.

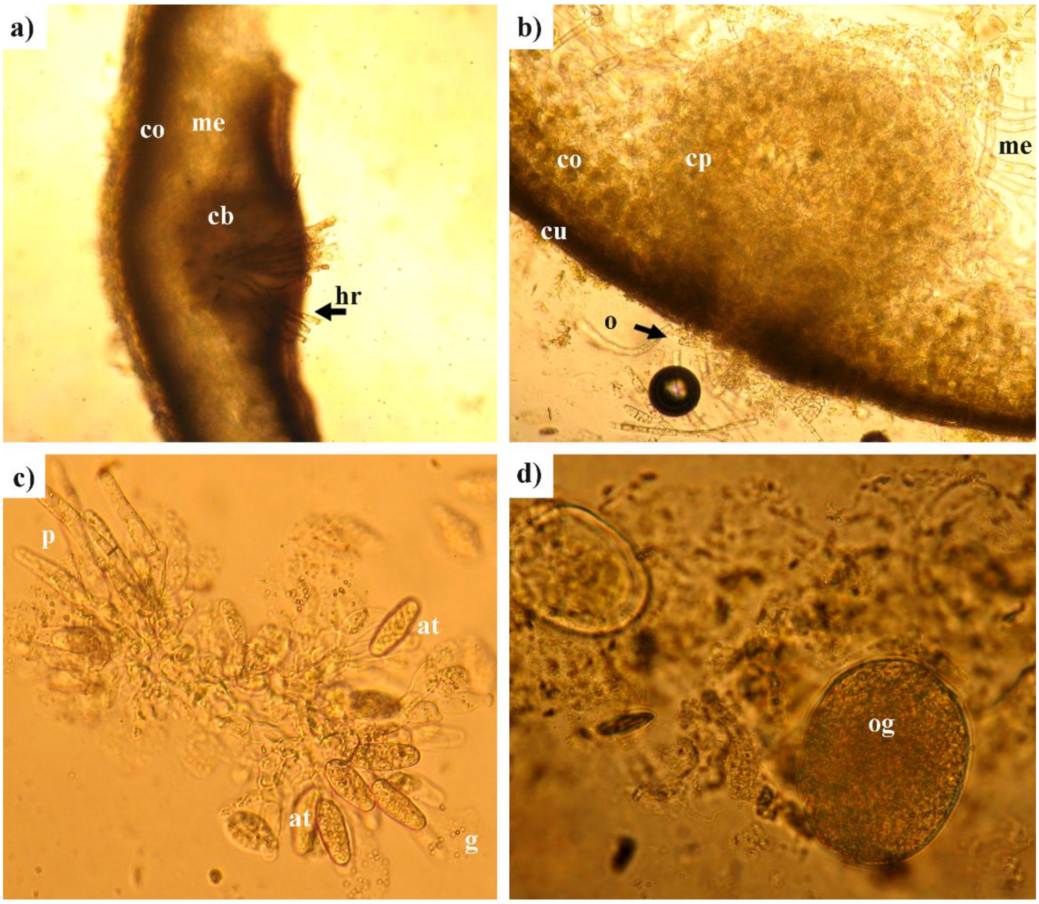

Figure 7. Light microscopic microphotographs of *F. vesiculosus* cross sections. **a)** Vegetative frond showing a cryptoblast (cb) with hairs (hr); **b)** Conceptacle (cp) in a receptacle from a mature frond, ostiole (o), cortex (co), and medulla (me); **c)** Detail of a male antheridia (at) with paraphysis (p), and released gametes (g); and **d)** Detail of an female oogonium (og). Scale = a-b) 10x; c-d) 40x.

3.2.2. *Delesseria sanguinea* (Hudson) Lamouroux 1813 (Ceramiales, Delesseriaceae) is a common subtidal red macroalga, endemic for Europe and distributed from northern Norway and Iceland to northwest Spain and Portugal (Lüning 1990, Potin *et al.* 1992). It is a perennial macroalga and may live around 5-6 years. *Delesseria* grows usually on rocks, stones or shells or epiphytic on other algae (Pankow 1971, Rueness 1977).

The fronds are red-to-pink, till 15 cm high and 1-5 cm wide (Fig. 8a), attached from a thickened discoid holdfast of 300 mm long. They have a short cylindrical stipe (cauloid) that expands forming foliaceous monostromatic blades and pinnately branched (and polystromatic) midribs, characteristic of the species (Fig. 8b, 9b). Apices have an evident apical cell (Lin *et al*. 2001, Guiry & Guiry 2011). Life history is isomorphic and typical for Ceramiales (Figure in S03, Supplementary information). The gametophytes are dioecious; the reproductive structures are placed in small oval, stalked blades, borne on midribs in winter (Guiry & Guiry 2011). This species exhibits a marked seasonality in its vegetative cycle (Fig. 10), which is controlled by photoperiod and temperature (Potin *et al.* 1992, Kain & Bates 1993). New fronds are formed in spring, and are well developed in early summer, but disappear in fall (Rueness 1977, Molenaar & Breeman 1997). More detailed structural information was given by Potter (1889), Maggs & Hommersand (1993) and Lin *et al*. (2001). The common names for *D*. *sanguinea* are “sea beech” (UK) and “ribbeblad” (Sweden) (Guiry & Guiry 2011).

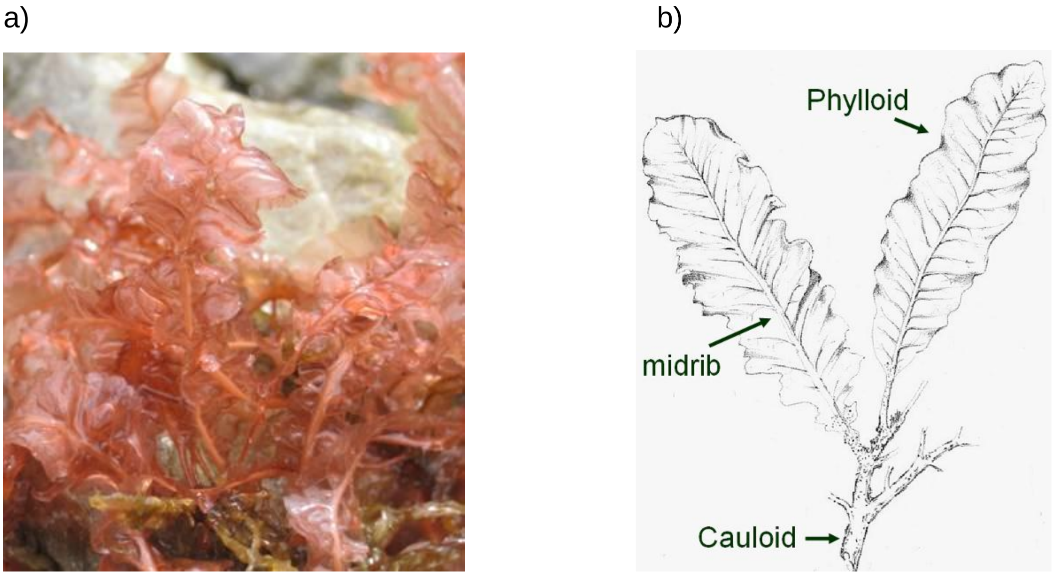

Figure 8. a) *Delesseria sanguinea* in the Baltic Sea. **b)** Schema of the habit of *D*. *sanguinea* modified after Potter (1889).

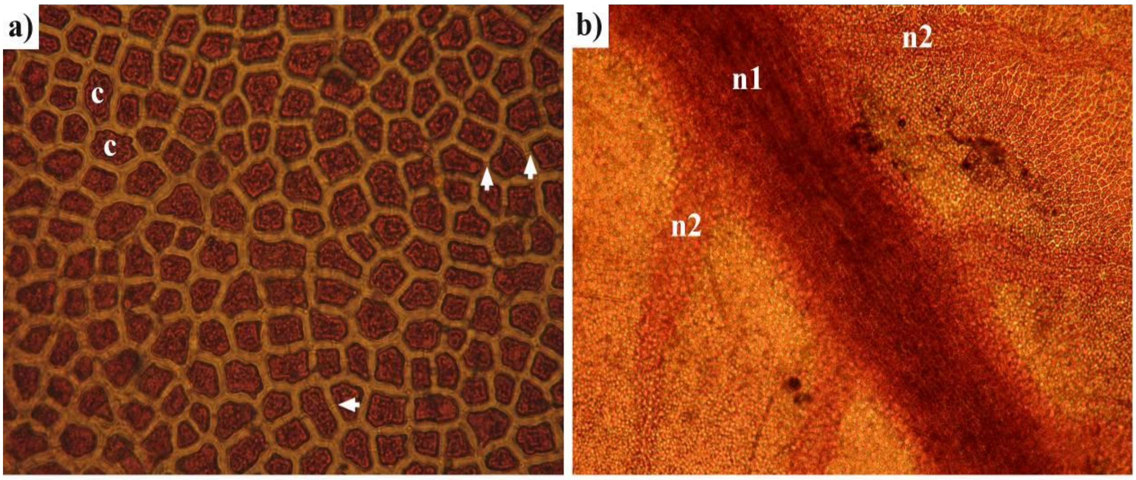

Figure 9. Light microscopic microphotographs of *D*. *sanguinea*. **a)** Detail of the phylloid with the uniform distribution of the cells (c) observed by light microscopy. Red algal pit-connections are visible between cells (arrows); **b)** Detail of the central polystromatic midrib (n1) and the secondary ones (n2) in between the monostromatic phylloid (clear zones). Scale bar= a) 10x; and b) 10x.

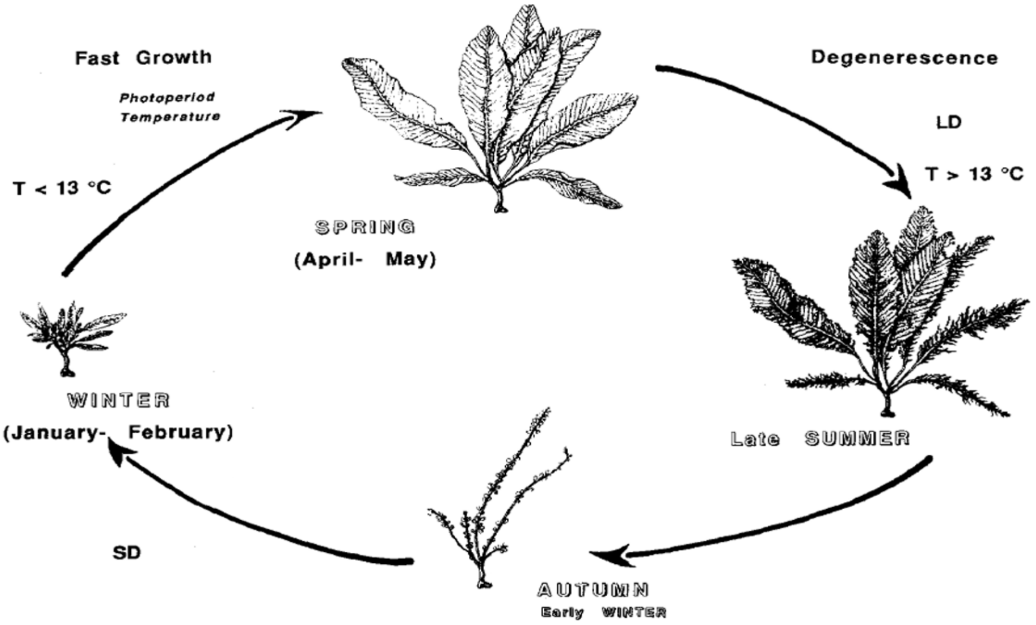

Figure 10. *D. sanguinea* exhibits a marked seasonality in its vegetative cycle controlled by photoperiod and temperature (modified from Potin *et al*. 1992).

3.2.3. *Durvillaea antarctica* (Chamisso) Hariot 1892 is a large brown seaweed which belongs to the order Fucales (Phaeophyceae) (Cho *et al*. 2006, Fig. 11). It has a subantarctic distribution limited only to the Southern Hemisphere, specifically South America, New Zealand and subantarctic islands (Ramírez & Santelices 1991; Hoffmann & Santelices 1997). There, this genus plays an important role in the occupation of habitats and the structuring of coastal communities. *Durvillaea* species frequently dominate the intertidal and shallow subtidal flora in regions with a stable, rocky substratum and exposed to wave force (Cheshire *et al*. 1995). There, it is the dominant primary producer and repository of organic material and energy (Santelices *et al*. 1980).

Together with *Lessonia nigrescens* Bory (Phaeophyceae), *D. antarctica* forms conspicuous belts in high-energy rocky shores, where its long, floating fronds can reach a length of more than 15 m (Lawrence 1986; Westermeier *et al*. 1994). Like other large brown algae in temperate coasts of the world, they modify the microenvironments providing much of the vertical structure inhabited by smaller species from all domains of life (Taylor & Schiel 2000; Jaramillo *et al*. 2006). Its holdfast by itself constitutes temporal or permanent habitats for a rather large number of different species of invertebrates, giving them shelter, conforming spawning and habitat substrates, and minimizing wave and predation pressure (Vásquez & Buschmann 1997).

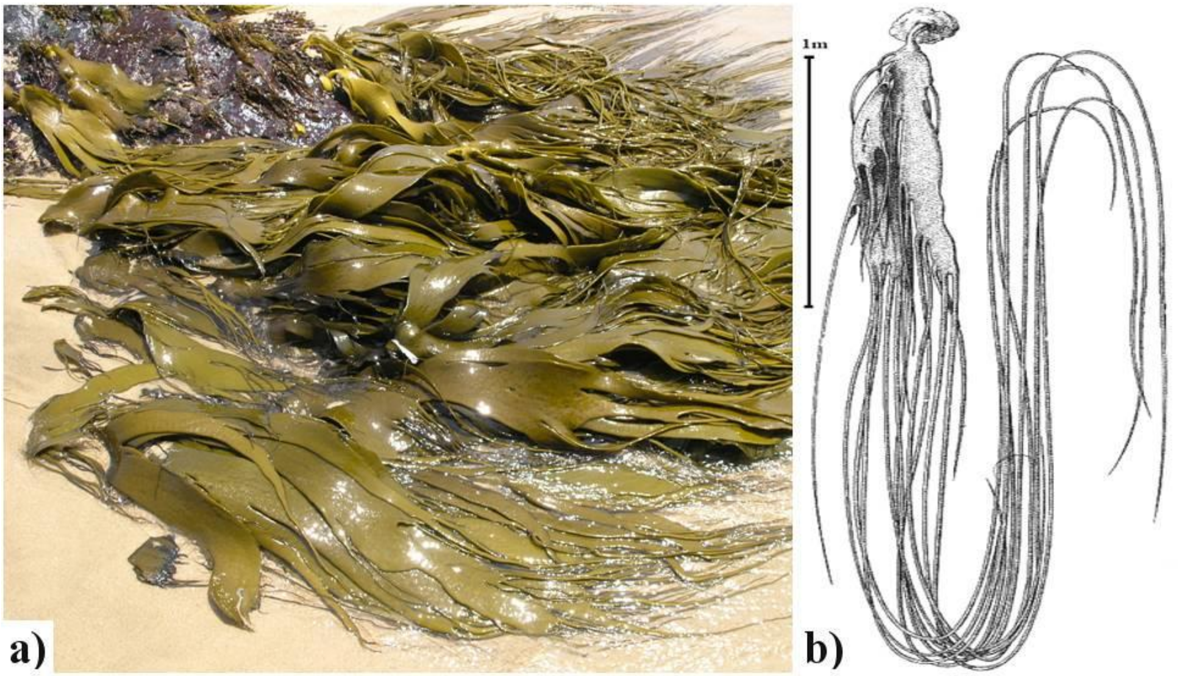

Figure 11. a) *Durvillaea antarctica* in Chile, Pacific Ocean. **b)** Schema of an adult frond of *D. antarctica* after Hoffmann & Santelices (1997)

Healthy fronds of the macroalgae were characterized by firm, elastic, smooth, and shiny thalli, in which the color varied normally from brown till dark-olive tones (Fig. 11). In cross sections a meristoderm, a cortex and a medulla was observed (Fig. 12b). As described by Naylor (1949), meristoderm consisted of a layer of 5-6 small, polyhedral, brick-shaped cells (Fig. 12c-d). The cortical zone was formed by regular radial rows of 8-15 elongate cells, and the medullary zone of irregularly interwoven hyphae (Fig. 12b, d), which originated air-filled cavities separated by septa (as described by Hoffmann and Santelices 1997; Collantes *et al*. 2002). Gametes in antheridia and oogonia (in a male or female fronds respectively), differentiated inside ovoid conceptacles that develop in the cortical zone with a small ostiole that opens to the surface (Fig. 12d) as described by Hoffmann and Santelices (1997). In transversal sections of a reproductive frond, one layer of conceptacles was observed (Fig. 12b,d).

More detailed structural information on morphology, reproduction (Fig. S04 in Supplementary information), and cytology of *D. antarctica* is given by Herriott (1923), Naylor (1949), Roberts (1979), and Collantes *et al*. (2002). The common names for *D. antarctica* are “cochayuyo”, “ulte” and “huilte” (Chile) as well as bull kelp (UK) (Hoffmann & Santelices 1997).

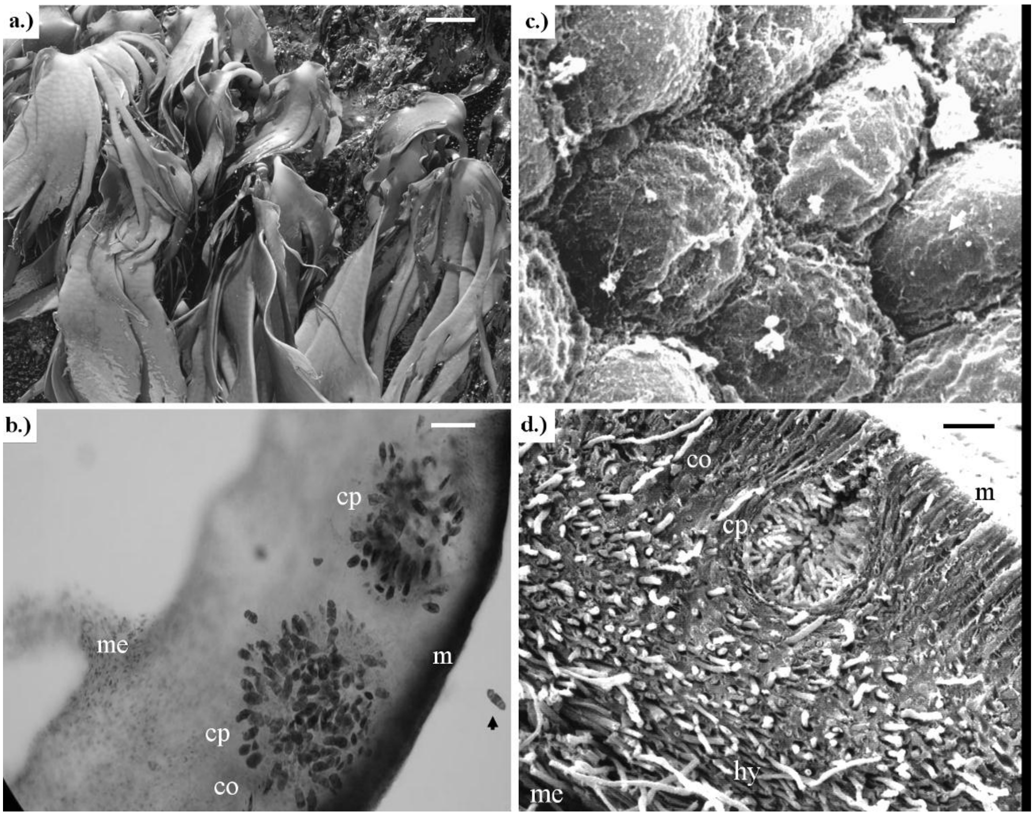

Figure 12. a) *Durvillaea antarctica* in the natural environment in Chile; **b)** Light microscopic microphotograph of a cross-section of a dioecious frond of *D. antarctica* (stained with aniline blue) showing two female conceptacle (cp) with one free oogonium (black arrow), meristoderm (m), cortical (co) and medullary (me) zones in a normal frond (picture: A. Núñez); **c)** Scanning electron microphotograph (SEM) with details of the surface of the thallus, and cells disposition in the algal surface (arrow shows one cell); **d)** Detail of a cross-section of a thallus using SEM showing early stages in conceptacle development (cp), meristoderm (m), cortical (co) and medullar (me) tissue with normal swift hyphae (hy). Scale bar: a) 10 cm; b) 100µm; c) 2µm; and d) 50µm.

4 CHAPTER I

Phylogenetic relationships among bacteria described from algae: A distinct source of new taxa

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ABSTRACT

Bacteria are an inherent part of the physical environment of algae. Recent investigations have determined that bacterial communities associated with algae are highly specific to their host. Worldwide several new bacterial species and genera have been isolated from algae, but the phylogenetic relationships between these bacteria remains unresolved. We conducted a phylogenetic study based on 16S rRNA gene sequences available in GenBank of 101 bacterial species (type strains only) which have been described as new species and came from eukaryotic macro- and micro-algal sources. We observed a clear representation of 6 major bacterial lineages. The major lineage corresponded to Bacteroidetes with 42 newly described bacterial species, followed by Proteobacteria (including Alpha and Gammaproteobacteria) with 36 species. Firmicutes, Actinobacteria, and Verrucomicrobia contributed in a lesser extent. Based on the information of each strain description, 32% of all new bacterial species were able to decompose macroalgal polysaccharides, especially by member of Bacteroidetes and Gammaproteobacteria. On the other hand, most of the bacteria described from marine microalgae grouped into the *Roseobacter* clade (Alphaproteobacteria), indicating that some members of this group are well adapted to living in close association with phytoplankton. We confirmed algae as a distinct source for new bacterial taxa. Although such associations can be random or specific, they could be explained by evolutionary adaptations through metabolic pathways, niche specificity or mutualistic relationships. We are starting to discover the diversity the algae-bacteria relationship in nature. Algae are key components of the aquatic environments, not only as microhabitats for invertebrates and fishes, but also as substrates for millions of microorganisms waiting to be discovered.

INTRODUCTION

Seawater contains up to millions of viruses and bacteria, thousands of fungi and microalgae, and hundreds of microscopic larvae and spores per mL (Harder 2009). Many of those microorganisms have biofouling potential or interact with marine macroorganisms, in pathogenic or nonpathogenic ways. Algae are especially susceptible to epibiosis being covered by diverse marine microbial communities. The algae and their associated communities form complex and highly dynamic ecosystems, consisting of a diverse range of organisms (Holmström et al. 2002). Bacteria are dominant among the primary colonizers of algal surfaces (Lachnit et al. 2009). Nevertheless, biofouling is not a simple process: Strain-specific preferences for certain substrates and strain-specific pro- or antifouling activities of algal metabolites play an important role in formation of specific communities (Wahl et al. 2010; Sneed and Pohnert 2011). In recent investigations it has been suggested that different specific ecological associations have evolved between algae and bacteria (i.e. Biegala et al.

2002; Croft et al. 2005; Dimitrieva et al. 2006; Grossart and Simon 2007; Amin et al. 2009; Gärdes et al. 2010; Goecke et al. 2010; Hollants et al. 2011; Wagner-Dobler et al. 2011), and that we are still far from an understanding of the ecological role both host and epibionts play in nature.

Specific bacterial communities associated to algae

Phylogenetic studies of epiphytic bacteria provided an insight into the complex bacterial communities associated with algae (Penesyan et al. 2009). Although comprehensive assessments of whole bacterial communities on algal surfaces are relatively few (Burke et al. 2011), the available data have suggested that bacterial communities associated with algae are specific to the host. These results are based on 16S rRNA gene sequencing clone libraries, denaturing gradient gel electrophoresis (DGGE), or restriction fragment length polymorphism (RFLP) fingerprinting, and show a difference of number and generic composition of the algal communities to the surrounding seawater (Grossart et al. 2005; Staufenberger et al. 2008; Bengtsson et al. 2010; Burke et al. 2011), from other living surfaces (Longford et al. 2007), and even between different algal species (Hold et al. 2001; Meusnier et al. 2001; Jasti et al. 2005; Lachnit et al. 2009; Hengst et al. 2010; Namba et al. 2010; Nylund et al. 2010; Sneed and Pohnert 2011). Different species of marine algae in the same habitat showed different bacterial communities (Lachnit et al. 2009; Nylund et al. 2010), while the same algal species, even from different localities, demonstrated high similarities in the composition of the associated microbial communities (Staufenberger et al. 2008; Sneed and Pohnert 2011). Hence, certain bacteria are consistently found as algal epiphytes suggesting that they associate highly specific (see Table S1, supporting information).

Worldwide, several new bacterial species, genera and orders have been described after isolation from algal materials, suggesting that algae represent a distinct source for new bacterial taxa, even if the origin does not necessarily indicates a specific association (Goecke et al. 2010). To proof that algae are in fact a distinct habitat of microorganisms, i.e. some bacterial groups are better represented in algal communities than others from the surrounding environment, a comprehensive analysis is necessary. We decided to conduct a phylogenetic study of all those bacteria that were described to be isolated from macro- and microalgae from all eukaryotic algal divisions. The phylogenetic study was based on 16S rRNA gene sequences available in GenBank in September 2011.

MATERIAL AND METHODS

Sequence handling. A set of 101 16S rRNA gene sequences from bacterial species isolated and described from algal sources (meaning the eukaryotic algal Divisions Rhodophyta, Ochrophyta and Chlorophyta but excluding prokaryotic Cyanobacteria) was selected and downloaded from GenBank taxonomy browser on 20 September 2011. The selection criteria applied were bacteria isolated and described from any eukaryotic algal sources including both micro- and macroalgae from marine and limnic environment, which include planktonic and benthonic species, either from identified or unknown species, algal mats, entire plants or pieces of algae, from natural populations or stranded and degraded algal material, as well as from blooms or phycosphere environment. The focus was set on newly described bacterial species (including proposed new species in process for confirmation) instead of a metagenomic analysis with all the information available in GenBank to avoid redundancy of the data, and simplify the phylogenetic threes. This focus further enabled the use of strain specific information for the analysis of possible associations, i.e. polysaccharide degradation ability (see below in database mining).

A list of the type strains and their respective nucleotide accession numbers, as well as the host, is provided in Table 2 and 3 in appendixes. The sequences were aligned using ClustalW implemented in Bioedit version 7.0.9.0 (Hall 1999). Phylogenetic analysis based on Maximum Likelihood method was conducted using the Mega5 package (Tamura et al. 2011). The phylogenetic analysis was conducted using the General Time Reversible (GTR) model with Gamma distributed rates among sites (4 discrete Gamma categories). All positions based on the partial deletion treatment with a site coverage cutoff of 95% were included in the phylogenetic calculations. The phylogeny was tested by bootstrap method with 100 bootstrap replications. Nearest Neighbor-Interchange (NNI) was used as ML Heuristic Method based on an automatically generated initial tree.

Database mining. The ability of the bacterial species to decompose major algal polysaccharides was considered. The information was obtained from strain descriptions and recent reviews on alginase, carrageenase and agarase activities (see Wong et al. 2000; Michel et al. 2006; Fu et al. 2010; and Goecke et al. 2010). Additionally, the Braunschweig Enzyme Database BRENDA (Technische Universität Braunschweig, Germany) was searched for the mentioned enzymes plus ulvanases, laminarases and fucanases, available online http://www.brenda-enzymes.org (Schomburg et al. 2004).

RESULTS

According to the selection criteria, 16 S rRNA gene sequences of 101 bacterial species were identified in GenBank, which were described as new species, genus or higher taxon after isolation from algal species.

Algal sources of the new species. The originating algae comprised more than 42 algal species (16 spp. that belong to Ochrophyta, 11 to Chlorophyta and 8 to Rhodophyta, 6 to Dinoflagellata and 1 to Cryptophyta). Most of the newly described bacterial species were obtained from macroalgae (82 species) in contrast to microalgae, which were the source for 19 new species. Chlorophyta was the source of most newly described bacteria with 30% of all (Fig. 1). Just within the macroalgae (comprising members of Chlorophyta, all Phaeophyceae and all Rhodophyta), Chlorophyta were the source for most of the new species (27 spp.), followed by Phaeophyceae (Ochrophyta, 23 bacterial species) and Rhodophyta (12 spp.). 20 species were retrieved from unidentified macroalgae. Within the microalgae (comprising members of Chlorophyta, Dinoflagellata, Cryptophyta and the rest of Ochrophyta but excluding from them Phaeophyceae, and no Rhodophyta), Ochrophyta were the source for 4 bacterial species isolated from the algal classes Bacillariophyceae. 8 bacterial species were isolated from Dinoflagellata and 1 new bacterial species was isolated from Cryptophyta (Table 3 in appendixes). Microalgae from the Division Chlorophyta were the source of 4 new bacterial species (21%) as well, and 2 newly bacterial species (11%) were isolated from unspecified phytoplanktonic sources.

The 101 bacterial taxa corresponded to 71 genera from which 36 were also newly described. One species, *Kiloniella laminariae* conformed also a new taxonomical family (Kiloniellaceae), and new order (Kiloniellales) in the Alphaproteobacteria (Wiese et al. 2009a).

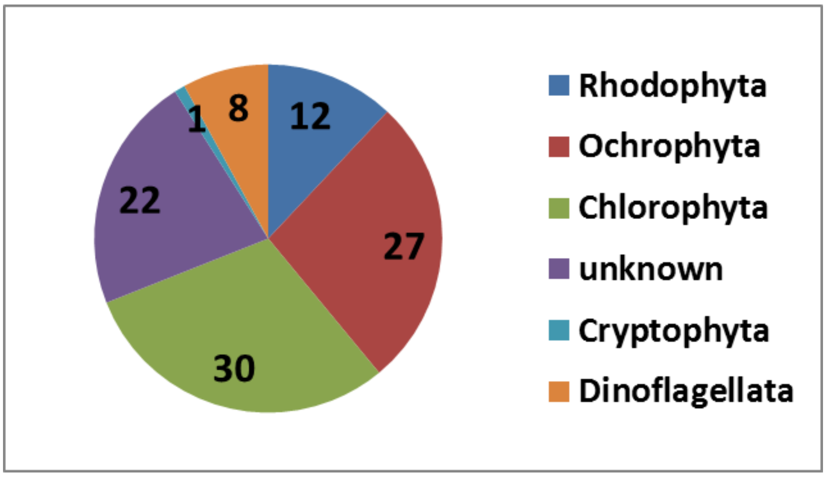


Fig. 1. Percentage of the origin of newly described species of bacteria from algal sources. The three main eukaryotic groups of algae are represented. Chl = Chlorophyta, Cr = Cryptophyta, Di = Dinoflagellate, Och = Ochrophyta, Rho = Rhodophyta, and unk = unidentified algae.

Phylogenetic analysis. The 16S rRNA sequences of those 101 bacterial species were utilized to construct the phylogenetic relationship among them. Six major bacterial domains were represented: Actinobacteria, Alpha- and Gammaproteobacteria, Bacteroidetes, Firmicutes, and Verrucomicrobia. From the phylogenetic analysis, we observed that the major cluster corresponded to Bacteroidetes (42%), seconded by Proteobacteria (35%), and to a lesser extent Firmicutes (10%), Actinobacteria (8%), and Verrucomicrobia (5%) (Fig. 2).

From the Gram-negative bacteria, the Bacteroidetes cluster contained 42 new bacterial species affiliating to the genera *Aequorivita*, *Algibacter**, *Algoriphagus*, *Aquimarina*, *Arenibacter**, *Aureispira**, *Cellulophaga**, *Croceitalea**, *Flagellimonas**, *Flavobacterium*, *Formosa**, ‘*Fucobacter*’, *Kordia**, *Kriegella**, *Lacinutrix*, *Maribacter*, *Mesonia**, *Persicivirga*, *Pibocella**, *Pseudozobellia**, *Roseivirga**, *Tenacibaculum*, *Ulvibacter**, *Winogradskyella**, and *Zobellia**. Of these 25 genera, 16 (64%) were first described based on the algae derived isolates (marked with an asterisk).

The Proteobacteria cluster contained 36 bacterial species. Alphaproteobacteria contained 19 species affiliating to 17 genera: *Brevundimonas*, *Dinoroseobacter**, *Erythrobacter**, *Hoeflea*, *Huaishuia**, *Kiloniella**, *Labrenzia**, *Lentibacter**, *Marinovum**, *Maritalea*, *Marivita**, *Nitratireductor*, *Oceanicaulis**, *Paracoccus*, *Planktotalea**, *Roseibium**, and *Roseovarius*. Gammaproteobacteria contained 17 species affiliating to *Agarivorans*, *Algicola**, *Ferrimonas*, *Leucothrix*, *Marinobacter*, *Microbulbifer*, *Pseudoalteromonas*, *Pseudomonas*, *Shewanella*, and *Vampirovibrio** species (11 genera). 13 genera (11 for Alpha- and 2 for Gammaproteobacteria) were newly described for Proteobacteria based on the algae derived strains (marked with an asterisk).

The Verrucomicota cluster contained five bacterial species affiliating to the four genera *Haloferula*, *Luteolibacter*, *Phycisphaera**, and *Roseibacillus*. One genus was newly described based on an alga derived species (marked with an asterisk).

From the Gram-positive bacteria, the Firmicutes cluster contained 10 bacterial species affiliating to *Alkalibacterium*, *Bacillus*, *Halolactibacillus**, *Leuconostoc*, *Oceanobacillus*, *Paraliobacillus**, and *Salinicoccus* species. 2 of the 7 genera were newly described based on the algal derived species. The Actinobacteria cluster contained 8 bacterial species affiliating to *Aeromicrobium*, *Agrococcus*, *Brevibacterium*, *Demequina*, *Koreibacter**, *Labedella**, *Phycicoccus**, and *Phycicola** species. From which 4 (of 8) genera were newly described based on the algae derived strains (marked with an asterisk).

Based on the information of each strain description, supported by recent reviews on microbial polysaccharide degradation and BRENDA database, 32% of all bacterial taxa (i.e. 32 species) originated from algal sources are able to decompose at least one of the major algal polysaccharides (i.e. agar, alginate, carrageenan, fucan, laminaran and ulvan) (Table 1,

Fig. 3). 30 species corresponded to bacteria isolated from macroalgae and just 2 from bacteria isolated from microalgal sources (Table 3 in appendixes).

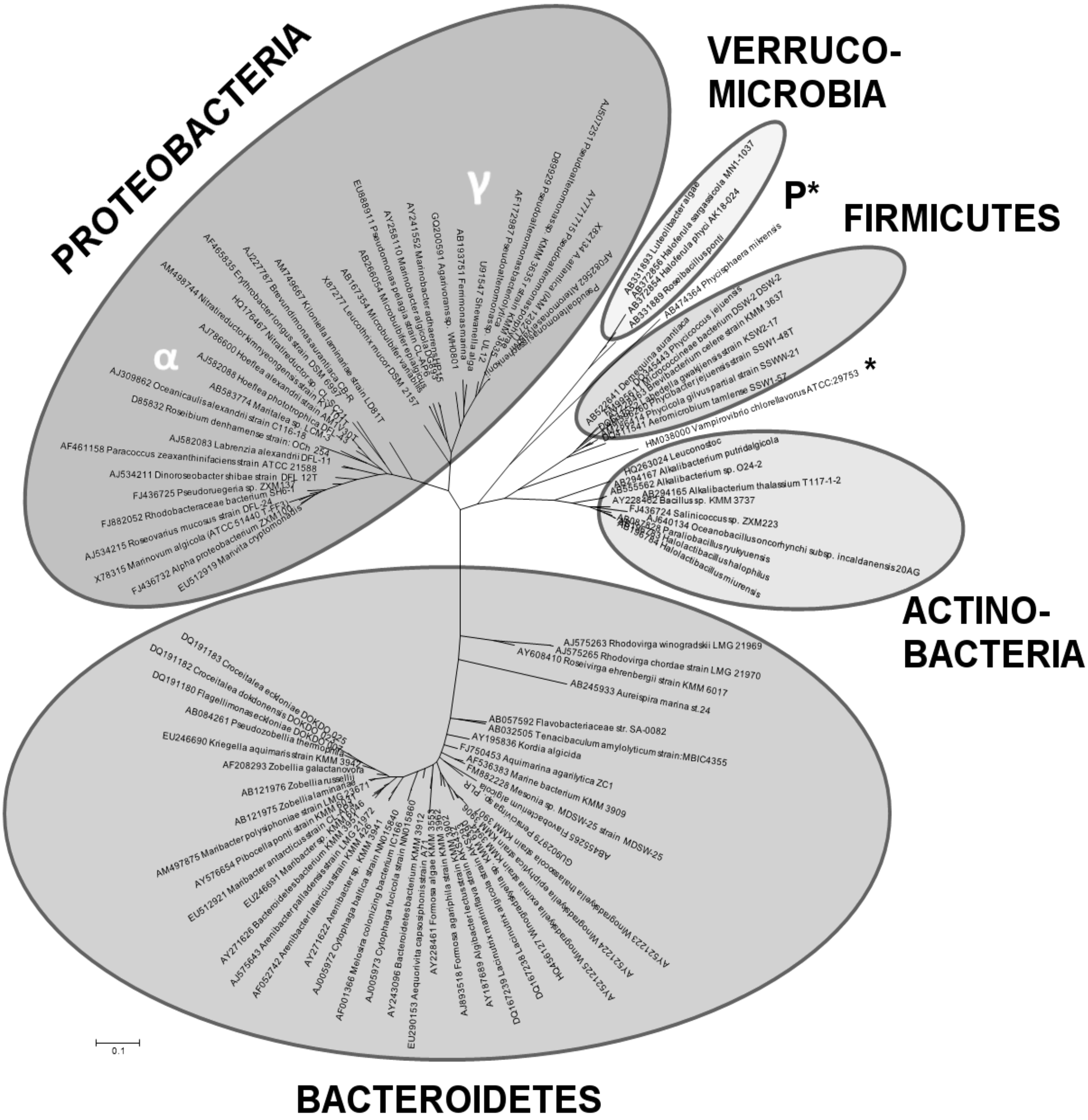
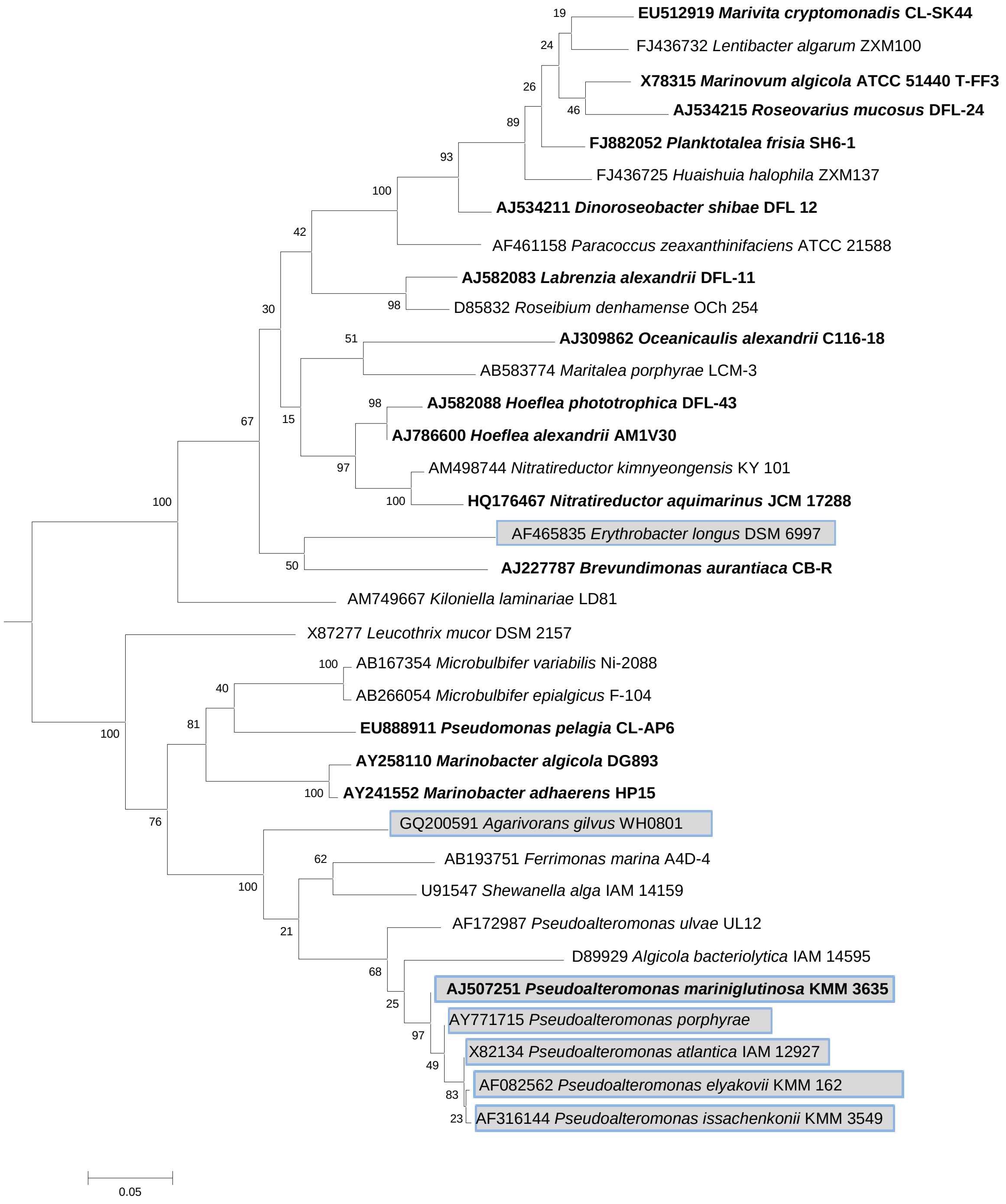


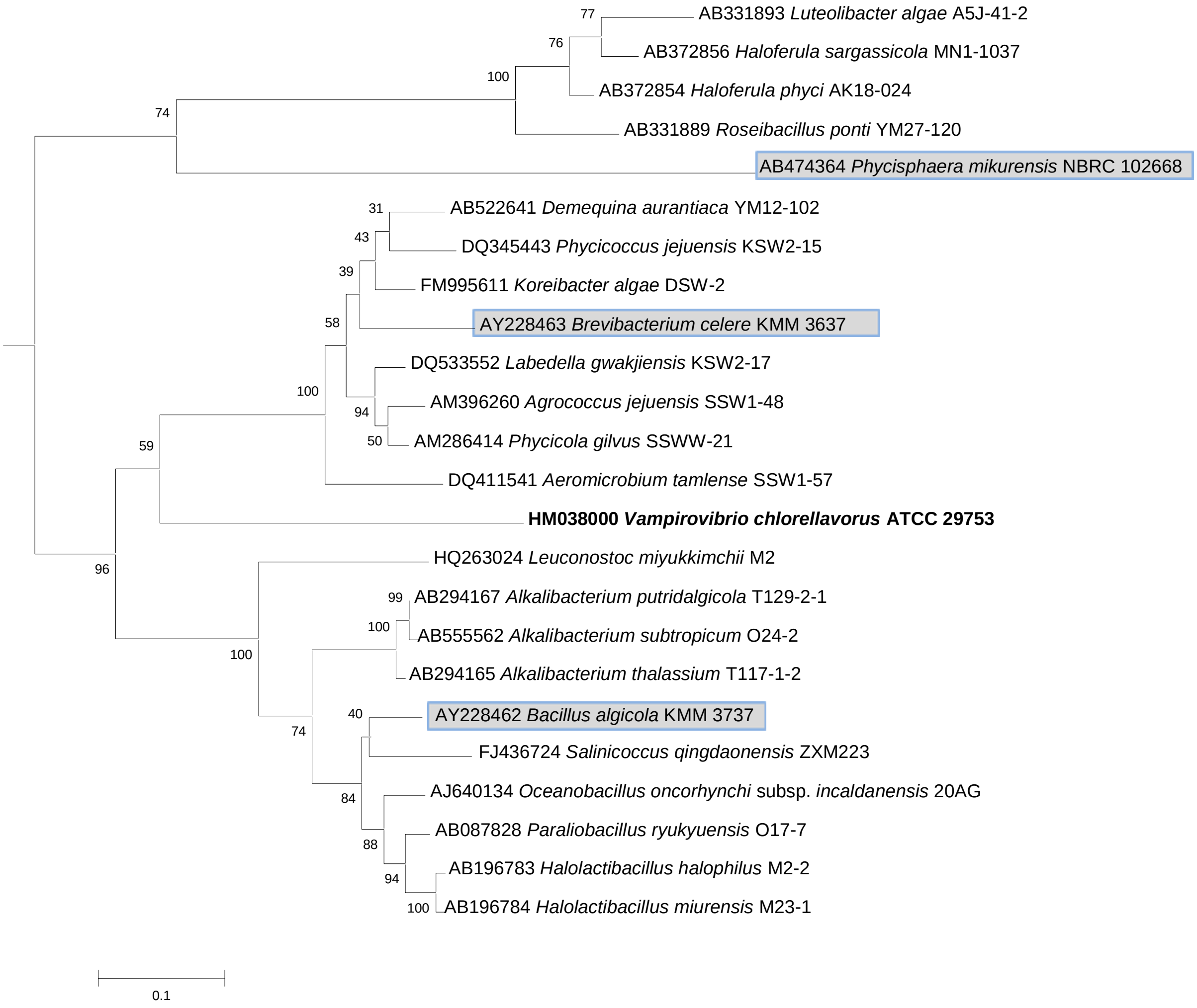
Fig. 2. Phylogenetic tree of the 16S rRNA gene sequences from bacterial species described as new after isolation from algae. A clear representation of 6 major bacterial phyla was observed: Bacteroidetes, Proteobacteria (with representatives of Alpha and Gammaproteobacteria), Verrucomicrobia, Firmicutes, and Actinobacteria. One member representing Planctomycetes (P*) was also observed. Apparently, one bacterial taxon (*Vampirovibrio*) needs further taxonomical revision (marked with an asterisk). The phylogenetic analysis based on Maximum Likelihood method was conducted using the Mega5 package. The phylogeny was tested by Bootstrap method with 100 bootstrap

replications. Nearest Neighbor-Interchange (NNI) was used as ML Heuristic Method based on an automatically generated initial tree. The scale bar represents 10% divergence.

a) Proteobacteria group



b) Verrucomicrobia, Planctomycetes, Actinobacteria and Firmicutes group



c) Bacteroidetes group

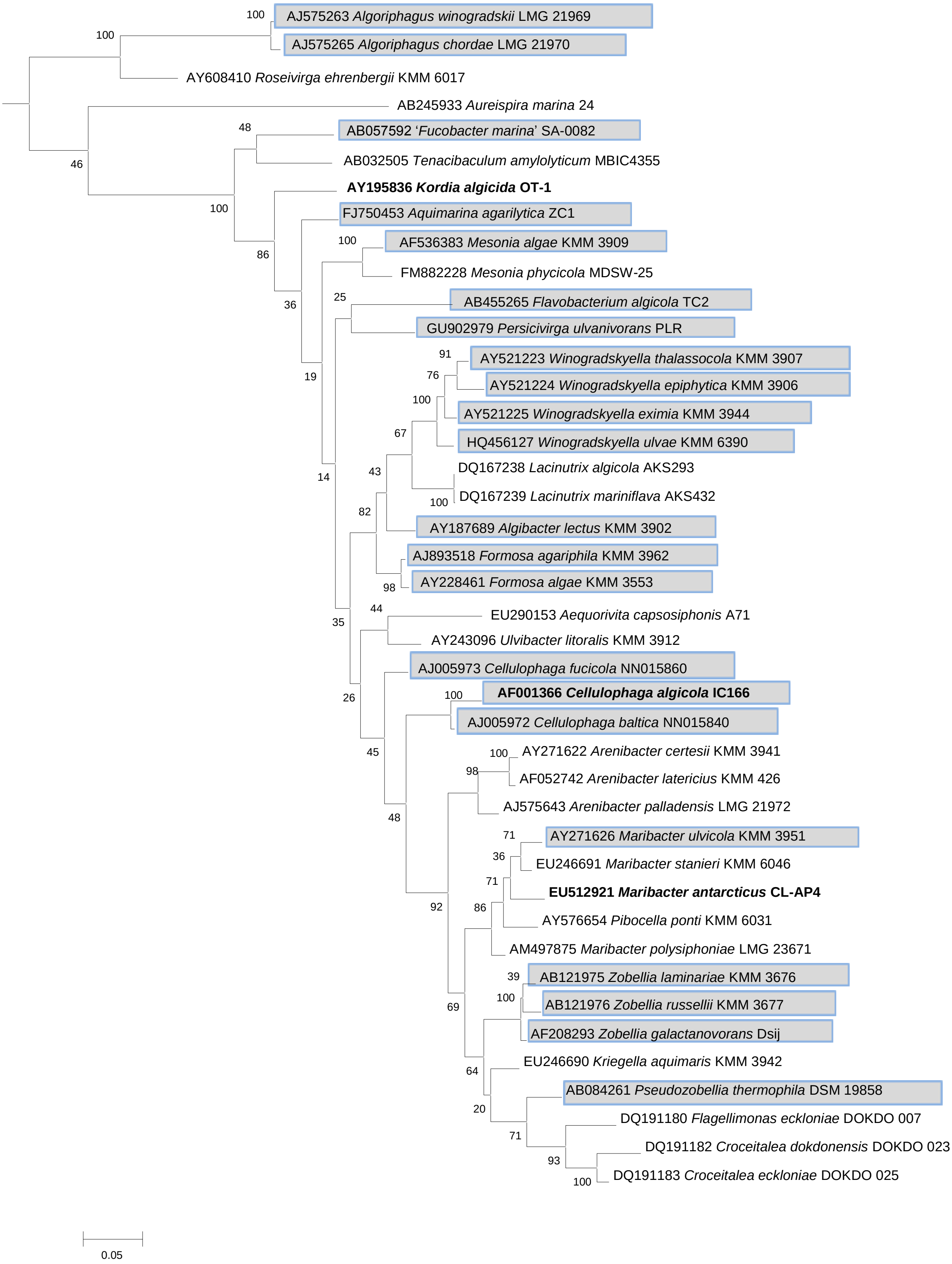


Fig. 3. Detail of the phylogenetic tree of the 16S rRNA gene sequences from bacterial species isolated from algae. The phylogenetic tree of sequences belonged to Proteobacteria

are represented by subtree **a)**, the phyla Verrucomicota, Plactomycetes, Firmicutes and Actinobacteria are prepresented in subtree **b)**, and Bacteroidetes in **c)**. Strains which have presented the ability to degrade algal polysaccharides (i.e. agar, alginate, carrageenan, laminaran, fucan, and ulvan) are presented in grey boxes. Sequences obtained from microalgal sources are displayed in bold. NCBI accession numbers and strain name are given, the bar represent 5% divergence.

Table 1 Number of bacterial strains which have presented the ability to degrade algal polysaccharides according to the algal origin where they were described. The eukaryotic algal divisions are represented by Chl (Chlorophyta), Och (Ochrophyta), Rho (Rhodophyta), and "unk" for the unidentified algae. The organizational form of the algal of source was given as macroalga (Macro) or microalga (Micro). The presence of the enzyme β-glucosidase (*) in part responsible of cellulose degradation is also given.

	Chl	**Och**	**Rho**	**Cr**	**Di**	**Unk**	**Macro**	**Micro**
Agar	8	9	3	0	0	1	19	2
Alginate	6	8	0	0	0	1	15	0
Carrageenan	0	3	2	0	0	0	4	1
Fucan	1	2	0	0	0	0	3	0
Laminaran	0	2	0	0	0	0	2	0
Ulvan	1	0	0	0	0	0	1	0
Cellulose*	4	6	1	0	2	6	17	2

DISCUSSION

The presented research was carried out in order to contribute towards further understanding of algae-bacteria relationships. To proof that algae are in fact a distinct habitat of microorganisms, i.e. some bacterial groups are better represented in algal communities as others from the surrounding environment, a comprehensive analysis is necessary. For this purpose we focused on newly described bacterial species (with available 16S rRNA gene sequences), which were obtained from algal sources in previous studies.

An evaluation of 16S rRNA gene sequences in GenBank revealed the description of 101 new bacterial species that have been isolated from algal sources, confirmating that algae represent a source for new bacterial taxa (Table 2 in appendixes). Although many of the algal sources were not specified, there is undoubly a large fraction of algae that have not been studied yet. Only 42 algal species were identified as a source for isolation of microbes, from which 17 genera belong to Ochrophyta (brown algae), followed by 8 from Chlorophyta (green algae), and 6 from Rhodophyta (red algae). Macroalgae have been in focus for isolation of associated bacteria: 81% of the newly described bacterial species originated from macroalgal sources and just 19% from microalgae (Table 2). Despite the higher diversity of microalgae, which would indicate a higher diversity of associated bacteria, and due to the absence of adequate sampling methods (Schäfer et al. 2002), the availability of sufficient algal material may favour the preference to work with macroorganisms. Most of the new bacterial descriptions concentrated in few common algal genera, e.g. the cosmopolitans *Ulva* (*Enteromorpha*), *Porphyra*, *Prorocentrum* and *Alexandrium*, and, as well as other typical algae like *Fucus* and *Saccharina* spp.

By a phylogenetic analysis of cultivated bacteria isolated and described worldwide from algal sources, we observed a clear representation of 6 major bacterial phyla (Fig. 2). The major cluster corresponded to Bacteroidetes and Proteobacteria (including Alpha and Gammaproteobacteria), and in a lesser extent Firmicutes, Actinobacteria, and Verrucomicrobia. Bacteria belonging to those lineages are usually founded in aquatic environments (Longford et al. 2007; Bruckner et al. 2008; Tujula et al. 2010; Lachnit et al. 2011).

Proteobacteria and Bacteroidetes are dominant in aquatic environments (Cottrell and Kirchman 2000a; b; Biegala et al. 2002; Simonato et al. 2010), and it seems to be reflected also in the discovering of new bacterial taxa from algal sources (together 77% of all). A prevalence of sequences from these two bacterial groups has been reported in cultivation and DGGE based studies of phytoplankton (Riemann et al. 2000; Knoll et al. 2001; Schäfer et al. 2002; Makk et al. 2003; Green et al. 2004; Jasti et al. 2005; Bruckner et al. 2008), green macroalgae (Burke et al. 2011, Hollants et al. 2011), and brown macroalgae (Wiese et al. 2009b; Bengtsson et al. 2010; Lachnit et al. 2011), and hence appear to emerge as important associates of algae in general (Tujula et al. 2010).

Bacteroidetes is one of the major bacterial lineages in marine systems, among the most abundant microbes in coastal marine waters, and usually one of the dominant clades during phytoplankton blooms (Alonso et al. 2007; Simonato et al. 2010). Members of this phylum have been reported as living associated with algae from different parts of the world (Kong and Chan 1979; Shiba and Taga 1980; Beleneva and Zhukova 2006; Staufenberger et al. 2008). Bacteriodetes are increasingly regarded as specialists for the degradation of high molecular weight organic matter (Cottrell and Kirchman 2000a). Members of this group are very versatile in the range of biopolymers they can use as carbon and energy source, especially in the form of polysaccharides and proteins (Thomas et al. 2011) (Table 3 in appendixes). Indeed, many marine species use cell-wall components and exudates from macroalgae as nutrient sources (Johansen et al. 1999), which may explain the high number of Bacteroidetes species (42 spp.) that have been found and described from algal sources.

Both, the fact that Bacteroidetes are often abundant in nutrient rich waters where biomacromolecules accumulate (Cottrell and Kirchman 2000a), and that macroalgae release large amounts of organic carbon into the surrounding environment (Wada et al. 2007), could partly explain the possible associations between those bacteria and the alga. Interestingly, several diseases of macroalgal species such as the ‘anaaki disease’ on *Porphyra yezoensis* Ueda 1932, the ‘ice-ice disease’ on *Eucheuma denticulatum* (Burman) Collins and Hervey 1917 and *Kappaphycus alvarezii* (Doty) ex Silva 1996, the ‘spot-rotting disease’ and the ‘shot hole disease’ on *Undaria pinnatifida* (Harvey) Suringar 1873, are caused by members of Bacteroidetes (Thomas et al. 2011). Also, members of the genera *Zobellia*, *Cellulophaga*, and *Kordia* have algicidal activities, and have been referred to as a possible control for blooms of microscopic algae (Sohn et al. 2004; Thomas et al. 2011).

Proteobacteria is the second major bacterial group of new bacterial taxa in the presented phylogenetic analysis (Fig. 2). This group is the largest and most physiologically diverse of all Bacteria, including many phototrophic organisms (Kersters et al. 2005). Fluorescent *in situ* hybridation (FISH) and 16S RNA gene cloning experiments have indicated Proteobacteria to be dominant members of bacterioplankton communities in surface waters, where in terms of abundance Alphaproteobacteria was the dominant group, followed by Gammaproteobacteria (González and Moran 1997; Cottrell and Kirchman 2000b, Moran et al. 2007). Alphaproteobacteria are known to be associated ubiquitously with diatoms, independent of the habitat of the algae (Riemann et al. 2000; Knoll et al. 2001; Makk et al. 2003; Grossart et al. 2005; Buchan et al. 2006; Bruckner et al. 2008). Especially the *Roseobacter* clade (Alphaproteobacteria) has been enumerated as the most abundant group within the bacterial assemblages associated with some marine algal cultures and phytoplankton blooms in nature (Prokic et al. 1998; Alavi et al. 2001; Schäfer et al. 2002), indicating that members of this group are well adapted to living in close association with phytoplankton (Jasti et al. 2005). This correlates with the circumstance, that the majority (68%) of the new bacterial taxa that have been isolated and described from planktonic algal sources (especially diatoms and dinoflagellates) belong to Alphaproteobacteria (Fig. 3). In algal samples the order Roseobacterales was represented with 11 species.

A direct interaction between these algae and their associated bacteria is suggested by the fact that *Roseobacter* cells have been found attached to the surface of toxic *Pfiesteria* species (Alavi et al. 2001) or to *Prorocentrum lima* (Ehrenberg) Dodge 1975 (Wagner-Dobler et al. 2011) using molecular methods (FISH and CARD-FISH, respectively). Those phototrophic bacteria may obtain by epibiosis of phytoplanktonic organisms both, light and nutrients (Wagner-Dobler and Biebl 2006). A clear example was published recently with the study of *Dinoroseobacter shibae*, a bacterium associated with several phylogenetically diverse species of algae. It was shown that the strain can produce and provide the algae with vitamins B1 and B12 in exchange of photosynthate leaking from the host during photosynthesis, demonstrating a possible symbiotic relationship with microalgae (Wagner-

Dobler et al. 2011). Nevertheless, by molecular methods, stable populations of *Alphaproteobacteria* have also been observed living associated with macroalgae from different parts of the world as well (Staufenberger et al. 2008; Hengst et al. 2010; Namba et al. 2010; Tujula et al. 2010).

Members of the Gammaproteobacteria have been often isolated from different algal sources (Beleneva and Zhukova 2006; Wang et al. 2009; Wiese et al. 2009b), or detected by molecular methods associated with algae (Longford et al. 2007; Staufenberger et al. 2008; Tujula et al. 2010). Besides Alphaproteobacteria, Gammaproteobacterial *Marinobacter* clades appeared among the most notable members of bacterial community co-occurring with bloom forming dinoflagellates like *Gymnodinium catenatum* Graham 1943 or *Scrippsiella trochoidea* (Stein) Loeblich III 1976 (Green et al. 2004). Not only *G. catenatum* showed to require growth-stimulatory marine bacteria like *Marinobacter* spp. for post-germination survival and growth (Bolch et al. 2011), but also this bacterial genus demonstrated to promote algal assimilation (in *S. trochoidea*) of iron by facilitating photochemical redox cycling of this critical nutrient (Amin et al. 2009). Nevertheless, several studies have indicated that some taxa of the Gammaproteobacteria may represent bacteria with an opportunistic life strategy, with the capacity for fast growth in response to abundant inorganic nutrients and easily assimilated carbon sources in freshwater and marine environments (Pinhassi and Berman 2003). Indeed, several diseases of macroalgae such as the ‘hole-rotten disease’, the ‘red spot disease’, and the ’spot-wounded fronds’ in *Saccharina japonica* Lane et al. 2006 (Sawabe et al. 1998; 2000; Wang et al. 2008), the ‘green decay diseases’ in *Undaria pinnatifida* (Jiang et al. 1997), and the ‘green spot rotting’ in *Porphyra yezoensis* (Fujita et al. 1972), are caused by members of Gammaproteobacteria.

Interestingly, a large fraction (32%) of the strains investigated during this study were bacteria capable to decompose cell wall components from living algae or are capable of utilizing nutrients provided by their algal host, especially from the cluster of Bacteroidetes and Gammaproteobacteria (Barbeyron et al. 2001; Ivanova et al. 2004a; b; Michell et al. 2006; Table 1 and 3, Fig. 3). Conceptually, the surface chemistry of an alga is the sum of exuded secondary metabolites and extracellular exopolymeric substances (EPS) present on the thallus. This complex mixture of compounds is presumably in equilibrium with the ambient water body (Lachnit et al. 2010). Also, the cell/thallus morphology and microtopography of the algal surface play a role in the colonization and association with the microbiota and invertebrates (Wahl et al. 2010): Hence, each algal species can be described as a unique microenvironment. Utilization of substrates produced or released by the algae, including the mentioned structural polysaccharide components of algal cell walls, is an important aspect of surface colonization by bacteria (Goecke et al. 2010; Bengtsson et al. 2011). Bacteria may use the macroalga not only as surface for settlement but also as a substrate (Johansen et al.

1999), and probably this is one important reason for specific macroalga-bacteria interactions driven by the bacteria. The macromolecular composition of algal surface is characteristically different among the major evolutionary lineages of algae, linking specific life style or nutritional habits to specifically encountered biopolymers. Mostly of these algal macromolecules are polysaccharides, which occur in sulphated (carrageenans, fucans) or highly ionic (alginates) forms (Thomas et al. 2011). Those large macromolecules, such as agar and carrageenan in red algae, alginate, fucan and laminaran in brown algae, and ulvan in green algae, are produced almost exclusively by macroalgae compared to the microalgae.

In microalgae instead, dinoflagellates (i.e. *Alexandrium* spp., *Prorocentrum* sp.) have a cell wall consisting of cellulose, while those of the diatoms (*Melosira* sp., *Skeletonema* sp.) are build of silica frustules, and those of cryptophytes (*Cryptomonas* sp.) of organic plates underneath cell membrane (the periplast) (Jasti et al. 2005). Those differences in the cell wall biochemistry and compounds released by the divergent algal species are likely to select for different associated bacterial species that may rely on organic compounds available in the phycosphere (Jasti et al. 2005). And may explain for example, why a higher number of Bacteroidetes have been found and described from macroalgal sources. And why many *Roseobacter* bacteria, which can degrade (dimethylsulfoniopropionate) an osmoprotectant released in large quantities during algal blooms (Moran et al. 2007, Wagner-Dobler et al. 2011), are in higher number associated on microalgae such as phytoplankton (Fig. 3). In fact, a positive chemotaxis toward dinoflagellate products including DMSP and amino acids has been already described from *Silicibacter* sp. (strain TM1040 *member of the Roseobacter* clade), a bacterium isolated from a dinoflagellate culture of *Pfiesteria piscicida* Steidinger and Burkholder 1996 (Miller et al. 2004).

Bacteria are an inherent part of the physical environment of micro and macroalgae (Hold et al. 2001). The isolation source of a determined microorganism not necessary implies an exclusive origin and ecological associations *per se*. Despite methodological precautions taken, e.g. rinsing off loosely attached bacteria with sterile seawater, it is left open whether the bacterial strains originally lived associated with the algae, or are found also in seawater or any other component of the habitat of the alga. However, algal surface as a microbial habitat display several advantages. For example, seawater has relatively low nutrients concentrations, whereas living surfaces such as alga exude organic carbon and nutrients. Further, free living bacteria in the seawater must protect themselves from damaging ultraviolet radiation and predation, while microbial communities on living surfaces form biofilm structures shielding them from environmental stress (Burke et al. 2011). Algal mats, for example, can act as microbial reservoirs through adverse environmental conditions. Those mats may provide a favourable environment in which certain bacteria are able to persist by reducing or eliminating many of those common stresses bacteria face in the water column, as well as by providing a more stable habitat in which to exist (Englebert et al. 2008). Nevertheless, it seems that host characteristics influence the association of bacteria and algae. The repeated isolation of species (or strains) of a determined bacterial group from an algal taxon, or in general from algae (see Table S1, supporting information), could be explained by evolutionary adaptations, i.e. metabolic pathways, niche specificity, or even established mutualistic relationships (Amin et al. 2009; Bolch et al. 2011; Hollants et al. 2011; Wagner-Dobler et al. 2011).

No doubt, we are only starting to discover the relationships of algae and bacteria in nature. Interactions between bacteria and algae are thought to be important in controlling the dynamics of both communities and yet are only beginning to be understood at the species composition level (Grossart et al. 2005; Jasti et al. 2005). We need a deeper insight into these mutualistic interactions, if we are to understand and predict algal blooms formations, disease outbreaks, and the response of populations of algae and bacteria to changes in their environment (Amin et al. 2009). Following studies of the bacterial communities on different algal species that consider also different algal populations will contribute to the knowledge of such ecological associations and will surely raise the number of new bacterial species isolated from algae. Algae are key components of the aquatic environments, not only microhabitats for many invertebrates and fishes, but also for millions of microorganisms waiting to be discovered.

Appendix

Table 2 List of the newly described bacterial species including proposed new species (in process for confirmation) from algal sources in aquatic environments. The type strain (T), GenBank accession number (GB-nr), and the reference are given.

Nr.	Bacterial species (T)	GB-nr	Source
01	*Aequorivita capsosiphonis* A71, JCM 15070	EU290153	Park et al. 2009
02	*Aeromicrobium tamlense* SSW1-57	DQ411541	Lee and Kim 2007
03	*Agarivorans gilvus* WH0801, NRRL B-59247	GQ200591	Du et al. 2011
04	*Agrococcus jejuensis* SSW1-48, JCM 14256	AM396260	Lee 2008
05	*Algibacter lectus* KMM 3902, DSM 15365	AY187689	Nedashkovskaya et al. 2004e
06	*Algicola bacteriolytica* IAM 14595	D89929	Sawabe et al. 1998
07	*Algoriphagus chordae* KMM 3957, LMG 21970	AJ575265	Nedashkovskaya et al. 2004f
08	*Algoriphagus winogradskyi* KMM 3956	AJ575263	Nedashkovskaya et al. 2004f
09	*Alkalibacterium putrialgicola* DSM 19182	AB294167	Ishikawa et al. 2009
10	*Alkalibacterium subtropicum* O24-2	AB555562	Ishikawa et al. 2011
11	*Alkalibacterium thalassium* T117-1-2	AB294165	Ishikawa et al. 2009
12	*Aquimarina agarilytica* ZC1, NBRC 107695	FJ750453	Lin et al. 2011
13	*Arenibacter certesii* KMM 3941, KCTC 12113	AY271622	Nedashkovskaya et al. 2004a
14	*Arenibacter latericius* KMM 426, CIP 106861	AF052742	Ivanova et al. 2001
15	*Arenibacter palladensis* KMM 3961	AJ575643	Nedashkovskaya et al. 2006b
16	*Aureispira marina* 24, IAM 15389	AB245933	Hosoya et al. 2006
17	*Bacillus algicola* KMM 3737	AY228462	Ivanova et al. 2004a
18	*Brevibacterium celere* KMM 3637, DSM 15453	AY228463	Ivanova et al. 2004b
19	*Brevundimonas aurantiaca* DSM 4731, CB-R	AJ227787	Abraham et al. 1999
20	*Cellulophaga algicola* IC 166, ACAM 630	AF001366	Bowman 2000
21	*Cellulophaga baltica* NN015840	AJ005972	Johansen et al.1999
22	*Cellulophaga fucicola* NN015860, LMG 18536	AJ005973	Johansen et al.1999
23	*Croceitalea dokdonensis* DOKDO023	DQ191182	Lee et al. 2008b
24	*Croceitalea eckloniae* DOKDO025	DQ191183	Lee et al. 2008b
25	*Demequina aurantiaca* YM12-102	AB522641	Ue et al. 2011
26	*Dinoroseobacter shibae* DFL12, DSM 16493	AJ534211	Biebl et al. 2005b
27	*Erythrobacter longus* OCh101, IFO 14126	AF465835	Shiba and Simidu 1982
28	*Ferrimonas marina* A4D-4, DSM 16917	AB193751	Katsuta et al. 2005
29	*Flagellimonas eckloniae* DOKDO007	DQ191180	Bae et al. 2007
30	*Flavobacterium algicola* TC2, CIP 109574	AB455265	Miyashita et al. 2010
31	*Formosa agariphila* KMM 3962, DSM 15362	AJ893518	Nedashkovskaya et al. 2006a
32	*Formosa algae* KMM 3553, CIP 107684	AY228461	Ivanova et al. 2004c
33	‘*Fucobacter marina*’ SA-0082	AB057592	Sakai et al. 2002
34	*Haloferula phyci* AK18-024, KCTC 22200	AB372854	Yoon et al. 2008b
35	*Haloferula sargassicola* MN1-1037	AB372856	Yoon et al. 2008b
36	*Halolactibacillus halophilus* M2-2, DSM 17073	AB196783	Ishikawa et al. 2005
37	*Halolactibacillus miurensis* M23-1	AB196784	Ishikawa et al. 2005
38	*Hoeflea alexandri* AM1 V30, DSM 16655	AJ786600	Palacios et al. 2006
39	*Hoeflea phototrophica* DFL-43, DSM 17068	AJ582088	Biebl et al. 2006
40	*Huaishuia halophila* ZXM137, LMG 24854	FJ436725	Wang et al. 2011
41	*Kiloniella laminariae* LD81, NCIMB 14374	AM749667	Wiese et al. 2009
42	*Kordia algicida* OT-1, KCTC 8814P	AY195836	Sohn et al. 2004
43	*Koreibacter algae* DSW-2, DSM 22126	FM995611	Lee and Lee 2010
44	*Kriegella aquimaris* KMM 3665, DSM 19886	EU246690	Nedashkovskaya et al. 2008a
45	*Labedella gwakjiensis* KSW2-17, JCM 14008	DQ533552	Lee 2007
46	*Labrenzia alexandri* DFL-11, DSM 17067	AJ582083	Biebl et al. 2007
47	*Lacinutrix algicola* AKS 293, JCM 13825	DQ167238	Nedashkovskaya et al. 2008b
48	*Lacinutrix mariniflava* AKS 432, JCM 13824	DQ167239	Nedashkovskaya et al. 2008b
49	*Lentibacter algarum* ZXM100, LMG 24861	FJ436732	Li et al. 2011
50	*Leuconostoc miyukkimchii* M2, JCM 17445	HQ263024	Lee et al. 2011
51	*Leucothrix mucor* ATCC 25107, DSM 2157	X87277	Brock 1966, Ludwig et al. 1995
52	*Luteolibacter algae* A5J-41-2, KCTC 22040	AB331893	Yoon et al. 2008a
53	*Maribacter antarcticus* CL-AP4, JCM 15445	EU512921	Zhang et al. 2009

54	*Maribacter polysiphoniae* KCTC 22021	AM497875	Nedashkovskaya et al. 2007
55	*Maribacter stanieri* KMM 6046, KCTC 22033	EU246691	Nedashkovskaya et al. 2010
56	*Maribacter ulvicola* KMM 3951, DSM 15366	AY271626	Nedashkovskaya et al. 2004d
57	*Marinobacter adhaerens* HP15, DSM 23420	AY241552	Kaeppel et al. 2011
58	*Marinobacter algicola* DG893, DSM 16394	AY258110	Green et al. 2004, 2006
59	*Marinovum algicola* ATCC 51440	X78315	Martens et al.2006
60	*Maritalea porphyrae* LCM-3, NBRC 107169	AB583774	Fukui et al. 2011
61	*Marivita cryptomonadis* CL-SK44	EU512919	Hwang et al. 2009a
62	*Mesonia algae* KMM 3909, KCTC 12089	AF536383	Nedashkovskaya et al. 2003
63	*Mesonia phycicola* MDSW-25, DSM 21425	FM882228	Kang and Lee 2010
64	*Microbulbifer epialgicus* F-104, DSM 18651	AB266054	Nishijima et al. 2009
65	*Microbulbifer variabilis* Ni-2088	AB167354	Nishijima et al. 2009
66	*Nitratireductor aquimarinus* JCM 17288	HQ176467	Jang et al. 2011
67	*Nitratireductor kimnyeongensis* JCM 14851	AM498744	Kang et al. 2009
68	*Oceanicaulis alexandrii* C116-18, DSM 11625	AJ309862	Strömpl et al. 2003
69	*Oceanobacillus oncorhynchi incaldanensis* 20AG	AJ640134	Romano et al. 2006
70	*Paracoccus zeaxanthinifaciens* ATCC 21588	AF461158	Berry et al. 2003
71	*Paraliobacillus ryukyuensis* O17-7, DSM 15140	AB087828	Ishikawa et al. 2003
72	*Persicivirga ulvanivorans* PLR, DSM 22727	GU902979	Barbeyron et al. 2011
73	*Phycicoccus jejuensis* KSW2-15, KCCM 42315	DQ345443	Lee 2006
74	*Phycicola gilvus* SSWW-21, DSM 18319	AM286414	Lee et al. 2008a
75	*Phycisphaera mikurensis* KCTC 22515	AB474364	Fukunaga et al. 2009
76	*Pibocella ponti* KMM 6031, KCTC 12262	AY576654	Nedashkovskaya et al. 2005a
77	*Planktotalea frisia* SH6-1, DSM 23709	FJ882052	Hahnke et al. 2011
78	*Pseudoalteromonas atlantica* IAM 12927	X82134	Akagawa-Matsushita et al. 1992
79	*Pseudoalteromonas elyakovii* KMM 162	AF082562	Sawabe et al. 2000
80	*Pseudoalteromonas issachenkonii* KMM 3549	AF316144	Ivanova et al. 2002
81	‘*Pseudoalteromonas porphyrae*'	AY771715	Dimitrieva et al. 2006
82	*Pseudoalteromonas ulvae* UL12, NCIMB 13762	AF172987	Egan et al. 2001
83	*Pseudolateromonas mariniglutinosa* NCIMB1770	AJ507251	Romanenko et al. 2003
84	*Pseudomonas pelagia* CL-AP6, JCM 15562	EU888911	Hwang et al. 2009b
85	*Pseudozobellia thermophila* DSM 19858	AB084261	Nedashkovskaya et al. 2009
86	*Roseibacillus ponti* YM27-120, KCTC 12987	AB331889	Yoon et al. 2008a
87	*Roseibium denhamense* OCh254, JCM 10543	D85832	Suzuki et al. 2000
88	*Roseivirga ehrenbergii* KMM 6017	AY608410	Nedashkovskaya et al. 2005b
89	*Roseovarius mucosus* DFL-24, DSM 17069	AJ534215	Biebl et al. 2005a
90	*Salinicoccus qingdaonensis* ZXM223	FJ436724	Qu et al. 2011
91	*Shewanella alga* IAM 14159	U91546	Simidu et al. 1990
92	*Tenacibaculum amylolyticum* DSM 13766	AB032505	Suzuki et al. 2001
93	*Ulvibacter litoralis* KMM 3912, KCTC 12104	AY243096	Nedashkovskaya et al. 2004c
94	*Vampirovibrio chlorellavorus* ATCC 29753	HM038000	Gromov and Mamkayeva 1980
95	*Winogradskyella epiphytica* KMM 3906	AY521224	Nedashkovskaya et al. 2005c
96	*Winogradskyella eximia* KMM 3944	AY521225	Nedashkovskaya et al. 2005c
97	*Winogradskyella thalassocola* KMM 3907	AY521223	Nedashkovskaya et al. 2005c
98	*Winogradskyella ulvae* KMM 6390	HQ456127	Nedashkovskaya et al. 2011
99	*Zobellia galactanovorans* Dsij, DSM 12802	AF208293	Barbeyron et al. 2001
100	*Zobellia laminariae* KMM 3676, LMG 22070	AB121975	Nedashkovskaya et al. 2004b
101	*Zobellia russellii* KMM 3677, LMG 22071	AB121976	Nedashkovskaya et al. 2004b

Table 3 List of the newly described bacterial species including proposed new species (in process for confirmation) from algae. The type of algal source (macroalgae = Ma, microalgae = Mi), Division (C = Chlorophyta, CR = Cryptophyta, DI = Dinoflagellata, O = Ochrophyta, R = Rhodophyta), Class (cr = Cryptophyceae, df = Dinophyceae, di = Bacillariophyceae, and P = Phaeophyceae) is given. In the table is mentioned the ability to degrade the main algal polysaccharides, for agar (AG), alginate (AL), carrageenan (CA), fucan (FU), laminaran (LA), and ulvan (UL). The presence of enzymes capable of degrading cellulose is also given (B).

Nr.	Bacterial species	Algal source	Degrade	Host
01	*A. capsosiphonis*	*Capsosiphon fulvescens*	0	Ma-C
02	*A. tamlense*	Unidentified macroalgae	0	Ma
03	*A. gilvus*	Unidentified macroalgae	AG B	Ma
04	*A. jejuensis*	Unidentified macroalgae	0	Ma
05	*A. lectus*	*Acrosiphonia sonderi*	AG AL	Ma-C
06	*A. bacteriolytica*	*Saccharina japonica*	0	Ma-OP
07	*A. chordae*	*Chorda filum*	AG AL	Ma-OP
08	*A. winogradskyi*	*Acrosiphonia sonderi*	AG AL	Ma-C
09	*A. putrialgicola*	Unidentified macroalgae	0	Ma
10	*A. subtropicum*	*Galaxaura* sp.	0	Ma-R
11	*A. thalassium*	Unidentified macroalgae	0	Ma
12	*A. agarilytica*	*Porphyra haitanensis*	AG	Ma-R
13	*A. certesii*	*Ulva fenestrata*	0	Ma-C
14	*A. latericius*	*Chorda filum*	B	Ma-OP
15	*A. palladensis*	*Ulva fenestrata*	B	Ma-C
16	*A. marina*	Unidentified macroalgae	0	Ma
17	*B. algicola*	*Fucus evanescens*	AL	Ma-OP
18	*B. celere*	*Fucus evanescens*	AL LM	Ma-OP
19	*B. aurantiaca*	*Chlorella* culture	0	Mi-C
20	*C. algicola*	*Melosira* sp.	AG	Mi-Odi
21	*C. baltica*	*Fucus serratus*	AG AL CA B	Ma-OP
22	*C. fucicola*	*Fucus serratus*	AG AL CA B	Ma-OP
23	*C. dokdonensis*	*Ecklonia kurome*	B	Ma-OP
24	*C. eckloniae*	*Ecklonia kurome*	0	Ma-OP
25	*D. aurantiaca*	Unidentified macroalgae	B	Ma
26	*D. shibae*	*Prorocentrum lima*	0	Mi-DI
27	*E. longus*	*Enteromorpha linza*	AL	Ma-C
28	*F. marina*	Unidentified macroalgae	0	Ma
29	*F. eckloniae*	*Ecklonia kurome*	B	Ma-OP
30	*F. algicola*	Unidentified macroalgae	AL	Ma
31	*F. agariphila*	*Acrosiphonia sonderi*	AG	Ma-C
32	*F. algae*	*Fucus evanescens*	0	Ma-OP
33	‘*F. marina*’	*Kjellmaniella crassifolia*	FU	Ma-OP
34	*H. phyci*	Unknown green alga	0	Ma-C
35	*H. sargassicola*	*Sargassum thunbergii*	0	Ma-OP
36	*H. halophilus*	Unknown macroalga	0	Ma
37	*H. miurensis*	Unknown macroalga	0	Ma
38	*H. alexandri*	*Alexandrium minutum*	B	Mi-DI
39	*H. phototrophica*	*Prorocentrum lima*	0	Mi-DI
40	*H. halophila*	*Enteromorpha prolifera* bloom	AL B	Ma-C
41	*K. laminariae*	*Saccharina latissima*	0	Ma-OP
42	*K. algicida*	red tide bloom	0	Mi
43	*K. algae*	Unidentified macroalgae	0	Ma
44	*K. aquimaris*	*Ulva fenestrata*	0	Ma-C
45	*L. gwakjiensis*	Unidentified macroalgae	B	Ma
46	*L. alexandri*	*Alexandrium lusitanicum*	0	Mi-DI
47	*L. algicola*	Gigartinaceae	0	Ma-R
48	*L. mariniflava*	Gigartinaceae	0	Ma-R

49	*L. algarum*	*Enteromorpha prolifera*	0	Ma-C
50	*L. miyukkimchii*	*Undaria pinnatifida*	0	Ma-OP
51	*L. mucor*	*Monostroma* sp.	0	Ma-C
52	*L. algae*	Unidentified red algae	0	Ma-R
53	*M. antarcticus*	*Pyramimonas gelidicola*	0	Mi-C
54	*M. polysiphoniae*	*Polysiphonia japonica*	0	Ma-R
55	*M. stanieri*	*Ulva fenestrata*	0	Ma-C
56	*M. ulvicola*	*Ulva fenestrata*	AG B	Ma-C
57	*M. adhaerens*	*Thalassiosira weissflogii*	0	Mi-Odi
58	*M. algicola*	*A. tamarense, G. catenatum*	0	Mi-DI
59	*M. algicola*	*Prorocentrum lima*	B	Mi-DI
60	*M. porphyrae*	*Porphyra yezoensis*	0	Ma-R
61	*M. cryptomonadis*	*Cryptomonas* sp.	0	Mi-CR
62	*M. algae*	*Acrosiphonia sonderi*	FU	Ma-C
63	*M. phycicola*	Unidentified macroalgae	0	Ma
64	*M. epialgicus*	*Caulerpa* sp.	0	Ma-C
65	*M. variabilis*	*Pocockiella* sp.	0	Ma-OP
66	*N. aquimarinus*	*Skeletonema costatum*	0	Mi-Odi
67	*N. kimnyeongensis*	Unidentified macroalgae	0	Ma
68	*O. alexandrii*	*Alexandrium tamarense*	0	Mi-DI
69	*O. oncorhynchi incal.*	Unidentified macroalgae	0	Ma
70	*P. zeaxanthinifaciens*	Unidentified macroalgae	B	Ma
71	*P. ryukyuensis*	Unidentified macroalgae	0	Ma
72	*P. ulvanivorans*	*Ulva* (*Aplysia* faeces)	UL	Ma-C
73	*P. jejuensis*	Unidentified macroalgae	B	Ma
74	*P. gilvus*	Unidentified macroalgae	B	Ma
75	*P. mikurensis*	*Porphyra* sp.	AG B	Ma-R
76	*P. ponti*	*Acrosiphonia sonderi*	0	Ma-C
77	*P. frisia*	Phytoplankton bloom	0	Mi
78	*P. atlantica*	*Lessonia* sp.	AG AL	Ma-OP
79	*P. elyakovii*	*Saccharina japonica*	AL LM	Ma-OP
80	*P. issachenkonii*	*Fucus evanescens*	AL FU	Ma-OP
81	‘*P. porphyrae*'	*Porphyra yezoensis*	CA	Ma-R
82	*P. ulvae*	*Ulva lactuca*	0	Ma-C
83	*P. mariniglutinosa*	*Chaetoceros lauderi*	AG CA	Mi-Odi
84	*P. pelagia*	*Pyramimonas gelidicola*	0	Mi-C
85	*P. thermophila*	*Ulva fenestrata*	AG AL B	Ma-C
86	*R. ponti*	*Padina* sp.	0	Ma-OP
87	*R. denhamense*	*Botryocladia* sp.	0	Ma-R
88	*R. ehrenbergii*	*Ulva fenestrata*	0	Ma-C
89	*R. mucosus*	*Alexandrium ostenfeldii*	0	Mi-DI
90	*S. qingdaonensis*	*Ulva* sp. bloom	0	Ma-C
91	*S. alga*	*Jania* sp.	0	Ma-R
92	*T. amylolyticum*	*Avrainvillea riukiuensis*	0	Ma-C
93	*U. litoralis*	*Ulva fenestrata*	0	Ma-C
94	*V. chlorellavorus*	*Chlorella vulgaris* culture	0	Mi-C
95	*W. epiphytica*	*Acrosiphonia sonderi*	AG	Ma-C
96	*W. eximia*	*Saccharina japonica*	AG	Ma-OP
97	*W. thalassocola*	*Chorda filum*	AG B	Ma-OP
98	*W. ulvae*	*Ulva fenestrata*	AG	Ma-C
99	*Z. galactanovorans*	*Delesseria sanguinea*	AG CA	Ma-R
100	*Z. laminariae*	*Saccharina japonica*	AG	Ma-OP
101	*Z. russellii*	*Acrosiphonia sonderi*	AG AL	Ma-C

5 CHAPTER II

Phylogenetic analysis and antibiotic activity of epibiotic bacteria associated with two co-occurring macroalgae from the Baltic Sea.

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ABSTRACT

Bacteria associated with *Fucus vesiculosus* and *Delesseria sanguinea*, two macroalgae from the Kiel Fjord (Baltic Sea, Germany) were investigated seasonally over two years by cultivation based methods. A total of 166 bacteral strains were isolated from the macroalgae. By phylogenetic analysis of 16S rRNA gene sequences they affiliated to 82 phylotypes according to sequence similarities of >99.0%. Bacteria belonged to *Actinobacteria, Alphaproteobacteria*, *Bacilli*, *Betaproteobacteria*, *Flavobacteria*, *Gammaproteobacteria*, and *Sphingobacteria*. Following the assumption that chemical interactions rule the bacteria-macroalga associations, we tested the antibiotic activity of the bacterial strains against a panel comprising four standard (strains commonly used in bioactivity tests) and four ecologically relevant microorganisms (two pathogens and two surface associated strains). Organic extracts of more than 60% of the bacteria inhibited the growth of at least one of the tested microorganisms. Over 54% of the strains derived respectively from *F. vesiculosus* and *D. sanguinea*, showed antimicrobial activity against macroalga-associated bacteria, as compared to the standard set of microorganisms (19 and 28% in *F. vesiculosus* and *D. sanguinea*, respectively). A higher number of active isolates were found in *D. sanguinea* in comparison with the brown alga. And some of those isolates associated specifically but seasonally with the host. High antibacterial activity against macroalgal pathogens and bacterial competitors support the assumption that complex chemical interactions shape the bacteria-macroalga relationships, and that epibiotic bacteria are a rich source of antimicrobial metabolites.

INTRODUCTION

Surfaces submerged in the ocean are rapidly covered by biofilms (Harder 2009). Especially the surfaces of marine organisms provide attractive habitats for microorganisms (Boyd et al. 1999). Epibiotic bacteria are fast colonizers, highly adaptative and capable of rapid metabolization of algal exudates, and therefore play a key role in the colonization and biofouling processes on macroalgae (Corre and Prieur 1990). Different species of marine macroalgae in the same habitat support different bacterial communities (Lachnit et al. 2009; Nylund et al. 2010), while the same macroalgal species even from different localitites demonstrated high similarities in the composition of the associated microbial communities (Staufenberger et al. 2008; Lachnit et al. 2009; Sneed and Pohnert 2011). The production of biologically active compounds by the algal host might influence those relationships between the bacteria and macroalgae (Goecke et al. 2010; Persson et al. 2011). Recently it was shown, that the Baltic brown alga *Fucus vesiculosus* mediated bacterial surface colonization by compounds excreted from the thallus (Lachnit et al. 2010; Saha et al. 2011). But the possible effect natural products produced by associated microbiota on alga-bacteria interactions still remain unknown.

Different studies have focused on antifouling and antibiotic activities of macroalga-associated bacteria (Boyd et al. 1999; Egan et al. 2000; Zheng et al. 2005; Dobretsov et al. 2006; Kanagasabhapathy et al. 2006; 2008; Penesyan et al. 2009; Wiese et al. 2009; Villarreal-Gómez et al. 2010). These cultivation-based studies indicated that epibiotic bacteria exhibit a great potential to produce bioactive compounds, which inhibit the growth of environmental microbes, laboratory strains and also human pathogens. Space and nutrient limitation as well as a highly competitive environment, forces surface dwelling microorganisms to evolve particular adaptive responses as well as antagonistic strategies to prevent colonization or growth of potential competitors (Egan et al. 2008). Therefore, the production of antimicrobial chemical defenses by symbiotic microorganisms may represent an added level of complexity to the mechanisms by which marine plants avoid the deleterious effects of microbial pathogens (Engel et al. 2002). Unfortunately, assessments of the antibacterial activity exhibited by marine organisms in the natural products literature has focused on biomedically-relevant strains, using standard microbial strains from terrestrial origin or of medical relevance (Engel et al. 2006; Hughes and Fenical 2011).

In the present study we investigated for the first time epiphytic bacteria associated with the red macroalga *Delesseria sanguinea* and we compared them with the ones associated with *Fucus vesiculosus*. Those perennial macroalgae from the Baltic Sea have been shown by molecular methods to ‘harbor’ different associated microbial communities (Lachnit et al. 2009). Samples of both algae were processed in order to isolate associated bacteria, which were classified by phylogenetic analysis based on 16S rRNA gene sequences. We analyzed the potential of the associated microorganisms in shaping the microbial communities, by testing their crude extracts against macroalga-associated microorganisms and algal pathogens as well as against a standard set of bacteria often used for bioactivity tests.

MATERIALS AND METHODS

Sampling of the macroalgae

Samples of the macroalgae were taken from a distinct site of the Strande Beach, in the Kiel Fjord, Western Baltic Sea, Germany (54°25.5'N, 10°12'E). The brown macroalga *Fucus vesiculosus* Linneaeus (Fucales, Phaeophyceae) and the red macroalga *Delesseria sanguinea* Lamouroux (Ceramiales, Rhodophyta) were collected between two to six m depth in July 2008 (summer I), January 2009 (winter I), July 2009 (summer II) and January 2010 (winter II). The algae were removed carefully from the substrate with a knife and transferred into sterile plastic bags. Until processing within three hours after collection, the samples were stored in the dark at ambient seawater temperature using coolers. In the laboratory, the macroalgae were rinsed three times with sterile Baltic Sea water to remove associated debris, planktonic and loosely attached microorganisms (Penesyan et al. 2009). Part of the macroalgae was fixed in 4% formaldehyde for its taxonomic identification. Algae were identified by examination of their thallus architecture and special morphological characters: Fronds, branching, presence of air bladders, and reproductive structures (Pankow 1971). Voucher specimen were deposited in the Herbarium of Museo de Historia Natural, Santiago, Chile (code SGO).

Isolation and cultivation of bacteria

The sampling procedure for isolating bacteria associated with the surface of the macrolagae was performed according to Wiese et al. (2009). Pieces of the macroalgal tissue (one per species at each time point) were suspended in sterile Baltic seawater and homogenized using an Ultraturrax T25 (IKA Werke). The suspension was plated on five different nutrient media, which had the following composition (all amounts in g l^{-1}): TSB medium (12 g Difco tryptic soy broth, 10 g NaCl, 15 g Bacto agar, pH 7.2 in distilled water); SCA medium (10 g Bacto Trypton, 5 g yeast extract, 20 g NaCl, 1 g $MgSO_4$ x H_2O, 6 g Tris HCl, 5 ml glycerol, 15 g Bacto agar, pH 7.5 in distilled water), TM medium (1 g peptone from soymeal, 5 g yeast extract, 15 g Bacto agar, 30 g Tropic Marin Salt in distilled water), GPY medium (0.5 g peptone from soymeal, 0.1 g yeast extract, 15 g Bacto agar, 1 g glucose, pH 7.2 in sterile Baltic Sea water), and HWO medium according to Muscholl-Silberhorn et al. (2008) (0.1 g yeast extract, 15 g Bacto agar, in sterile Baltic Sea water). The plates were incubated at 22°C for 14 days. Colonies were sub-cultivated on individual agar plates by standard procedures. Preservation of the strains was performed according to Wiese et al. (2009).

16S rRNA gene sequence analysis

Genomic DNA of the strains was obtained either by freezing and boiling (-140°C for 30 min, 90°C for 5 min) or by using a Precellys 24 lysis and homogenization device, the Precellys^{TM} 24-cell mill and corresponding Precellys^{TM} 24 Kit VK05/VK01 (PEQLAB Biotechnologie GmbH, Erlangen, Germany). Samples were subjected to two times 6300 shakes min^{-1} for 20 s with an intermission of 20 s. After centrifugation for 10 min at 8,000x *g*, the supernatant was collected and the DNA extract was stored at -20°C. For the 16S rRNA gene amplifications (PCR) almost complete 16S rRNA gene fragments were obtained with the primers 27f and 1492r (Staufenberger et al. 2008). Subsequent sequencing was performed at the Institute for Clinical Molecular Biology (University Hospital Schleswig-Holstein, Kiel, Germany), and primers used for sequencing were: 534r, 342f, and 790f as described by Heindl et al. (2010). The 16S rRNA gene sequences obtained in this study were deposited in NCBI GenBank under the accession numbers FR718448, FR775436-FR775439, and FR821090-FR821249.

Phylogenetic analysis

Sequences were edited using CHROMASPRO 1.33 (Technelysium Pty. Ltd. Cologne, Germany). Results were compared with other sequences in the EMBL prokaryotes database using Basic Local Alignment Search Tool (BLAST), which is available online at the European Bioinformatics Institute homepage, as well as with the RDP database (Ribosomal Database Project; http://rdp.cme.msu.edu; Cole et al. 2009). Type strain relatives of all isolates were determined by comparison of 16S rRNA gene sequences in the EMBL prokaryotes database using BLAST (Altschul et al. 1990) and the online database LPSN (http://www.bacterio.net, Euzeby et al. 1997). The similarity matrix of the 16S rRNA gene sequences of all the strains was obtained using Phylogeny Interface Package (PHYLIP 3.9, Felsenstein 1989).

The strains were grouped into phylotypes by sequence similarities of >99.0%. One representative strain of each phylotype was selected for phylogenetic calculation and tree construction. In addition, all related type strains as well as selected non-type strains, were included in the phylogenetic analysis. Sequences were aligned using the CLUSTAL X program (Thompson et al. 1997) and manually corrected if necessary. The phylogenetic analysis was performed with the neighbour-joining (NJ) method (Saitou and Nei 1987) using the Kimura 2-parameter model (Kimura 1983) by the MEGA3.1 software package (Kumar et al. 2004). The topology of the trees was evaluated by bootstrap analysis (Felsenstein 1985) based on 1,000 resamplings (NJ). NJplot was used to display the phylogenetic trees (Perriere and Gouy 1996).

Preparation of cell extracts

The bacterial strains were plated on the nutrient media (30 ml), which were used for their isolation, and incubated as mentioned above. After two weeks, the plates were homogenized with an Ultraturrax T25 and extracted with 20 ml ethylacetate. The obtained extract was concentrated under reduced pressure in a Speedvac RVC 2-33 (Christ, Germany) until completely dry and resuspended in 1 ml methanol. All employed solvents were of analytical reagent grade and obtained from Merck.

Antimicrobial assays

The antimicrobial activity of the extracts (10 µl) of the isolated bacteria was tested against a panel of eight microorganisms comprising standard test strains and macroalga-associated bacteria. The following microorganisms were used: (i) Four microorganisms usually tested in standard laboratory tests of antibiotic activity (hereafter "standard set"): *Escherichia coli* DSM 498 (nutrient media: TSB12 = 12 g l^{-1} TSB in distilled water), as a Gram-negative strain; and *Bacillus subtilis* DSM 347 and *Staphylococcus lentus* DSM 6672 (both nutrient media: TSB3 = 3 g l^{-1} TSB in distilled water) as representatives of Gram-positive bacteria, and the yeast *Candida glabrata* DSM 6425 (nutrient media: 186/3 = 3 g l^{-1} yeast extract, 3 g l^{-1} malt extract, 5 g l^{-1} peptone from soymeal, 10 g l^{-1} glucose in distilled water), all obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). (ii) Four environmental strains that have been identified to be associated with macroalgae in previous studies (hereafter "macroalga-associated set"): iia) Two of our isolates from *F. vesiculosus* were used, which affiliated to other macroalga-associated bacteria (see Goecke et al. 2010) with >99% 16S rRNA gene sequence similarity to the as Gram-positive *Bacillus algicola* KMM 3737^T (AY228462) and Gram-negative *Formosa algae* KMM 3553^T (AY228461) (isolate AB423f and AB356f, accession numbers FR775437 and FR775436 respectively, see Supporting Information). iib) Two Gram-negative bacterial strains identified as macroalgal pathogens (Sawabe et al. 1998; 2000), *Algicola bacteriolytica* ATCC 700679^T ('red spot disease') and *Pseudoalteromonas elyakovii* ATCC 700519^T ('spot-wounded fronds'), originally isolated in Japan from diseased beds of *Saccharina japonica* (Areschoug) Lane et al. (formerly *Laminaria japonica*). Both were purchased from Institute Pasteur CIP (Paris, France), and grown on nutrient medium TM (5 g L^{-1} yeast extract, 1 g L^{-1} pepton, 30 g L^{-1} tropic marine sea salt in deionised water).

Assay mixtures were prepared by transferring 10 µl aliquots of methanolic solutions of extracts into a sterile 96-well microtiter plate and evaporating the solvent in a vacuum centrifuge (Schneemann et al. 2010). 200 µL overnight cultures of each test strain were diluted to an OD = 0.05 determined by spectrophotometry in the corresponding liquid media (see above). Cultures were incubated at 28°C for 24 h under constant shaking at 600 rpm. For evaluation of the cell viability, the reduction of resazurin to resorufin was assessed by measuring the intensity of fluorescence at 560Ex/590Em nm (Collins and Franzblau 1997). The tests were performed in three replicate treatments. The resulting values were compared to those for a positive control (100µg/well chloramphenicol for the bacteria, cycloheximide for *C. glabrata*), and respective negative controls wells: blank (extract, no bacteria), solvent (bacteria, no extract) and nutrient medium (no bacteria, no solvent) on the same plate (Schneemann et al. 2010).

RESULTS

Isolation and phylogenetic classification of bacteria

Altogether, 166 bacterial strains were isolated from the two macroalgae using samples taken between July 2008 and January 2010 in the Kiel Fjord (Baltic Sea, Germany). Strains with 16S rRNA gene sequences of >99.0% similarity were considered as one phylotype (based on specific level of sequence similarity and supported by the phylogenetic clustering, the most closely related type strains of all isolates are given in the Appendix). The strains affiliated to 82 phylotypes. Using one representative sequence for each phylotype, a phylogenetic tree was constructed (Fig. 1), which was used for detailed analyses. Phylogenetic analysis using the 16S rRNA gene sequences demonstrated that the bacteria isolated from *F. vesiculosus* and *D. sanguinea* were affiliated to six major classes of the bacterial domain (from four phyla), the Gram-positive *Actinobacteria* and *Bacilli*, and the Gram-negative *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Sphingobacteria* and *Flavobacteria* (Fig. 2). 57 phylotypes were associated with *D. sanguinea* (70%) and 43 phylotypes to *F. vesiculosus* (52%). 18 phylotypes were shared by both macroalgae, from which 7 were previously described as associated with algae (Table 1).

The detailed analyses revealed clear differences between the two algae with respect to the diversity of cultured bacteria (Table 2). The *Bacteroidetes* cluster contained 14 phylotypes (Fig. 1a) affiliating to *Algoriphagus*, *Cellulophaga*, *Cyclobacterium*, *Formosa*, *Maribacter*, *Olleya*, *Tenacibaculum*, *Winogradskyella*, and *Zobellia* species.

The *Alphaproteobacteria* contained 8 phylotypes (Fig. 1b) closely related to *Hoeflea*, *Labrenzia*, *Paracoccus*, *Pseudorhodobacter*, *Sulfitobacter*, and *Thalassobacter* species. The 25 phylotypes of *Gammaproteobacteria* (Fig. 1b) were closely related to species of *Aeromonas*, *Cobetia*, *Glaciecola*, *Marinomonas*, *Microbulbifer*, *Pantoea*, *Photobacterium*, *Pseudoalteromonas*, *Pseudomonas*, *Psychrobacter*, *Rheinheimera*, *Shewanella*, and *Vibrio* species (see Appendix). Only one single phylotype of *Betaproteobacteria* (gene sequence AB432f) was isolated and affiliated to a *Hydrogenophaga* species associated with the brown macroalga *Fucus vesiculosus* (Fig. 1b).

Among the Gram-positive bacteria, two main clusters were distinguishable, the *Bacilli* and the *Actinobacteria*. The *Bacilli* contained 19 phylotypes (Fig. 1c) with most sequences found to cluster in proximity to *Bacillus* and *Paenibacillus* species. The *Actinobacteria* contained 15 phylotypes (Fig. 1d) forming three branches which cluster in proximity to *Streptomyces*, *Nocardiopsis, Microbacterium* and *Salinibacterium* species.

Cluster analyses revealed that the isolated bacteria associated with both macroalgae comprised representatives of *Bacilli*, *Flavobacteria* and *Gammaproteobacteria* as stable components present over the seasons (winter and summer). *Alphaproteobacteria* were constantly found in association with *F. vesiculosus* (Fig. 2). Along these two years some phylotypes were repeatedly isolated by season (i.e. *Marinomonas dokdonensis* in summer, or *Zobellia galactanivorans*, *Microbulbifer thermotolerans* and *Photobacterium halotolerans* in winter), while other phylotypes were present during winter and summer, as *Bacillus amyloliquefaciens*, *B. licheniformis*, *B. hwajinpoensis*, *Cellulophaga baltica*, and *Olleya marilimosa* (see Appendix).

Antimicrobial activities

Antimicrobial activity was a common feature among the isolates (Fig.1). Extracts of 51 phylotypes (62%) comprising 85 isolates were active against at least one of the tested organisms (Table 2). The phylotypes that presented antimicrobial activity affiliated to *Bacillus algicola*, *Bacillus licheniformis*, *Bacillus hwajinpoensis*, *Bacillus pumilus*, *Bacillus subtilus*, *Cyclobacterium amurskyense*, *Formosa algae*, *Marinomonas polaris*, and *Microbulbifer thermotolerans*, *Photobacterium halotolerans*, *Salinibacterium amurskyense*, *Streptomyces fimicarius*, *Streptomyces* *griseorubens*, and *Vibrio rotiferianus* (Table 2, for detail see Table S1 in Supporting Information). Some of those bacteria exclusively associated with one of the two macroalgae interestingly often exhibited activity against macroalga-associated bacterial strains (Table 2). A particular high number of bioactive strains belong to the genera *Bacillus*, *Pseudoalteromonas* and *Streptomyces* (Table 3a, b).

The proportion of active strains isolated from *Delesseria sanguinea* was slightly higher (63%, 36 phylotypes) compared to *Fucus vesiculosus* (61%, 26 phylotypes) (Table 3a, b). Although there are changes between summer and winter in the occurence of associated epiphytic bacteria and therefore also in the individual capacity of production of bioactive compounds, a particular seasonal pattern of bioactivity during the two years was not observed neither in *Fucus* or *Delesseria* derived strains (see Table S2 in Supporting Information). An important result was that a higher portion of phylotypes was active against the macroalga-associated set of bacteria (58% of the *Fucus vesiculosus* and 54% of the *Delesseria sanguinea* phylotypes) as compared to the standard set of microorganisms (19% and 28% respectively, Table 3). As much as 16% of all phylotypes associated with *F. vesiculosus* and 19% with *D. sanguinea* were able to inhibit the growth of pathogens that affect brown macroalgae (Table 3a, b).

DISCUSSION

We investigated two co-occuring macroalgae *Fucus vesiculosus* and *Delesseria sanguinea* from the Kiel Fjord during summer and winter season for two years. We focussed on a cultivation-based approach for the determination of phylogenetic diversity and antimicrobial activity of bacterial isolates associated to them. In the same sampling area, both macroalgae have been shown by molecular methods to ‘harbor’ different associated microbial communities (Lachnit et al. 2009).

Phylogenetic analysis using the 16S rRNA gene sequences of the 82 bacterial phylotypes demonstrated that the bacteria isolated from *F. vesiculosus* and *D. sanguinea* were affiliated to four major phyla of the bacterial domain, *Actinobacteria*, *Proteobacteria*, *Firmicutes* and *Bacteroidetes* (Fig. 1 & 2). As in the present study, *Proteobacteria* and *Bacteroidetes* are usually dominant in aquatic environments (Cottrell and Kirchman 2000; Longford et al. 2007). A prevalence of sequences from these two bacterial groups has been reported in cultivation and molecular based studies of phytoplankton (Jasti et al. 2005), green macroalgae (Shiba and Taga 1980; Hengst et al. 2010; Burke et al. 2011; Hollants et al. 2011), and brown macroalgae (Kong and Chan 1979; Wiese et al. 2009; Lachnit et al. 2011), and hence appear to emerge as important associates of algae in general

(Longford et al. 2007; Tujula et al. 2010). Nevertheless, recently it has been demonstrated by molecular methods that the composition of the bacterial biofilm on Baltic Sea populations of *Fucus vesiculosus* varied with the season (Wahl et al. 2010; Lachnit et al. 2011), and as in the present results, demonstrates the importance of environmental impacts on the epiphytic microbial communities (see Appendix).

Among our 166 isolates, 39 strains affiliated to 14 type strains, which have been shown previously to be associated with algae (Table 1), indicating typical species to be associated with algae. Nevertheless, the fact that some bacteria were found only on one alga species (Table 2) indicates the presence of regulatory processes that act to selectively attract or eliminate specific phylotypes (Sneed and Pohnert 2011). The physiological and biochemical properties of the macroalgae predetermine the composition of the microbial communities adhering to their surfaces (Beleneva and Zhukova 2006; Hengst et al. 2010). The production of antibiotic and antifouling compounds by the algal host might influence relationships between the bacteria and macroalgae (Goecke et al. 2010; Persson et al. 2011). Nevertheless, it has been postulated that macroalgae without an important own chemical defense mechanism could rely on the secondary metabolites produced by their associated bacteria (Armstrong et al. 2001). Antimicrobial activity is widespread among alga-associated bacteria (Wiese et al. 2009, Table 3, see Table S2 in Supporting information for detail). The proportion of antibiotic active strains isolated from *Fucus vesiculosus* (60.5% of the phylotypes) and *Delesseria sanguinea* (63.2% of the phylotypes) is remarkably high (Table 3a, b): From the 18 bacterial phylotypes shared by both macroalgae a proportion of 72.2% was active against macroalga-associated bacteria (Table 3c).

Previous studies with *Fucus ceranoides* have shown that out 13% of bacteria associated with this alga (6 out of 45) were antibiotic active (Lemos et al. 1985). In another study, 17% of bacterial isolates associated with *Fucus serratus* were found to produce antibiotic substances (13 out of 77 isolates) (Boyd et al. 1999). There is no demonstration of antibiotic activity of bacteria associated with any species of *Delesseria*, though Skovhus et al. (2007) have identified several *Pseudoalteromonas* species associated with *D. sanguinea*, some of which grouped into a bacterial “antifouling subgroup”.

Although antimicrobial and antifouling metabolites are considered to selectively target marine microorganisms, the susceptibility of ecologically relevant bacteria has rarely been studied (Puglisi et al. 2007; Kanagasabhapathy et al. 2008; Vairappan et al. 2010). The specific action against possible environmental competitors is supported by the high portion (54-58%) of antimicrobial active bacteria observed against macroalga-associated bacteria (Table 3a, b). Particularly interesting is the high proportion of isolates active against pathogens affecting brown macroalgae (Fig. 3a, b).

Some of the phylotypes that presented antimicrobial activity were found associated exclusively with *Fucus vesiculosus* or only with *Delesseria sanguinea* (Table 2), which displayed antimicrobial activity especially against potential competitors or algal pathogen strains. In particular representatives of *Pseudoalteromonas* and *Bacillus* species were shown previously to be efficient producers of antimicrobial compounds and therefore highly successful colonizers of macroalgal surfaces (Egan et al. 2001; Holmström et al. 2002; Burgess et al. 2003; Kanagasabhapathy et al. 2006; Skovhus et al. 2007) (Fig. 1b, c, and see Table S2 in Supporting Information for detail).

A slightly higher number of antibacterial active phylotypes (and isolates) was found associated with *D. sanguinea* (Table 2, 3). If it is assumed that *F. vesiculosus* is better equipped for self-defense against bacterial attack than *D. sanguinea* (see Sandsdalen et al. 2003; Saha et al. 2011; Table S3 in Supporting Information), it can be expected that it depends to a lower degree on antibiotic active bacteria for its own defense against microbial attack. This is in line with our findings. However, it is not known, how the host alga selectively attracts and harbors such epibionts (Harder 2009).

Our study showed for the first time seasonal variations in occurrence and bioactivity patterns of different phylotypes associated with a brown and a red macroalga living at the same location. According to our results, we suggest a three dimensional factorial regulation of the epiphytic bacterial communities on algae: The host with its physiological and structural features, the environmental conditions, and the epiphytic bacteria itself. Further studies regarding the occurrence and quantification of the bioactive epiphytic strains on *F. vesiculosis* and *D. sanguinea*, respectively, using molecular-based approaches like FISH-analysis and the chemical analysis of the antibiotic compounds will contribute to a better understanding of the macroalgae-bacteria relationship.

Table 1 39 out of 166 isolates from *Fucus vesiculosus* (F) and *Delesseria sanguinea* (D) affiliated to 14 type strains previously described as associated with algae (source). The similarity (%) to the closest type strain and GenBank accession number is given. NI is the number of isolates.

Next related type strain (accession nr.)	**S%**	**Source**	**Sample**	**NI**	**Reference**
Bacillus algicola KMM 3737^{T} (AY228462)	99	*Fucus evanescens*	D & F	3	Ivanova et al. 2004a
Cellulophaga baltica NN 015840^{T} (AJ005972)	99	*Fucus serratus*	D & F	7	Johansen et al. 1999
Cellulophaga fucicola NN 015860^{T} (AJ005973)	99	*Fucus serratus*	D	1	Johansen et al. 1999
Cobetia marina DSM 4741^{T} (AJ306890)	99	*Fucus evanescens*	D & F	4	Ivanova et al. 2002
Formosa algae KMM 3553^{T}* (AY228461)	99	*Fucus evanescens*	F	2	Ivanova et al. 2004b
Hoeflea alexandrii AM1V30^{T} (AJ786600)	99	*Alexandrium minutum*	D	1	Palacios et al. 2006
Olleya marilimosa CAM 030^{T} (AY586527)	99	*Saccharina latissima*	D & F	3	Wiese et al. 2009
Microbulbifer epialgicus F-104^{T}* (AB266054)	99	*Caulerpa* sp.	D & F	2	Nishijima et al. 2009
Pseudoalteromonas atlantica IAM 12927 ^{T} (X82134)	99	*Lessonia* sp.	D & F	5	Akagawa-Matsushita et al. 1992
Pseudoalteromonas elyakovii KMM 162^{T} (AF116178)	99	*Saccharina japonica*	F	3	Sawabe et al. 2000
Pseudoalteromonas mariniglutinosa KMM3635^{T}* (AJ507251)	99	*Chaetoceros lauderi*	D & F	2	Romanenko et al. 2003
Pseudoalteromonas tunicata D2^{T} (Z25522)	99	*Ulva* spp.	D	1	Rao et al. 2007
Pseudoalteromonas ulvae UL12^{T} (AF172987)	99	*Ulva lactuca*	D	4	Egan et al. 2001
Zobellia galactanivorans Dsij^{T}* (AF208293)	99	*Delesseria sanguinea*	F	1	Barbeyron et al. 2001

*members of this species have been isolated only from algal sources.

Table 2 All different phylotypes (P-No.) with respective closest type strain (in parenthesys), that associated exclusively with *Fucus vesiculosus* or *Delesseria sanguinea*. They affiliated to 6 bacterial domain according 16S rRNA gene sequences. Only the phylotypes with antibiotic active bacteria are displayed with (*) against the standard set, (†) against macroalga-associated set, and (’) not determined.

Bacterial domain	***Fucus vesiculosus***	***Delesseria sanguinea***
Bacteroidetes	P27 (*Formosa algae*)* †	P2 (*Algoriphagus yeomjeoni*) †
	P34 (*Maribacter aquivivus*)’	P22 (*Cellulophaga fucicola*)
	P35 (*Marinomonas dokdonensis*)* †	P75 (*Tenacibaculum adriaticum*)
	P23 (*Cellulophaga tyrosinoxydans*)*	P81 (*Zobellia amurskyensis*) †
	P37 (*Marinomonas polaris*) †	
	P38 (*Marinomonas pontica*)	
	P80 (*Winogradskyella echinorum*) †	
α-Proteobacteria	P31 (*Labrenzia marina*)	P29 (*Hoeflea alexandrii*) †
	P46 (*Paracoccus marcusii*) †	P56 (*Pseudorhodobacter ferrugineus*)†
	P76 (*Thalassobacter arenae*)’	P57 (*Pseudorhodobacter ferrugineus*)
		P74 (*Sulfitobacter guttiformis*)
γ-Proteobacteria	P1 (*Aeromonas bivalvium*)	P33 (*Olleya marilimosa*)’
	P24 (*Cobetia marina*) †	P45 (*Pantoea agglomerans*)* †
	P28 (*Glaciecola mesophila*)	P48 (*Photobacterium halotolerans*)*†
	P51 (*Pseudoalteromonas mariniglutinosa*)*	P49 (*Photobacterium halotolerans*) †
	P55 (*Pseudomonas marincola*) †	P52 (*Pseudoalteromonas mariniglutinosa*)’
	P79 (*Vibrio rumoiensis*)’	P53 (*Pseudoalteromonas tunicata*)
		P54 (*Pseudoalteromonas ulvae*)* †
		P58 (*Psychrobacter maritimus*)* †
		P59 (*Rheinheimera pacifica*)*
		P61 (*Shewanella baltica*)
		P78 (*Vibrio rotiferianus*) †
β-Proteobacteria	P30 (*Hydrogenophaga taeniospiralis*)	-
Bacilli	P5 (*Bacillus altitudinis*)’	P3 (*Bacillus aerophilus*)
	P13 (*Bacillus infantis*) †	P9 (*Bacillus anthracis*)’
	P14 (*Bacillus licheniformis*)	P10 (*Bacillus aquimaris*)
	P17 (*Bacillus subtilis*) †	P11 (*Bacillus baekryungensis*)
		P16 (*Bacillus safensis*)*
		P43 (*Paenibacillus lautus*)* †
		P44 (*Paenibacillus xylanexedens*)
Actinobacteria	P19 (*Brevibacterium frigoritolerans*) †	P18 (*Brevibacterium frigoritolerans*)†
	P66 (*Streptomyces fimicarius*) †	P39 (*Microbacterium hatanonis*) †
	P67 (*Streptomyces griseinus*) †	P42 (*Nocardiopsis alba*) †
	P68 (*Streptomyces griseoflavus*)	P60 (*Salinibacterium amurskyense*) †
	P69 (*Streptomyces griseoplanus*) †	P62 (*Streptomyces alboviridis*)
		P63 (*Streptomyces chrysomallus*) †
		P64 (*Streptomyces ciscaucasicus*)*
		P65 (*Streptomyces drozdowiczii*)*
		P70 (*Streptomyces griseorubens*)*†
		P71 (*Streptomyces griseorubens*)*†
		P72 (*Streptomyces griseorubens*)*†
		P73 (*Streptomyces sampsonii*)

Table 3. Total number and percentage of phylotypes exhibiting antibiotic active members with respect to their origin: *Fucus vesiculosus* (3a) or *Delesseria sanguinea* (3b) as host, and the strain shared by both macroalgae. The specific activity against standard test strains: *Bacillus subtilis*, *Staphylococcus lentus*, *Escherichia coli*, and *Candida glabrata* ("Standard set"), surface-associated isolates: *Bacillus algicola* and *Formosa algae* ("Associated set"), and macroalgal pathogenic bacteria: *Algicola bacteriolytica* and *Pseudoalteromonas elyakovii* ("Pathogenic set") is given. The total active phylotypes represent the number of different phylotypes by algae that inhibited at least one test strain.

3a) *Fucus vesiculosus*

	Active against			
	Standard set	**Associated set**	**Pathogenic set**	**Assoc. or Path.**
Actinobacteria	0	3	1	3
Alphaproteobacteria	0	2	0	2
Betaproteobacteria	0	0	0	0
Gammaproteobacteria	3	5	2	6
Bacilli	3	4	3	8
Bacteroidetes group	2	6	1	6
No. of phylotypes with bioactive strains	8	20	7	25
	(18.6%)	(46.5%)	(16.2%)	(58.1%)
Total no. of phylotypes with bioactive strains: 26 out of 43 (60.5%)				

3b) *Delesseria sanguinea*

	Active against			
	Standard set	**Associated set**	**Pathogenic set**	**Assoc. or Path.**
Actinobacteria	5	7	3	7
Alphaproteobacteria	0	3	0	3
Betaproteobacteria	0	0	0	0
Gammaproteobacteria	6	7	4	10
Bacilli	3	6	3	7
Bacteroidetes group	2	3	1	4
No. of phylotypes with bioactive strains	16	26	11	31
	(28.1%)	(45.6%)	(19.3%)	(54.4%)
Total no. of phylotypes with bioactive strains: 36 out of 57 (63.2%)				

3c) Shared by both macroalgae

	Active against			
	Standard set	**Associated set**	**Pathogenic set**	**Assoc. or Path.**
Actinobacteria	0	0	0	0
Alphaproteobacteria	0	1	0	1
Betaproteobacteria	0	0	0	0
Gammaproteobacteria	1	3	1	4
Bacilli	3	4	4	5
Bacteroidetes group	2	3	1	3
No. of phylotypes with bioactive strains	6	12	6	13
	(33.3%)	(66.6%)	(33.3%)	(72.2%)
Total no. of phylotypes with bioactive strains: 13 out of 18 (72.2%)				

Fig. 1a Phylogenetic tree of sequences belonged to *Bacteroidetes*.

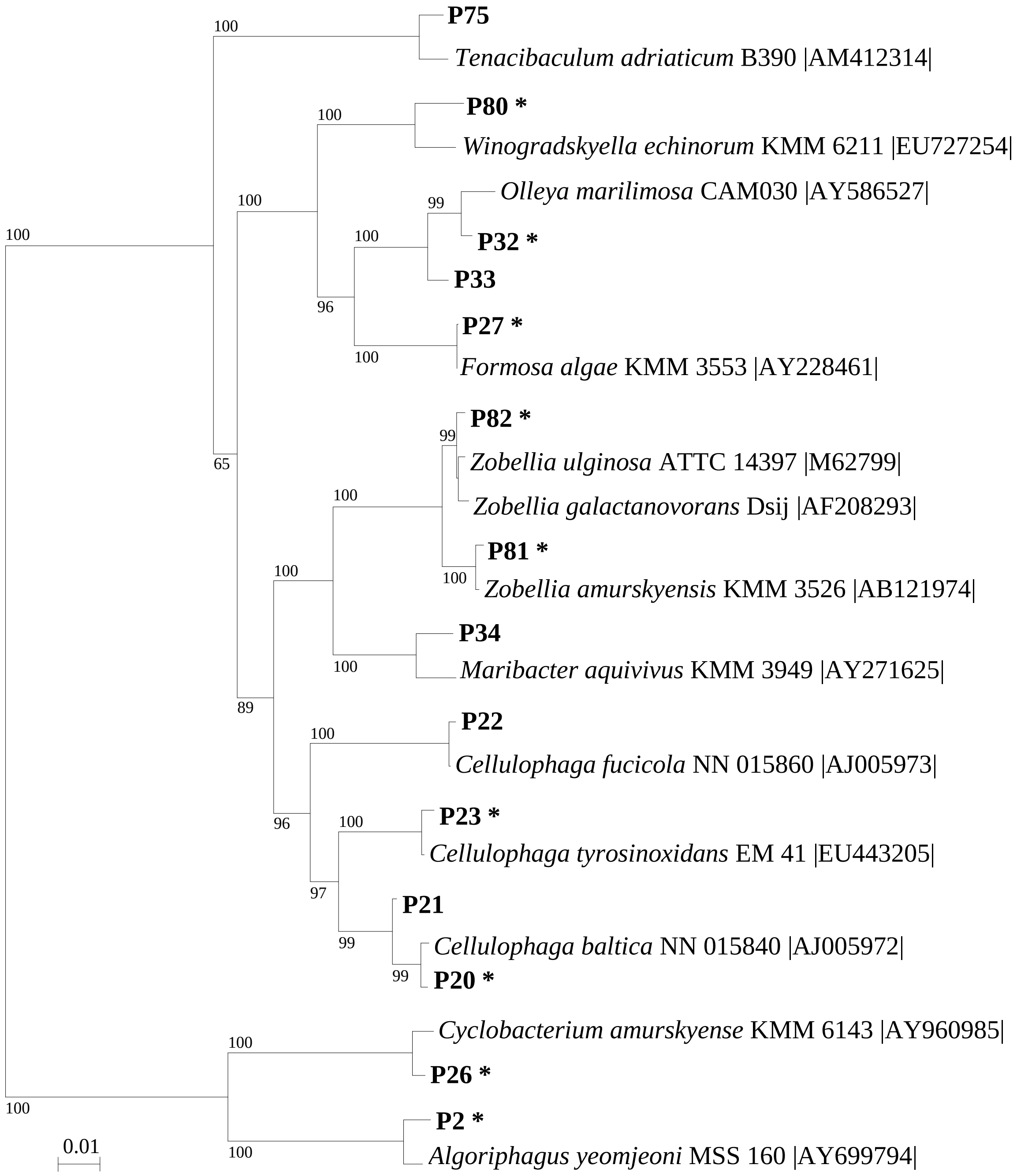

Fig. 1b Phylogenetic tree of sequences belonged to *Proteobacteria*.

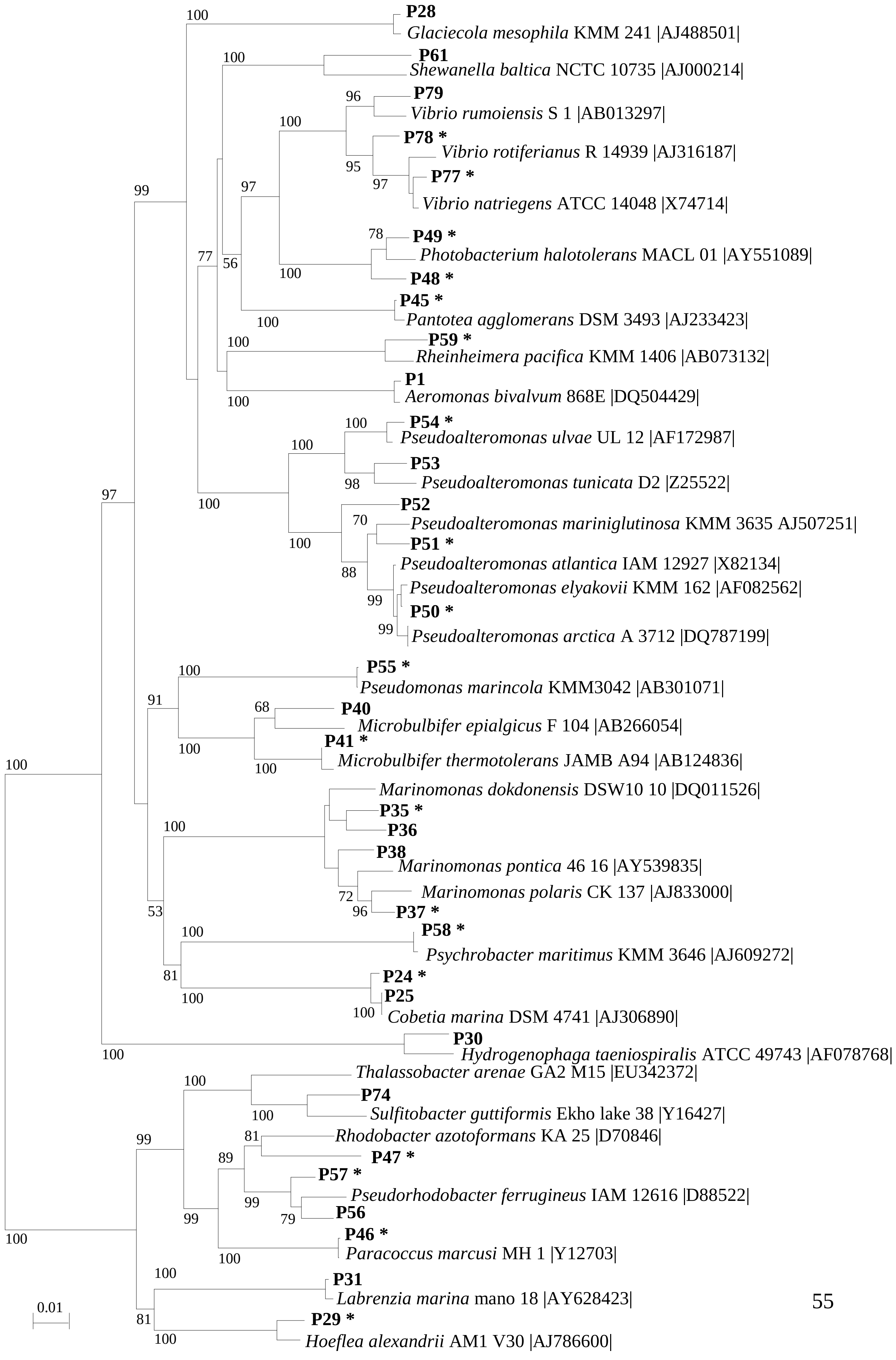

Fig. 1c Phylogenetic tree of sequences belonged to *Firmicutes*.

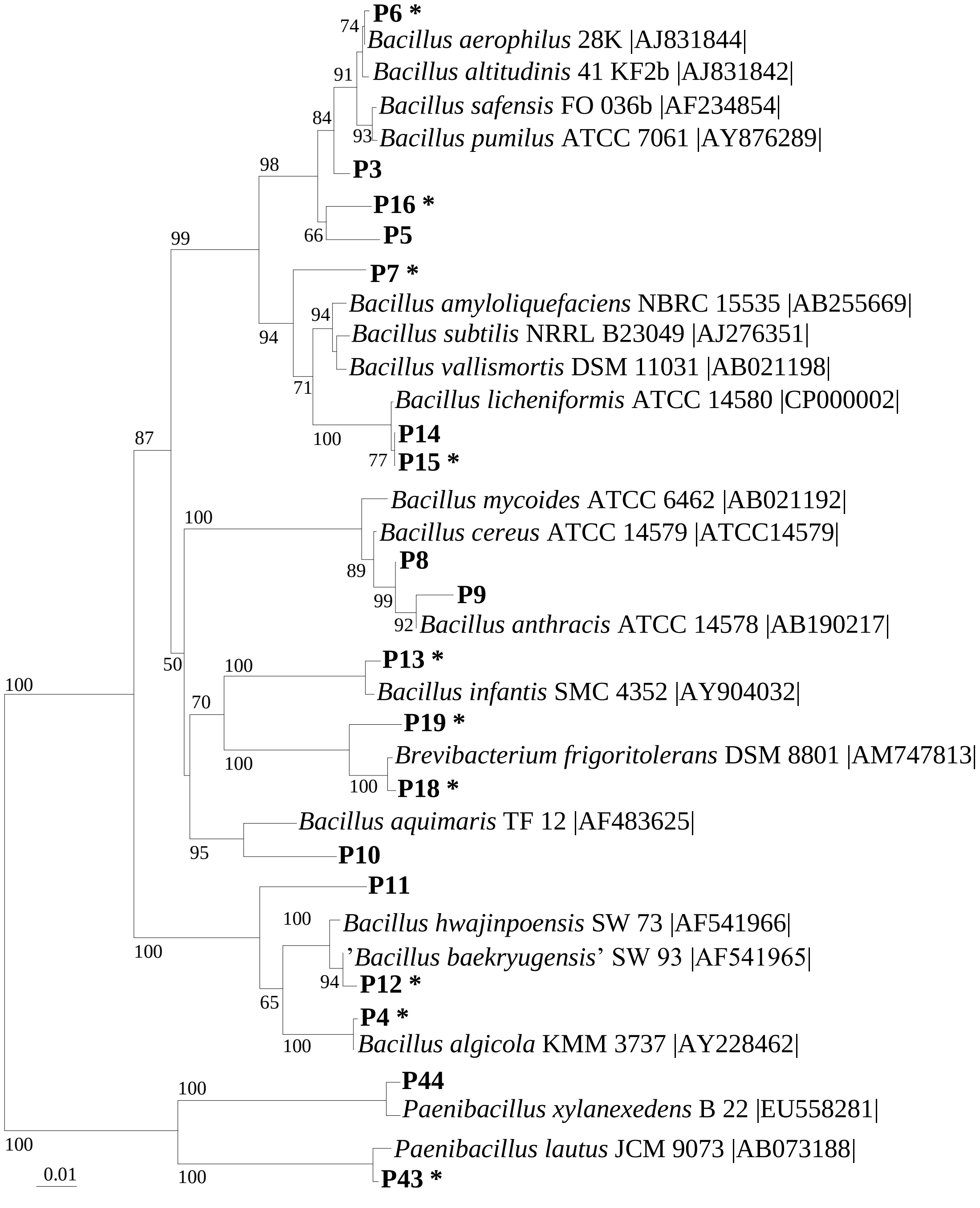

Fig. 1d Phylogenetic tree of sequences belonged to *Actinobacteria*.

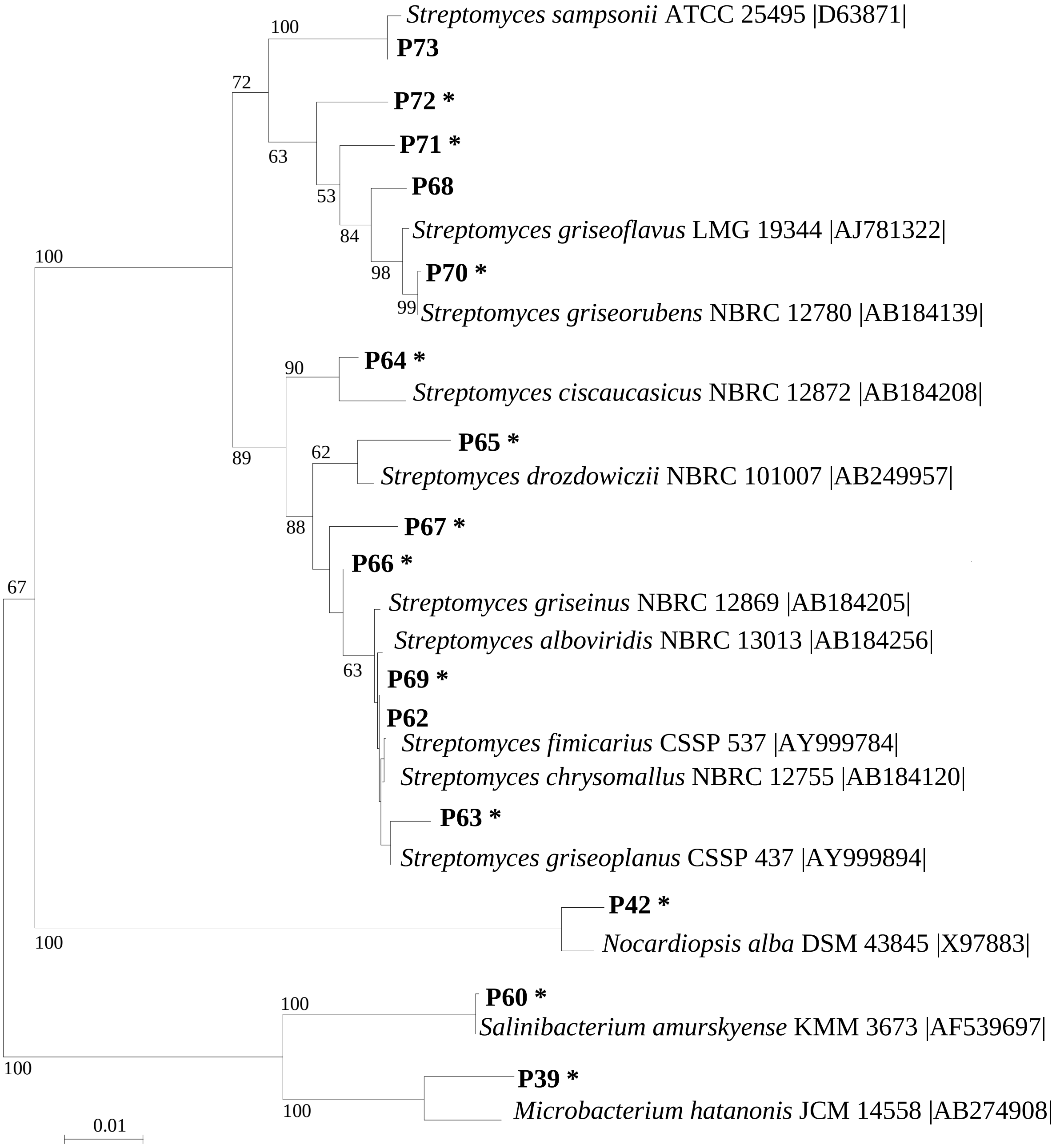

Figure 1. Phylogenetic relationships of bacteria associated with the two algae and their most closely related type strains (with NCBI accession number). Phylogenetic trees of sequences belonged to *Bacteroidetes* (Fig. 1a), *Proteobacteria* (Fig. 1b), *Bacilli* (Fig. 1c), and *Actinobacteria* (Fig. 1d) are given in separate. Neighbor-joining tree of phylogenetic relationships of the macroalga-associated bacteria are based on 16S rRNA gene sequences. Representatives of the phylotypes from this study are shown in bold type. Non-parametric bootstrapping analysis (1000 datasets) was conducted. Values equal to or greater than 50% are shown. The scale bar indicates the number of substitutions per nucleotide position. Only the phylotypes with antibiotic active bacteria are displayed with (*).

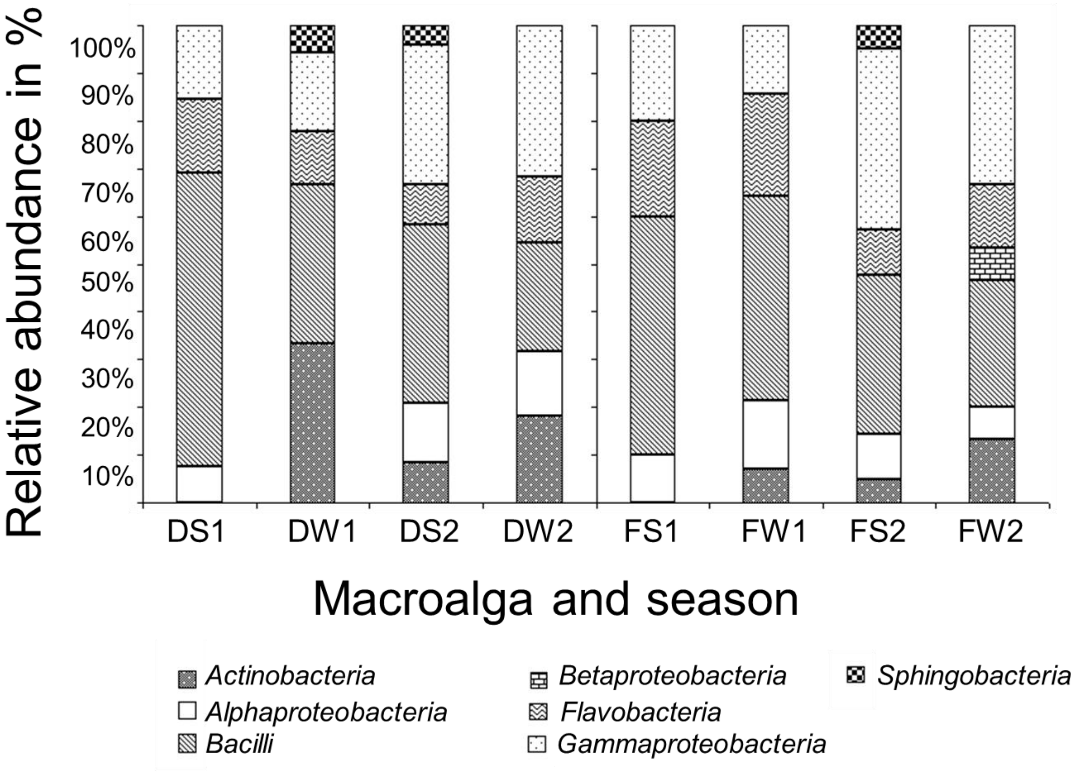

Figure 2. Relative abundance (in percentage) of the strains belonging to different bacterial classes regarding their origin: left, *Delesseria sanguinea* (D) and right, *Fucus vesiculosus* (F) in the different seasons (S1: July 2008, W1: January 2009, S2: July 2009, W2: January 2010).

Appendix

Classification of bacterial strains from the two macroalgae based on 16S rRNA gene sequences analysis. The affiliation to phylotypes (Ph) determined by similarity matrix (Phylip), the similarity (%) to the next closest type strain and GenBank accession number is given. *Fucus vesiculosus* (Fu), *Delesseria sanguinea* (De), GPY/TM/SCA/HWO correspond to the nutrient media; and the season is represented by (S1), summer I, (W1) winter 1, (S2) summer 2 and (W2) winter 2. The number of microorganisms inhibited by the strain is also given (No.Ac), where nd: not determined.

Ph	Isolate	Access. nr.	Bp	%	Next related type strain (accession nr.)	Sample	NoAc
P1	AB308f	FR718448	1396	99	*Aeromonas bivalvium* 868E^T (DQ504429)	FUGPY7 S2	0
P2	AB267d	FR821090	1345	98	*Algoriphagus yeomjeoni* MSS-160^T (AY699794)	DEGPY7 W1	1
P3	AB304d	FR821091	1417	98	*Bacillus aerophilus* 41KF2b^T (AJ831844)	DEGPY6 S2	0
P4	AB341d	FR821092	1396	99	*Bacillus algicola* KMM 3737^T (AY228462)	DETM5 S2	1
	AB357d	FR821093	1400	99	*Bacillus algicola* KMM 3737^T (AY228462)	DEHWO1 S2	0
	AB423f	FR775437	1376	99	*Bacillus algicola* KMM 3737^T (AY228462)	FUTM23 W2	1
P5	AB305f	FR821094	1463	98	*Bacillus altitudinis* 41KF2b^T (AJ831842)	FUSCA2 S2	nd
P6	AB335f	FR821095	1406	99	*Bacillus aerophilus* 28K^T (AJ831844)	FUTM5 S2	1
	AB196d	FR821096	1386	99	*Bacillus altitudinis* 41KF2b^T (AJ831842)	DETM2 S1	0
	AB243d	FR821097	1371	99	*Bacillus altitudinis* 41KF2b^T (AJ831842)	DEHWO13W1	nd
	AB244d	FR821098	1390	99	*Bacillus altitudinis* 41KF2b^T (AJ831842)	DEGPY1 W1	1
	AB247f	FR821099	1389	99	*Bacillus altitudinis* 41KF2b^T (AJ831842)	FUSCA2 W1	2
	AB249d	FR821100	1384	99	*Bacillus altitudinis* 41KF2b^T (AJ831842)	DEGPY2 W1	1
	AB412d	FR821101	1397	99	*Bacillus pumilus* ATCC 7061^T (AY876289)	DETSB15 W2	1
	AB438f	FR821102	1402	99	*Bacillus pumilus* ATCC 7061^T (AY876289)	FUTM48 W2	1
	AB200d	FR821103	1393	99	*Bacillus safensis* FO-036b^T (AF234854)	DETM6 S1	0
	AB208f	FR821104	1405	99	*Bacillus safensis* FO-036b^T (AF234854)	FUSCA5 S1	2
	AB224d	FR821105	1400	100	*Bacillus safensis* FO-036b^T (AF234854)	DEGPY2 S1	1
	AB235f	FR821106	1396	99	*Bacillus safensis* FO-036b^T (AF234854)	FUSCA2 S1	1
	AB212f	FR821107	1400	99	*Bacillus safensis* FO-036b^T (AF234854)	FUTM12 S1	nd
P7	AB202d	FR821123	1376	99	*Bacillus amyloliquefaciens* NBRC 15535^T (AB255669)	DETSB5 W1	0
	AB263f	FR821109	1323	99	*Bacillus amyloliquefaciens* NBRC 15535^T (AB255669)	FUSCA5W1	nd
	AB306f	FR821110	1394	99	*Bacillus amyloliquefaciens* NBRC 15535^T (AB255669)	FUGPY3 S2	0
	AB321d	FR821111	1402	99	*Bacillus amyloliquefaciens* NBRC 15535^T (AB255669)	DEGPY1 S2	1
	AB332d	FR821112	1394	99	*Bacillus amyloliquefaciens* NBRC 15535^T (AB255669)	DETM4 S2	0
	AB339d	FR821113	1390	99	*Bacillus amyloliquefaciens* NBRC 15535^T (AB255669)	DEHWO2S2	0
	AB363f	FR821114	1396	99	*Bacillus amyloliquefaciens* NBRC 15535^T (AB255669)	FUTMb S2	0
	AB256d	FR821115	1072	99	*Bacillus subtilis* NRRL B-23049^T (AF074970)	DETM11 W1	nd
	AB230d	FR821116	1399	99	*Bacillus subtilis* NRRL B-23049^T (AF074970)	DEGPY9 S1	2
	AB270f	FR821117	1375	99	*Bacillus vallismortis* DSM11031^T (AB021198)	FUGPY1 W1	0
	AB227d	FR821118	1404	99	*Bacillus subtilis* NRRL B-23049^T (AF074970)	DEGPY5 S1	1
	AB209f	FR821119	1396	99	*Bacillus subtilis* NRRL B-23049^T (AF074970)	FUSCA6 S1	1
	AB410d	FR821120	1392	99	*Bacillus subtilis* NRRL B-23049^T (AF074970)	DETSB12 W2	1
	AB194d	FR821121	1383	99	*Bacillus vallismortis* DSM11031^T (AB021198)	DESCA1 S1	1
	AB242d	FR821122	1362	99	*Bacillus vallismortis* DSM11031^T (AB021198)	DETM1 W1	0
	AB273d	FR821108	1376	99	*Bacillus vallismortis* DSM11031^T (AB021198)	DETM8 S1	nd
P8	AB289d	FR821127	1371	98	*Bacillus anthracis* ATCC 14578^T (AB190217)	DEHWO3 W1	0
	AB315f	FR821125	1375	99	*Bacillus anthracis* ATCC 14578^T (AB190217)	FUTSB1 S2	nd
	AB197d	FR821126	1372	99	*Bacillus cereus* ATCC 14579^T (AE016877)	DETM3 S1	0
	AB250d	FR821124	1391	100	*Bacillus mycoides* ATCC6462^T (AB021192)	DETM7 S2	0
P9	AB358d	FR821128	1371	99	*Bacillus anthracis* ATCC 14578^T (AB190217)	DETM10 S2	nd
P10	AB409d	FR821129	1084	98	*Bacillus aquimaris* TF-12^T (AF483625)	DETSB11 W2	0
P11	AB286d	FR821130	1067	98	'*Bacillus baekryungensis*' SW-93 (AF541965)	DEHWO4 S2	0
P12	AB471d	FR821131	1297	99	*Bacillus hwajinpoensis* SW-72^T (AF541966)	DESCA1 S2	1
	AB419f	FR821132	1397	99	*Bacillus hwajinpoensis* SW-72^T (AF541966)	FUTM29 W2	1
	AB309f	FR821133	1411	99	*Bacillus hwajinpoensis* SW-72^T (AF541966)	FUGPY5 S2	0
	AB257f	FR821137	1391	99	*Bacillus hwajinpoensis* SW-72^T (AF541966)	FUSCA6 W1	1
	AB342d	FR821135	1409	99	*Bacillus hwajinpoensis* SW-72^T (AF541966)	DEGPY7 S2	1
	AB352d	FR821136	1411	99	*Bacillus hwajinpoensis* SW-72^T (AF541966)	DEGPY13 S2	0
	AB343d	FR821134	1410	99	*Bacillus hwajinpoensis* SW-72^T (AF541966)	DESCA62 W2	nd
P13	AB217f	FR821139	1403	99	*Bacillus infantis* SMC 4352-1^T (AY904032)	FUTM1 W1	2
	AB265f	FR821138	1392	99	*Bacillus infantis* SMC 4352-1^T (AY904032)	FUTM7 S1	1
P14	AB213f	FR821140	1396	99	*Bacillus licheniformis* ATCC 14580^T (CP000002)	FUTM3 S1	0
P15	AB201d	FR821147	1382	99	*Bacillus licheniformis* ATCC 14580^T (CP000002)	DETM7 S1	1
	AB203d	FR821142	1395	99	*Bacillus licheniformis* ATCC 14580^T (CP000002)	DETM9 S1	1

	AB246d	FR821143	1392	99	*Bacillus licheniformis* ATCC 14580^{T} (CP000002)	DEHWO1 W1	1
	AB266f	FR821144	1380	99	*Bacillus licheniformis* ATCC 14580^{T} (CP000002)	FUSCA36 W2	2
	AB353f	FR821145	1383	99	*Bacillus licheniformis* ATCC 14580^{T} (CP000002)	FUHWO6 S2	0
	AB424f	FR821146	1415	99	*Bacillus licheniformis* ATCC 14580^{T} (CP000002)	FUGPY34 W2	0
	AB464f	FR821141	1315	99	*Bacillus licheniformis* ATCC 14580^{T} (CP000002)	FUSCA1 W1	0
P16	AB366d	FR821148	1076	99	*Bacillus safensis* FO-036b^{T} (AF234854)	DETSB6 bl S2	1
P17	AB297f	FR821149	1474	98	*Bacillus subtilis* NRRL B-23049^{T} (AJ276351)	FUTSB4 S2	1
P18	AB195d	FR821150	1404	99	*Brevibacterium frigoritolerans* DSM8801^{T} (AM747813)	DETM1 S1	1
P19	AB218f	FR821151	1073	98	*Brevibacterium frigoritolerans* DSM8801^{T} (AM747813)	FUTM8 S1	1
P20	AB252f	FR821156	1363	99	*Cellulophaga baltica* NN015840^{T} (AJ005972)	DESCA8 S2	2
	AB262f	FR821153	1368	99	*Cellulophaga baltica* NN015840^{T} (AJ005972)	FUTM2 W1	1
	AB405d	FR821154	1377	98	*Cellulophaga baltica* NN015840^{T} (AJ005972)	DEHWO7 W2	0
	AB420f	FR821155	1390	99	*Cellulophaga baltica* NN015840^{T} (AJ005972)	FUGPY31 W2	0
	AB359d	FR821152	1269	97	*Cellulophaga baltica* NN015840^{T} (AJ005972)	FUGPY4 W1	nd
P21	AB290f	FR821157	1046	99	*Cellulophaga baltica* NN015840^{T} (AJ005972)	DEGPY3 S2	0
	AB299d	FR821158	1063	99	*Cellulophaga baltica* NN015840^{T} (AJ005972)	FUTM9 S2	0
P22	AB261d	FR821159	1381	99	*Cellulophaga fucicola* NN015860^{T} (AJ005973)	DEHWO4 W1	0
P23	AB204d	FR821160	1391	99	*Cellulophaga tyrosinoxydans* EM41^{T} (EU443205)	DETM10 S1	1
P24	AB320f	FR821161	1351	98	*Cobetia marina* DSM 4741^{T} (AJ306890)	FUTM2 S2	1
P25	AB312d	FR821164	1062	99	*Cobetia marina* DSM 4741^{T} (AJ306890)	FUTM8 S2	0
	AB324d	FR821163	1377	99	*Cobetia marina* DSM 4741^{T} (AJ306890)	DEGPY2 S2	0
	AB350f	FR821162	1363	99	*Cobetia marina* DSM 4741^{T} (AJ306890)	DETSB2b S2	0
P26	AB294d	FR821166	1360	98	*Cyclobacterium amurskyense* KMM 6143^{T} (AY960985)	FUTM7 S2	1
	AB348f	FR821165	1365	98	*Cyclobacterium amurskyense* KMM 6143^{T} (AY960985)	DESCA4bS2	1
P27	AB322f	FR775436	1400	99	*Formosa algae* KMM 3553^{T} (AY228461)	FUSCA3 S2	1
	AB356f	FR821167	1391	98	*Formosa algae* KMM 3553^{T} (AY228461)	FUGPY4 S2	3
P28	AB437f	FR821168	1387	99	*Glaciecola mesophila* KMM 241^{T} (AJ488501)	FUTM47 W2	0
P29	AB205d	FR821169	1062	99	*Hoeflea alexandrii* AM1V30^{T} (AJ786600)	DEGPY1 S1	1
P30	AB432f	FR821170	1389	98	*Hydrogenoph. taeniospiralis* ATCC 49743^{T} (AF078768)	FUTSB42W2	0
P31	AB434f	FR821171	1341	98	*Labrenzia marina* mano18^{T} (AY628423)	FUGPY44 W2	0
P32	AB233d	FR821173	1353	99	*Olleya marilimosa* CAM030^{T} (AY586527)	FUSCA39 W2	1
	AB429f	FR821172	1386	99	*Olleya marilimosa* CAM030^{T} (AY586527)	DEHWO4 S1	1
P33	AB292d	FR821174	1068	98	*Olleya marilimosa* CAM030^{T} (AY586527)	DESCA12 S2	nd
P34	AB214f	FR821175	1362	98	*Maribacter aquivivus* KMM 3949^{T} (AY271625)	FUTM4 S1	nd
P35	AB216f	FR821176	1051	97	*Marinomonas dokdonensis* DSW10-10^{T} (DQ011526)	FUTM6 S1	2
P36	AB287f	FR821178	1391	97	*Marinomonas dokdonensis* DSW10-10^{T} (DQ011526)	DETSB6na S2	nd
	AB337d	FR821177	1382	97	*Marinomonas dokdonensis* DSW10-10^{T} (DQ011526)	FUSCA8 S2	0
P37	AB433f	FR821180	1364	99	*Marinomonas polaris* CK137^{T} (AJ833000)	FUSCA38 W2	1
	AB465f	FR821179	1267	99	*Marinomonas polaris* CK137^{T} (AJ833000)	FUTSB43 W2	nd
P38	AB314f	FR821181	1067	99	*Marinomonas pontica* 46-16^{T} (AY539835)	FUTSBbS2	0
P39	AB302d	FR821182	1377	99	*Microbacterium hatanonis* JCM 14558^{T} (AB274908)	DETSB4 S2	1
P40	AB443f	FR821184	1063	98	*Microbulbifer epialgicus* F-104^{T} (AB266054)	DETM71 W2	nd
	AB454d	FR821183	1380	99	*Microbulbifer epialgicus* F-104^{T} (AB266054)	FUTM53 W2	nd
P41	AB259d	FR821187	1354	99	*Microbulbifer thermotolerans* JAMB A94^{T} (AB124836)	DEHWO54W2	2
	AB425f	FR821186	1384	99	*Microbulbifer thermotolerans* JAMB A94^{T} (AB124836)	FUGPY35 W2	2
	AB470d	FR821185	1270	99	*Microbulbifer thermotolerans* JAMB A94^{T} (AB124836)	DEHWO5 W1	0
P42	AB240d	FR821188	1358	99	*Nocardiopsis alba* DSM 43845^{T} (X97883)	DETSB12 W1	1
P43	AB236d	FR821189	1343	99	*Paenibacillus lautus* JCM 9073^{T} (AB073188)	DESCA3 W1	2
	AB241d	FR775438	1356	99	*Paenibacillus lautus* JCM 9073^{T} (AB073188)	DETSB4 W1	2
P44	AB355d	FR821190	1387	99	*Paenibacillus xylanexedens* B22a^{T} (EU558281)	DETSB7 S2	0
P45	AB365d	FR821191	1309	99	*Pantoea agglomerans* DSM 3493^{T} (AJ233423)	DESCA5 S2	2
P46	AB219f	FR821192	1317	99	*Paracoccus marcusii* MH1^{T} (Y12703)	FUGPY1 S1	1
P47	AB277f	FR821196	1308	99	*Paracoccus marinus* KKL-A5^{T} (AB185957)	DEHWO9 W2	1
	AB307d	FR821194	1316	99	*Paracoccus marinus* KKL-A5^{T} (AB185957)	DETM1 S2	0
	AB300d	FR821195	1329	99	*Rhodobacter azotoformans* KA25^{T}(D70846)	DEGPY5 S2	1
	AB407d	FR821193	1328	99	*Rhodobacter azotoformans* KA25^{T}(D70846)	FUGPY6 W1	0
P48	AB238d	FR821199	1314	98	*Photobacterium halotolerans* MACL01^{T} (AY551089)	DETSB6 W1	nd
	AB248d	FR821198	1364	97	*Photobacterium halotolerans* MACL01^{T} (AY551089)	DESCA6 W1	4
	AB272d	FR821197	1360	97	*Photobacterium halotolerans* MACL01^{T} (AY551089)	DEGPY3 W1	nd
P49	AB408d	FR821200	1368	99	*Photobacterium halotolerans* MACL01^{T} (AY551089)	DETSB10 W2	1
P50	AB416d	FR821209	1410	99	*Pseudoalteromonas arctica* A 37-1-2^{T} (DQ787199)	FUTM68 W2	1
	AB293f	FR821202	1413	99	*Pseudoalteromonas atlantica* IAM 12927^{T} (X82134)	FUSCA4 S2	nd
	AB296d	FR821203	1414	99	*Pseudoalteromonas atlantica* IAM 12927^{T} (X82134)	DESCA2 S2	4
	AB330f	FR821204	1403	99	*Pseudoalteromonas atlantica* IAM 12927^{T} (X82134)	FUHWO5 S2	0
	AB333f	FR821205	1398	99	*Pseudoalteromonas atlantica* IAM 12927^{T} (X82134)	FUTSB3 S2	4
	AB360f	FR821206	1409	99	*Pseudoalteromonas atlantica* IAM 12927^{T} (X82134)	FUHWO2 S2	0
	AB462f	FR821207	1297	99	*Pseudoalteromonas elyakovii* KMM162^{T} (AF082562)	FUTM52 W2	0
	AB466f	FR821208	1309	99	*Pseudoalteromonas elyakovii* KMM162^{T} (AF082562)	FUSCA40 W2	2

	AB474f	FR821201	1318	99	*Pseudoalteromonas elyakovii* KMM162^T (AF082562)	DESCA26W2	nd
P51	AB276f	FR821210	1376	99	*Pseudoalteromonas mariniglutinosa* (AJ507251)	FUSCA4W1	1
P52	AB291d	FR821211	1068	99	*P. mariniglutinosa* KMM 3635^T (AJ507251)	DETSB3 S2	nd
P53	AB231d	FR821212	1387	99	*Pseudoalteromonas tunicata* D2^T (Z25522)	DEHWO2 S1	0
P54	AB198d	FR821216	1370	98	*Pseudoalteromonas ulvae* UL12^T (AF172987)	DEGPY8 S1	3
	AB199d	FR821214	1386	99	*Pseudoalteromonas ulvae* UL12^T (AF172987)	DETM5 S1	nd
	AB228d	FR821215	1365	99	*Pseudoalteromonas ulvae* UL12^T (AF172987)	DEGPY6 S1	2
	AB229d	FR821213	1388	98	*Pseudoalteromonas ulvae* UL12^T (AF172987)	DETM4 S1	3
P55	AB251f	FR775439	1378	99	*Pseudomonas marincola* KMM 3042^T (AB301071)	FUTSB2 W1	1
P56	AB319d	FR821218	1331	99	*Pseudorhodobacter ferrugineus* IAM12616^T (D88522)	DETM9 S2	0
	AB347d	FR821217	1323	99	*Pseudorhodobacter ferrugineus* IAM12616^T (D88522)	DETM8 S2	1
P57	AB415d	FR821219	1021	97	*Pseudorhodobacter ferrugineus* IAM12616^T (D88522)	DESCA24 W2	0
P58	AB260d	FR821220	1368	99	*Psychrobacter maritimus* KMM 3646^T (AJ609272)	DESCA4 W1	2
P59	AB402d	FR821221	1393	97	*Rheinheimera pacifica* KMM 1406^T (AB073132)	DEGPY2 W2	1
P60	AB271d	FR821222	1365	99	*Salinibacterium amurskyense* KMM3673^T (AF539697)	DESCA2 W1	2
P61	AB411d	FR821223	1047	98	*Shewanella baltica* NCTC 10735^T (AJ000214)	DETSB13 W2	0
P62	AB274d	FR821236	1341	99	*Streptomyces alboviridis* NBRC 13013^T (AB184256)	DEGPY8 W1	0
P63	AB275d	FR821237	1140	96	*Streptomyces chrysomallus* NBRC 12755^T (AB184120)	DEHWO8 W1	1
P64	AB453d	FR821238	1014	97	*Streptomyces ciscaucasicus* NBRC 12872^T (AB184208)	DETSB69 W2	3
P65	AB254d	FR821239	922	93	*Streptomyces drozdowiczii* NBRC 101007^T (AB249957)	DEGPY9 W1	1
P66	AB450f	FR821240	1050	99	*Streptomyces fimicarius* CSSP537^T (AY999784)	FUSCA66 W2	3
P67	AB368f	FR821241	1031	99	*Streptomyces griseinus* NBRC 12869^T (AB184205)	FUTM4 S2	2
P68	AB431f	FR821242	1358	99	*Streptomyces griseoflavus* LMG 19344^T (AJ781322)	FUTSB41 W2	0
P69	AB255f	FR821243	1357	100	*Streptomyces griseoplanus* CSSP437^T (AY999894)	FUHWO4 W1	1
P70	AB269d	FR821244	1339	99	*Streptomyces griseorubens* NBRC 12780^T (AB184139)	DEGPY10 W1	nd
	AB281d	FR821245	1348	99	*Streptomyces griseorubens* NBRC 12780^T (AB184139)	DESCA12 W1	2
P71	AB448d	FR821246	1035	98	*Streptomyces griseorubens* NBRC 12780^T (AB184139)	DETM64 W2	5
P72	AB288d	FR821247	608	96	*Streptomyces griseorubens* NBRC 12780^T (AB184139)	DETSB5 S2	4
P73	AB460d	FR821249	1206	98	*Streptomyces sampsonii* ATCC25495^T (D63871)	DEGPY4 W2	0
	AB457d	FR821248	1262	99	*Streptomyces sampsonii* ATCC25495^T (D63871)	DETSB14 W2	0
P74	AB445d	FR821224	1316	99	*Sulfitobacter guttiformis* Ekho Lake-38^T (Y16427)	DEGPY55 W2	0
P75	AB401d	FR821225	1378	98	*Tenacibaculum adriaticum* B390^T (AM412314)	DEGPY1 W2	0
P76	AB346f	FR821226	1317	100	*Thalassobacter arenae* GA2-M15^T (EU342372)	FUSCA12 S2	nd
P77	AB344f	FR821227	1385	99	*Vibrio natriegens* ATCC 14048^T (X74714)	FUSCA9 S2	nd
	AB334d	FR821228	1186	96	*Vibrio rotiferianus* R-14939^T (AJ316187)	DETSB2 S2	0
	AB336d	FR821229	1402	99	*Vibrio rotiferianus* R-14939^T (AJ316187)	DESCA9 S2	1
	AB394f	FR821230	1325	99	*Vibrio rotiferianus* R-14939^T (AJ316187)	FUSCA13 S2	nd
P78	AB310d	FR-	620	97	*Vibrio rotiferianus* R-14939^T (AJ316187)	DEGPY15 S2	1
P79	AB367f	FR821231	1405	97	*Vibrio rumoiensis* S-1^T (AB013297)	FUSCA7 S2	nd
P80	AB222f	FR821232	1389	97	*Winogradskyella echinorum* KMM 6211^T (EU727254)	FUHWO3 S1	1
P81	AB264d	FR821233	1370	98	*Zobellia amurskyensis* KMM 3526^T (AB121974)	DETM3 W1	1
P82	AB237f	FR821234	1372	98	*Zobellia uliginosa* ATCC 14397^T (M62799)	DETM57 W2	1
	AB446d	FR821235	1399	98	*Zobellia galactanivorans* Dsij^T (AF208293)	FUGPY3 W1	1

6 CHAPTER III

Observation of bacteria over the surface of released oogonia from *Fucus vesiculosus* L (Phaeophyceae)

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ABSTRACT

The distribution of microorganisms over the surface of the brown alga *Fucus vesiculosus* was observed by scanning electron microscopy. For the first time, bacteria over the surface of oogonia of one plant were noticed. Apparently, the oogonia were at the moment of their release from the conceptacle. In contrast, oogonia covered by the exochiton as shown in cross sections of conceptacles of the same alga were apparently clean of epibiosis. The role microorganism play in macroalgae at this developing stage is still unknown, although degradative and pathogenic effects of microbes on adult macroalgae have been already proved. The impact of microorganisms over the life cycle and colonization process may be important factors regulating algal populations that should be investigated, since fucoid algae play an important role in coastal environments as habitat-forming and base of food webs.

INTRODUCTION

Bacteria are an inherent part of the physical environment of micro and macroalgae (Hold *et al.* 2001). Many of those microorganisms have biofouling potential or interact with algae in pathogenic or non-pathogenic ways. Algae are especially susceptible to epibiosis being covered by diverse marine microbial communities (Goecke *et al.* 2010). Interactions between bacteria and algae are thought to be important in controlling the dynamics of both communities and yet are only beginning to be understood at the species composition level (Jasti *et al.* 2005). Although some of the macroalga-bacteria interactions have been discussed earlier, the ecological relevance of most naturally occurring bacterial communities on macroalgae remains unclear and in most cases the bacterial species involved have not yet been identified (Ivanova *et al.* 2002).

Fucus vesiculosus Linnaeus 1753 (Fucales, Phaeophyceae) is a common intertidal and habitat-forming brown alga widespread in the Northern Hemisphere (Lüning 1990). As in most fucoids, reproductive tissue develops as apical muscilagineous swellings termed receptacles, within which form many spherical conceptacles containing the male gametangia (antheridia) and/or female gametangia (oogonia) (Pearson & Serrao 2006). Oogonia, which present initially a globular form, are placed between abundant hair cell filaments inside the conceptacle (Fig. 1). Oogonia of all stages of development can be found in the same conceptacle. The mature oogonium contains eight haploid eggs arranged in a characteristic manner (Naylor 1953). More detailed structural information was given by Moss (1950) and McCully (1968). Gametes in *Fucus* spp. are oogamous and sperms are attracted to eggs by pheromones (fucoserraten) (Lee 2008). The free eggs sizes range from about 70 µm in diameter and the laterally-biflagelatte sperm about 10 µm in diameter (Brawley *et al.* 1999).

Structurally, in this genus, the wall of the oogonium is composed of three layers, the exochite (thin outer layer), the mesochite (thick middle layer), and the endochite (thin inner layer). When the oogonium is mature, the exochite ruptures, releasing the packet of eggs, still surrounded by the other two wall layers, and the oogonium remains within the conceptacle (Naylor 1953, Lee 2008). Once free of the restricting exochiton, and on access of seawater, the egg packet as a whole increases in size, and the oospheres lose their angular shape and round off, so that the eight chambered nature of the endochiton can be clearly seen (Naylor 1953, Fig. 2). Each conceptacle has an ostiole opening to the surface through which the gametangia are released during spawning (Pearson & Serrao 2006). Gametes are released through the ostiole while still enclosed inside oogonia and antheridia. A compression of the oogonia during their discharge from conceptacle has been observed (Brawley *et al.* 1999). The mesochite ruptures apically, slips backward, and exposes the eggs within the endochite (Lee 2008). In seawater at moderate temperature, the polysaccharide investments that encase the eggs and sperm dissolve rapidly to release free gametes. In the case of antheridia, this occurs within about a minute of discharge from the conceptacle, eggs release from oogonia can require 10 min in calm culture dishes, but may rupture sooner in nature (Brawley *et al.* 1999).

Eggs of *Fucus* spp. are bounded only by a plasma membrane, but following fertilization there is a rapid secretion of a cell wall (alginic acid, cellulose and suphated fucan) (Callow *et al.* 1978). Settlement directly follows release since fucoids lack a planktonic stage and have negatively buoyant eggs (Pearson & Serrao 2006).

In a study investigating the associations from bacteria with macroalgae in the Baltic Sea, we observed the distribution of microorganisms on the surface of different macroalgal species including *Fucus vesiculosus* with scanning electron microscopy. Although bacteria are present by millions of individuals per millilitres seawater (Goecke *et al.* 2010), this is the first observation of bacterial associations to the earlier stages of life of *F. vesiculosus*. The effect microorganisms have at this stage is yet completely unknown.

METHODS

Sampling of the macroalgae

Samples of different macroalgae were taken from a distinct site of the Strande Beach, in the Kiel Fjord, Western Baltic Sea, Germany (54°25.5’N, 10°12’E). They were collected in summer and winter between two to six m depth between 2008 and 2011. The macroalgae were removed carefully from the substrate with a knife and transferred into sterile plastic bags. Until processing within three hours after collection, the samples were stored in the dark at ambient seawater temperature using coolers. In the laboratory, the macroalgae were rinsed three times with sterile Baltic Sea water to remove associated debris, planktonic and loosely attached microorganisms. Voucher specimen were deposited in the Herbarium of Museo de Historia Natural, Santiago, Chile (code SGO).

Scanning electron microscopy (SEM)

Different individuals were investigated by scanning electron microscopy to observe the distribution of microorganisms on the surface of the macroalgae according to Heindl *et al.* (2010). Briefly, samples were prepared by fixation with 1% formaldehyde in seawater. After dehydration in a gradient ethanol series (30%, 50%, 70%, 90%, and 100%; v/v) the samples were critical point dried with carbon dioxide (Balzers CPD030) and sputter coated with gold–palladium (Balzers Union SCD004). The specimens were examined in triplicate in the phylloid area (thallus blade) and in the cauloid area (algal stalk) per sample with a scanning electron microscope (Zeiss DSM960). Pictures were taken with a Contax SLR camera.

RESULTS

Under scanning electron microscopy (SEM) the surface of the phylloid of *Fucus vesiculosus* was covered by a diverse biofilm. In cross sections of the conceptacles, oogonia were apparently clean of epibiosis, and still covered by the exochiton (Fig. 1). In one plant, at the ostiole area, few oogonia already liberated from the exochiton where observed presenting bacteria associated to their surface (Fig. 2).

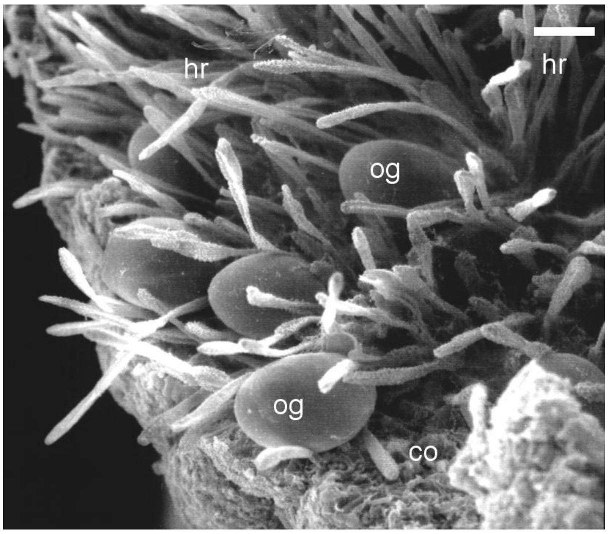

Fig. 1. SEM microphotograph of a cross section of a single female conceptacle on a reproductive frond. Inside de conceptacle a different number of globular female oogonia (og) were placed between large number of hairs (hr) on the cortical cells of the plant (co). Each female oogonium covered with the exochiton contained 8 ovules inside. Scale bar = 50 µm.

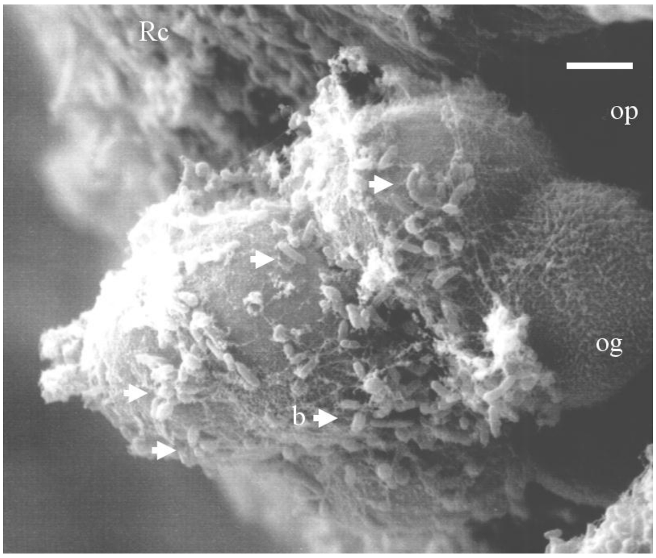

Fig. 2. SEM microphotograph with the detail of an oogonium (og) previously liberated from its exochite and now being released of a conceptacle by the ostiole (op) in a reproductive *Fucus vesiculosus* plant. In the figure, six oospheres (of eight) of the oogonia are shown and already presented bacteria on the surface (arrows). Scale bar = 5 µm.

DISCUSION

We observed for the first time bacteria over the surface of oogonia of one plant of *Fucus vesiculosus* at the moment of their release from the conceptacle (Fig. 2), although bacterial ‘contamination’ has been mentioned previously on an experiment with eggs and embryos of the same species (see Peterson & Torrey 1968). If those bacteria play a role on algal ecology is completely unknown. The rupture of the inner layers of the oogonium for the release of eggs has been described as a fast process and driven by mechanical factors (Naylor 1953, Brawley *et al.* 1999), which does not seem to need an external degradation as the case of some seeds in land plants (Howard & Elliott 1988).

Heterotrophic bacteria play a key role in regulating accumulation, export, remineralisation and transformation of the largest part of organic matter in aquatic ecosystems (Mudryk & Skórczewski 2006); nutrients that can be taken up again by algae for new growth. Furthermore, it was discovered that bacterial biofilms can play a role in spore release, germination and subsequent colonization of new substrates by algae (reviewed by Goecke *et al.* 2010). Marine macroorganisms can be expected to display the whole spectrum of host-bacterial associations on their surface, ranging from passive and random epibiosis to highly specific and obligate symbiosis (Bengtsson *et al.* 2011). Many of these bacterial strains can produce extracellular enzymes that enable them utilize algal polysaccharide as a carbon source (Ivanova *et al.* 2002). Although degradative and pathogenic effects of microbes on adult macroalgae have been already proved (Gachon *et al*. 2010), the effect of bacteria over algal gametes and spores remain extremely neglected. The impact of microorganisms over the life cycle and colonization process may be important factors regulating algal populations that should be investigated. The recent development of new detection and identification technologies is supporting the recognition of the role of parasites and diseases in food web dynamics and ecology of the aquatic environments (Neuhauser *et al.* 2011). Pathologies seem to be much more common and important in regulating algal populations as previously thought. Due the important role fucoids algae play in coastal environments as habitat forming and base of food webs, we considered important to report this observation.

7 CHAPTER IV

Biological activity of macroalgal extracts on selected bacterial strains

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ABSTRACT

It is assumed, that the nature of the bacterial strains used in bioactivity tests has influence on antibacterial activities. Therefore, the selection of bacterial test strains may rush conclusions on the effect of macroalgal extracts and metabolites on bacteria. To proof this assumption, we have analysed the biological activities of crude extracts of 16 macroalgae from the coastal waters of Kiel Fjord (Germany), and tested their effect against a panel of ten microorganisms comprising 5 standard test strains of bacteria and 5 macroalga-associated bacteria. 14 macroalgae (88%) displayed antibacterial activity against at least one of the test strains. Despite the high proportion of extracts exhibiting antimicrobial activity, only 3 members of the standard set were susceptible to macroalgal extracts and the overall activities were low (less than 80% of inhibition). Most of active extracts inhibited *Bacillus subtilis*, while no inhibition effects were found against *Erwinia amylovora*, *Escherichia coli*, and the macroalga-associated bacteria. In contrast, all extracts produced stimulatory growth effects of at least two of the tested bacteria. While growth stimulation of standard set of bacteria was rare (22.5% of total tests) with exception of plant pathogen *Erwinia amylovora* and two cases of *Staphylococcus lentus*, it was common among bacteria associated with macroalgae (77.5%), especially *Bacillus algicola*, *Pseudomonas marincola* and both algal-pathogenic bacteria. This study demonstrates that macroalgal extracts can display different effects on growth of bacteria, especially on bacteria associated with macroalga, displaying inhibitory effects on some and stimulatory effects on other bacteria.

INTRODUCTION

Secondary metabolites mediate numerous biological interactions and play a particular important role in mediating host-microbe associations in the ocean (Lane *et al*. 2010). Recently, it has been addressed that chemical interactions regulate the bacteria-macroalga relationships and may cause specific associations (Goecke *et al*. 2010, Sneed & Pohnert 2011a). Indeed, different species of marine macroalgae growing in the same habitat under the same environmental conditions support different bacterial communities (Lachnit *et al*. 2009, Nylund *et al*. 2010). The biological active compounds (both deterrents and attractants) produced by macroalgae as well as those from the associated bacteria may be involved in shaping these epiphytic bacterial communities (Egan *et al*. 2000, Lachnit *et al*. 2010).

Macroalgal chemistry is rich and diverse, spanning most natural product classes and including functional group characteristics found from no other source (Maschek & Baker 2008). The antibacterial activity of different extracts of macroalgae from almost all groups has been described in many studies around the world (Sridhar & Vidyavathi 1991, Hellio *et al*. 2000, Magallanes *et al*. 2003, Freile-Pelegrin & Morales 2004, Engel *et al*. 2006, Lane *et* *al*. 2009, Muñoz-Ochoa *et al*. 2010). Although several metabolites with antimicrobial activities have been already characterized from macroalgae, results of different studies on antibacterial activities of crude extracts of one and the same species are equivocal: While some studies reported antibacterial activities others did not (Sandsdalen *et al*. 2003). It is well known that biotic factors such as reproductive state, age of the thallus of the macroalgae, as well as abiotic factors such as seasonality and geographic location have influence on the bioactivity of macroalgal extracts (Hellio *et al*. 2004, Paul & Puglisi 2004, Arunkumar *et al*. 2010). Furthermore there are discrepancies related to the different extraction procedures and to the target microorganisms used in the bioassays (Sridhar & Vidyavathi 1991, Kanagasabhapathy *et al*. 2006). Unfortunately, assessments of the antibacterial activity exhibited by macroalgae in the natural products literature has focused on biomedically-relevant strains, using standard microbial strains from terrestrial origin or of medical relevance (Engel *et al*. 2006, Paul *et al*. 2006, Hughes & Fenical 2011). Bacteria from the marine habitat were rarely included, although bioactivity against these would make possible to draw conclusions in regard to the ecological role of the substances in the macroalga-bacteria interactions (Jormalainen & Honkanen 2008).

In order to prove if a selection of ecologically relevant bacteria produces different responses in the bioactivity tests as compared to a standard set of bacteria, we studied the effect of extracts from different macroalgae of the Kieler Fjord (Germany) upon bacterial growth.

MATERIALS AND METHODS

Sampling of the macroalgae

Samples of 16 macroalgae were taken from distinct sites in the Kiel Fjord, Western Baltic Sea, Germany (54°25.5’N, 10°12’E) (Table 1). The macroalgae were collected between one to six metres depth. Until processing within three hours after collection, the samples were stored in the dark at ambient seawater temperature using coolers. In the laboratory, the macroalgae were manually cleaned from sand, epiphytes and animals, and rinsed with sterile and filtered Baltic Sea water to remove associated debris, planktonic and loosely attached microorganisms.

Part of the macroalgae was fixed in 4% formaldehyde for its taxonomic identification. Algae were identified by examination of their thallus architecture and special morphological characters: Fronds, branching, and reproductive structures (Pankow 1971, Maggs & Hommersand 1993, Braune 2008). For the filamentous macroalgae histological cuts were performed and observed by light microscopy. The names of the species were used according to Guiry & Guiry (2011). Voucher specimen were deposited in the Herbarium of Museo Nacional de Historia Natural, Santiago, Chile (code SGO).

Macroalgal extract preparation

10 g of the macroalgae were extracted by immersing them with 200 ml dichloromethane (DCM) at room temperature and shaking them by hand (modified method from Nylund *et al*. 2005). The extracts were centrifuged at 4000 rpm for 10 min and filtered through Whatman 542 filter paper (Freile-Pelegrin & Morales 2004). All extracts were concentrated separately under reduced pressure in a Speedvac RVC2-33 (Christ, Germany) until completely dry, weighed and kept at +4°C. For further tests, 1 mg of the solid residue was resuspended in 1 ml methanol.

Antimicrobial testing

The antimicrobial activity of crude extracts of macroalgae was tested against a panel of ten microorganisms comprising standard test strains and macroalga-associated bacteria. The following microorganisms were used:

(i) Five microorganisms usually tested in standard laboratory tests of antibiotic activity (hereafter “standard set”): *Erwinia amylovora* DSM 50901 (nutrient medium M1 = 5 g l^{-1} peptone, 3 g l^{-1} meat extract in distilled water, pH 7), *Escherichia coli* DSM 498 and *Pseudomonas aeruginosa* DSM 50071 (both nutrient media TSB12 medium = 12 g l^{-1} Difco tryptic soy broth, 10 g l^{-1} NaCl, pH 7.2 in distilled water), as Gram-negative strains; and *Bacillus subtilis* DSM 347 and *Staphylococcus lentus* DSM 6672 (both nutrient media: TSB12) as representatives of Gram-positive bacteria. All strains were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany).

(ii) Five environmental strains that have been identified to be associated with macroalgae in previous studies (hereafter “macroalga-associated set”): iia) 3 isolates were utilized which were obtained from the surface of Baltic Sea macroalgae *Fucus vesiculosus* and *Delesseria sanguinea*. The sampling procedure for isolating strains of bacteria associated with the macroalgae was performed according to Staufenberger *et al*. (2008). 16S rRNA gene amplifications (PCR) and subsequent sequencing was performed at the Institute for Clinical Molecular Biology (University Hospital Schleswig-Holstein, Kiel, Germany). Phylogenetic analysis was performed as described by Heindl *et al*. (2010). 16S rDNA sequences of these strains (isolates AB423f, AB236d and AB251f) were deposited at NCBI under the accession numbers FR775437-FR775439. The isolates affiliated with >99% 16S rRNA gene sequence similarity to other macroalga-associated bacteria the Gram-positive *Bacillus algicola* KMM 3737^T (AY228462) (grown on TM: 5 g l^{-1} yeast extract, 1 g l^{-1} peptone, 30 g l^{-1} tropic marine sea salt in distilled water), *Paenibacillus lautus* JCM 9073^T (AB073188), and the Gram-negative *Pseudomonas marincola* KMM 3042^T (AB301071) (grown on TSB12); iib) Two Gram-negative bacterial strains identified as macroalgal pathogens (by Sawabe *et al*. 1998, 2000), *Algicola bacteriolytica* ATCC 700679^T ('red spot disease') and *Pseudoalteromonas elyakovii* ATCC 700519^T ('spot-wounded fronds'), originally isolated in Japan from diseased beds of *Saccharina japonica* (Areschoug) Lane *et al*. (formerly *Laminaria japonica*). Both were purchased from Institute Pasteur CIP (Paris, France), and grown cultivated on nutrient medium TM.

The bioactivity tests were modified according to Schneemann *et al*. (2010). Assay mixtures were prepared by transferring 10 µl aliquots of methanolic solutions of extracts into a sterile 96-well microtiter plate and evaporating the solvent in a vacuum centrifuge. 200 µl overnight cultures of each test strain were diluted to an optical density (OD) of 0.03 determined by spectrophotometry in the corresponding cultivation media (see above). Cultures of the standard set were incubated at +36°C for 5 h under constant shaking at 200 rpm except *E*. *amylovora* at +28°C. The macroalga-associated strains were cultivated at +28°C for 20 h; and the final OD was determined. We corrected the natural absorbance of the extract fractions by subtracting initial extract-only blank values from values obtained for treatments according to Lane *et al*. (2009). The tests were performed in three replicate treatments. By bacterial set a total of 240 tests were performed (16 macroalgal extracts x 5 bacterial strains x triplicate). The resulting values were compared to those for a positive control (100 µg ml^{-1} chloramphenicol) and respective negative controls wells: solvent ('no extract') and nutrient medium ('no extract, no solvent') on the same plate.

RESULTS

The effect of crude extracts of 16 species belonging to 11 families of marine macroalgae growing in the German coastal zone of the Baltic Sea (Table 1) was tested upon growth of a standard panel of bacteria as well as on selected macroalga-associated (surface-associated and pathogenic) bacteria. Interestingly both, growth stimulating as well as growth inhibitory effects, were found.

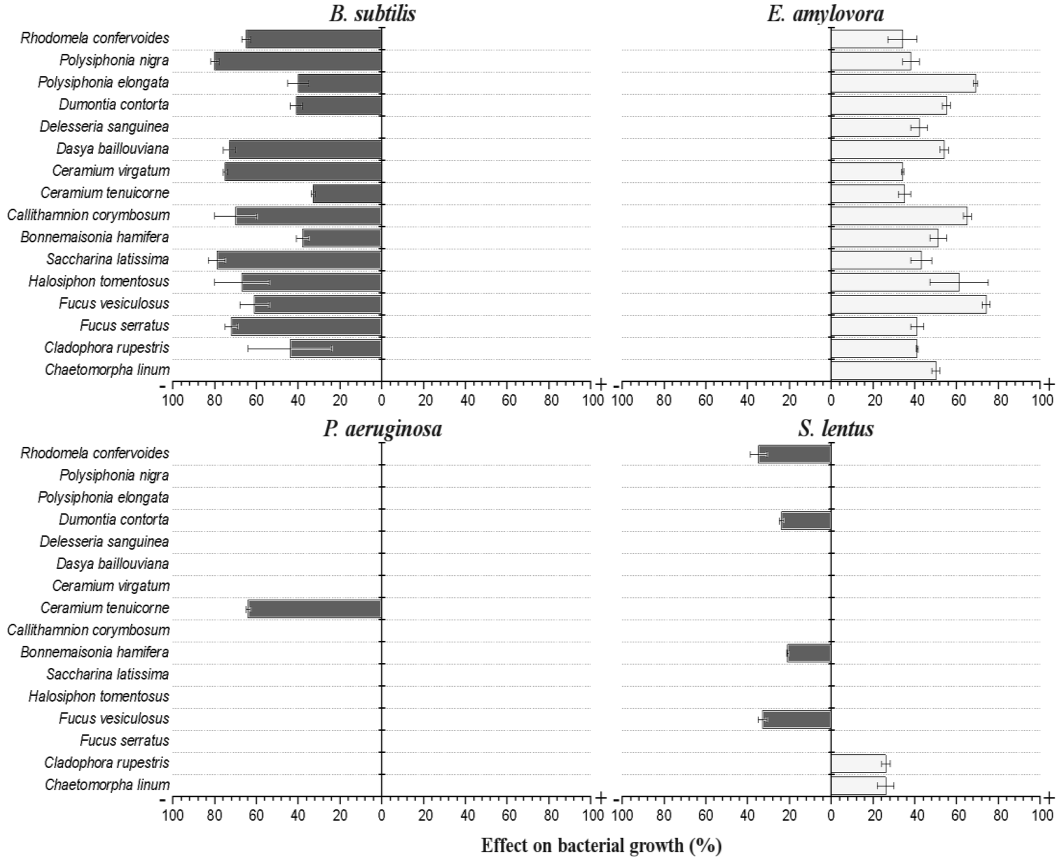
The antimicrobial assay showed that extracts of 14 from 16 macroalgal species (88% of the total) inhibited at least one of the tested organisms (Figs. 1a, b). Antimicrobial activity was demonstrated in members of the three phylogenetic divisions of macroalgae (Chlorophyta, Heterokontophyta and Rhodophyta, Table 2). Concerning the test organisms, Gram-positive bacteria and in particular *B*. *subtilis* were most susceptible (Fig. 2). From the standard set *E*. *amylovora* and *E*. *coli* were not inhibited by any extract tested (Table 2, Fig. 2). Also, no extract presented inhibition of the algal pathogens or the other strains of the macroalga-associated set (Fig. 1b). The extracts of *P*. *nigra* and *S*. *latissima* presented the strongest inhibitory effect on the bacterial strains with around 80% growth inhibition of *B*. *subtilis* (Table 2, Fig. 1a). No particular trend in the biological activity was observed by taxonomical macroalgal division (Fig. 3). Although growth inhibitory effects were generally weak (only against 3 bacterial strains and less than 80% of growth inhibition, Fig. 1a), there was a higher proportion of macroalgal species which inhibited the standard test strains (23.8% of the total tests performed) compared to species associated with macroalga (0%, Fig. 2). Only 12% of the algal species did not present any antibiotic activity (Fig. 1, 3).

Interestingly, the present study revealed significant growth stimulation of macroalga-associated bacteria (Table 2, Fig. 1b). The totality of the extracts stimulated the growth of at least two of the tested bacteria (Fig. 3). While growth stimulation of the standard set of bacteria was rare (22.5% of the total tests) - with the exception of plant pathogen *E*. *amylovora* and just two cases of *S*. *lentus* (Fig. 2) - it was common among bacteria associated with macroalgae (77.5%), especially *B*. *algicola* and *P*. *marincola*. Quite interestingly, also growth of the macroalgal pathogens *A*. *bacteriolytica* and *P*. *elyakovii* was stimulated by most of the macroalgal extracts (both 81.3% of the total), and surprisingly none of the macroalgal extracts inhibited these two strains (Fig. 1, 2). The extract of *D*. *baillouviana* presented the strongest stimulatory growth effects on the bacterial strains with around 200% growth stimulation of *A*. *bacteriolytica* (Fig. 1b).

FIGURES

Biological activity of macroalgal extracts according to different target strains.

a)



b)

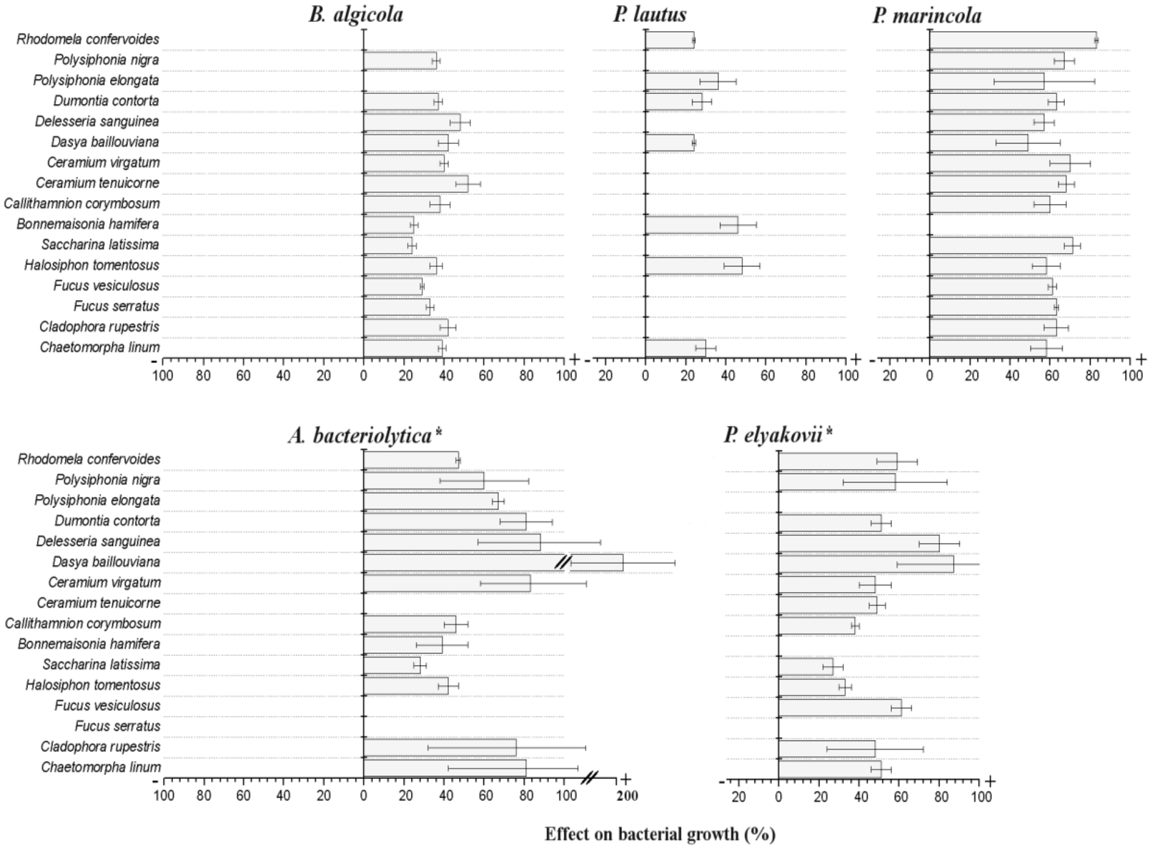


Figure 1 Effect of macroalgal extracts on the growth of the microorganisms tested in this study. Positive values represent enhancement of growth in comparison to the controls (clear bars), and negative values inhibition of growth (dark bars). Average ± SE (n = 3). Values between -20% and 20% were not contemplated. a) Standard set of test strains (without *Escherichia coli*, which were not affected by any extract). b) Macroalga-associated set with 3 surface-associated bacteria and two pathogenic strains (marked with asterisk).

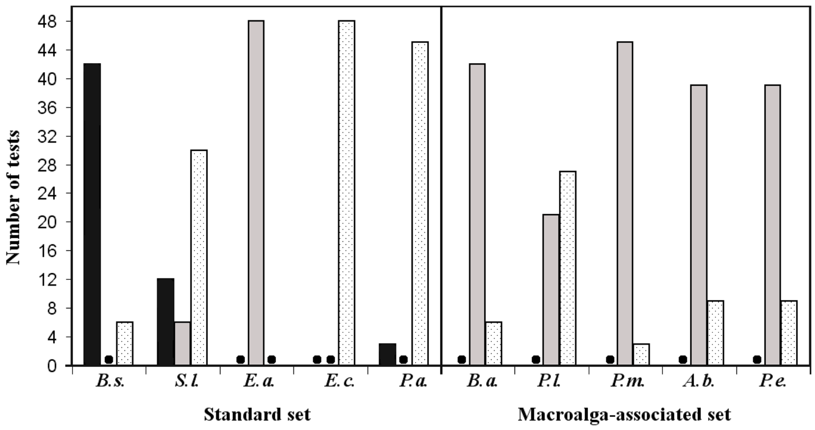


Figure 2 Summary by bacterial strain of the effect on the growth performed with the macroalgal extracts tested in this study. To the left – standard set: B.s.: *Bacillus subtilis*, E.a.: *Erwinia amylovora*, E.c.: *Escherichia coli*, P.a.: *Pseudomonas aeruginosa*, and S.l.: *Staphylococcus lentus*; and to the right – macroalga-associated set: B.a.: *Bacillus algicola*, P.m.: *Pseudomonas marincola*, P.l.: *Paenibacillus lautus*, A.b.: *Algicola bacteriolytica*, and P.e.: *Pseudoalteromonas elyakovii*. The bars represent enhancement of growth (grey), inhibition of growth (dark stripes) and no significant variation of growth (points) in comparison to negative controls. Absence of inhibitory/stimulatory activity is designated with a black dot. A total of 48 tests were performed for each strain.

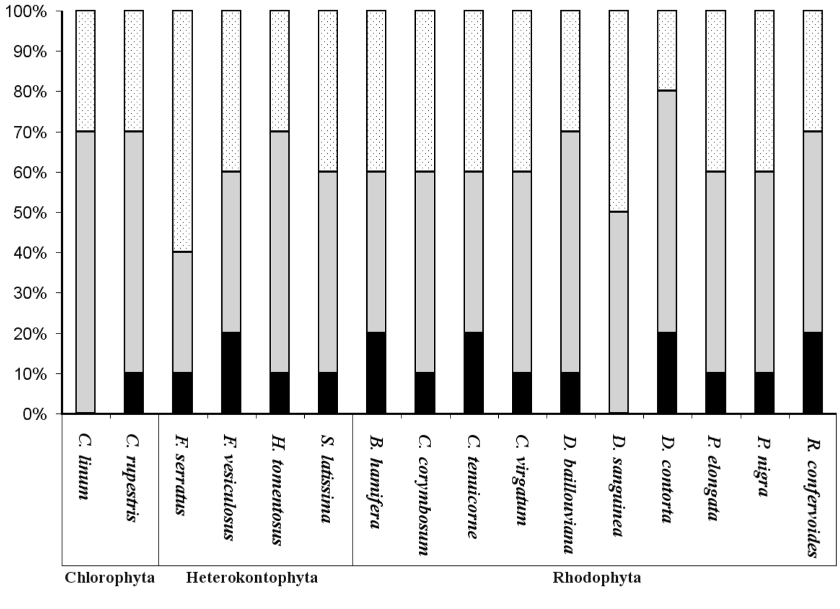


Figure 3 Proportion of biological activity according the different macroalgal species investigated in the present study. The bars represent inhibition of growth (dark stripes), enhancement of growth (grey), and no significant variation of growth (points) in comparison to negative controls. No particular trend in the biological activity was observed by taxonomical macroalgal division. Extracts of some macroalgal species usually associated with high antimicrobial activity as *Rhodomela confervoides* and *Bonnemaisonia hamifera* were active in the present study too.

DISCUSSION

In the majority of prior studies, bacterial growth inhibiting activities of different macroalgal extracts were investigated on human pathogens or standard terrestrial strains (Dubber & Harder 2008). In the present study we investigated the different effects macroalgal extracts can display over the growth of bacteria especially by testing ecologically relevant microorganisms. Hughes & Fenical (2011) suggested recently that antibacterial activity can only be evaluated in the context of the bacteria strains that are selected. We demonstrated that one macroalgal extract can display different effects on growth of microorganisms especially by testing macroalga-associated bacteria in comparison with a standard set of bacteria.

In the present study, 88% of the macroalgae displayed antibacterial activity against at least one of the test strains. The antibiotic effect was observed only against 3 bacterial strains from the standard set and less than 80% of inhibition. Extracts of some macroalgal species usually associated with high antimicrobial activity as *Rhodomela confervoides* (Glombitza 1969) and *Bonnemaisonia hamifera* (Nylund *et al*. 2005, Persson *et al*. 2011; Fig. 1a) were active too. *B*. *subtilis* from the standard set was the most susceptible to the macroalgal extracts (Fig. 2) in accordance with other studies on macroalgae (Freile-Pelegrin & Morales 2004, Dubber & Harder 2008, Sánchez-Saavedra *et al*. 2010).

Just a few studies have investigated the biological activities of macroalgal extracts of the German coasts, and most of them have used standard bacteria. For example, Roos (1957) investigated 27 species of macroalgae of the Kiel Fjord, and tested them against standard strains including *Staphylococcus aureus*, *B*. *subtilis* and *E*. *coli*. Most species (82%) were active against at least one of the tested microorganisms. As in the present study, Roos also found antibacterial activity in *F*. *serratus*, *F*. *vesiculosus*, *P*. *elongata*, *R*. *confervoides* (as *R*. *subfusca*), and *S*. *latissima*. Later, Glombitza (1969) confirmed some of these results during a study with 41 macroalgae from the coastal zone of the Helgoland Island in the German North Sea including *C*. *rupestris*, *D*. *contorta* (as *D*. *incrassata*) *S*. *latissima*, *F*. *vesiculosus*, and *R*. *confervoides*. The author also used standard bacteria in the tests (i.e. *Bacillus cereus*, *B*. *subtilis*, *E*. *coli*, *Proteus vulgaris*, *Sarcina lutea*, and *S*. *aureus*). From the same German island, Duber & Harder (2008) tested macroalgal extracts of *Ceramium rubrum*, *Mastocarpus stellatus* and *Laminaria digitata* against 7 fish pathogenic bacteria and 12 bacteria from marine sediments. Extracts of those 3 macroalgae presented high activity against growth of different strains. In another study, extracts of 4 German North Sea macroalgae (*C*. *rubrum*, *Hypoglossum hypoglossoides*, *S*. *latissima*, and *Plocamium cartilagineum*) were tested against 5 fish pathogenic strains, inhibiting all the macroalgae at least one bacterial strain, from which *S*. *latissima* was active only against *Vibrio anguillarum* (Bansemir *et al*. 2006).

Although these studies clearly showed that macroalgae commonly contain active metabolites with antibacterial properties, it is not known whether the metabolites have an active role within the ecological interactions with their natural enemies (Jormalainen & Honkanen 2008), and/or symbionts. Important variations in the effect of different macroalgal extracts on different marine fouling microorganisms have been shown by Hellio *et al*. (2000, 2001). In a study in the Caribbean Sea, the marine bacterial strains (including *P*. *elyakovii*) selected were most sensitive to extracts of *Sargassum polyceratium* compared to a standard bacterial strains, suggesting that defence strategies of this brown alga are specific (Thabard *et al*. 2011). Such targeted defence strategies have been described for some algal species before (see Paul & Puglisi 2004).

Different crude extracts tested in the present study have significantly stimulated the growth of bacteria, especially ecologically relevant strains (both surface associated and pathogenic strains, Table 2). One of the extract, of the introduced species *D*. *baillouviana*, presented the strongest stimulatory effects on the bacterial strains with approx. 200% growth stimulation of the pathogen *A*. *bacteriolytica* (Fig. 1b). Although several studies have shown stimulation of bacterial growth by marine algae, unfortunately, they have been based generally on exudates rather than extracts, and usually of phytoplanktonic sources (see Bell *et al*. 1974, Larsson & Hagström 1979, Brock & Clyne 1984, Murray *et al*. 1986, Coveney & Wetzel 1989).

Algal exudates, unknown or partially specified, have been shown to significantly affect the community structure of bacteria in biofilms and in the pelagic zone near the macroalgae (see Dobretsov *et al*. 2006, Lam & Harder 2007, Lachnit *et al*. 2010, Persson *et al*. 2011, Saha *et al*. 2011, Sneed & Pohnert 2011a, b). It is well known that macroalgae release large amounts of organic carbon into the surrounding environment, providing nutrients for microorganisms (Koop *et al*. 1982, Wada *et al*. 2007). Heterotrophic bacteria can directly utilize products excreted by algae as growth substrates (Larsson and Hagström 1979, Brock & Clyne 1984). Excreted compounds may also trigger chemotactic behaviour and stimulate growth (Goecke *et al*. 2010). These compounds are quite selective in their stimulation of bacteria, because different bacteria differ considerably in their ability to respond to these products (Bell *et al*. 1974). The strain-specific preferences for certain substrates and strain specific pro- or antifouling activities of algal metabolites play an important role in establishing ecological associations (Wahl *et al*. 2010). It has been suggested that chemical defences may affect marine communities by promoting some microbes on algal surfaces while deterring others (Lane & Kubanek 2008). However, after more than 20 yrs of research on this topic, there is still no experimental evidence demonstrating if or how host organisms selectively attract and harbour their epibionts (Harder 2009); especially because the studies focussed on inhibitory activities of extracts or metabolites and rarely were concerned with stimulatory effects on growth of the microorganisms.

The bacterial growth stimulatory effect may have different explanations. As mentioned recently, those organic extracts and compounds may rather resemble dead algal material available for microbial degradation (Bengtsson *et al*. 2011). Nevertheless, the possibility of the presence of certain algal substances that specifically stimulate a selected array of species or even strains has also been suggested recently (see Sneed & Pohnert 2011b).

In the present study, we tested activities of macroalgal extracts against two macroalgal pathogens: *A*. *bacteriolytica* (formerly *Pseudoalteromonas bacteriolytica*) and *P*. *elyakovii*. All studied macroalgae revealed no inhibitory activities against these pathogens; on the contrary, growth stimulatory activities were in general displayed (Fig. 1b). The extract of *S*. *latissima*, which was the only studied brown macroalga that belong to Laminariales, stimulated growth of both macroalgal pathogens (but <30%). Members of this genus were originally affected by those pathogens (Sawabe *et al*. 1998, 2000). Recently, in tropical environments, extensive investigations of different macroalgal extracts have shown high biological activity against *A*. *bacteriolytica* (Engel *et al*. 2006, Puglisi *et al*. 2007, Lane *et al*. 2010). This indicates highly variable amounts and different composition of active compounds, probably depending on the biotic and abiotic pressures onto the macroalgae. It is accepted that chemical defences are elaborated to a greater extent and are more important in tropical than temperate or cold areas as in the German coast (see Pereira & da Gama 2008). Unfortunately, with the exception of one study using causative agents of the macroalgal ice-ice disease (by Vairappan *et al*. 2010) and *P*. *elyakovii* (by Thabard *et al*. 2011), bioactivity tests of algal extracts or compounds against other known bacterial pathogens of macroalgae were rarely performed.

As recommended by Engel *et al*. (2006) caution must be exercised about drawing ecological conclusions of the role of secondary metabolites on the observed biological activity. The concentration used in our assays followed standard procedure (see Bansemir *et al*. 2006, Muñoz-Ochoa *et al*. 2010, Sánchez-Saavedra *et al*. 2010, Villarreal-Gómez *et al*. 2010), and crude extracts are usually complex mixtures of compounds. Therefore it is unknown which substances at which concentration exhibited the bioactivity in nature (Persson *et al*. 2011).

Despite the inconveniences using crude extracts of whole organism already mentioned by several authors (Paul *et al*. 2006, Lane *et al*. 2009, Nylund *et al*. 2010), there is no doubt that such experiments provide insights into the potential interactions mediated by algal metabolites, especially by using environmental test strains. We confirmed that macroalgal extracts exert effects of growth inhibition and stimulation according to the nature of the bacterial strains selected. We also demonstrated that macroalgal extracts have growth stimulant effects on macroalga-associated bacteria including algal pathogens.

Table 1 List of macroalgae sampled from the Kiel Fjord (Baltic Sea, Germany). Names according to Guiry & Guiry (2011)

Macroalgal Species	**Order**	**Family**	**Sampling Site at Kiel Fjord**	**Sampling Date**
Chlorophyta				
Chaetomorpha linum (Müller) Kützing 1849	Cladophorales	Cladophoraceae	Strande	21.10.2009
Cladophora rupestris (Linnaeus) Kützing 1843	Cladophorales	Cladophoraceae	Strande	21.10.2009
Heterokontophyta				
Fucus serratus Linnaeus 1753	Fucales	Fucaceae	Stohl	19.01.2010
Fucus vesiculosus Linnaeus 1753	Fucales	Fucaceae	Bülk	14.07.2009
Halosiphon tomentosus (Lyngbye) Jaasund 1957	Tilopteridales	Halosiphonaceae	Falkenstein	24.04.2011
Saccharina latissima (L.) Lane *et al*. 2006	Laminariales	Laminariaceae	Tonnenhof	14.07.2009
Rhodophyta				
Bonnemaisonia hamifera Hariot 1891 (tetrasporoph.)	Bonnemaisoniales	Bonnemaisoniaceae	Falkenstein	20.11.2010
Callithamnion corymbosum (S.) Lyngbye 1819	Ceramiales	Callithamniaceae	Falkenstein	20.11.2010
Ceramium tenuicorne (Kützing) Waern 1952	Ceramiales	Ceramiaceae	Strande	24.04.2011
Ceramium virgatum Roth 1797	Ceramiales	Ceramiaceae	Strande	21.10.2009
Dasya baillouviana (Gmelin) Montagne 1841	Ceramiales	Dasyaceae	Strande	21.10.2009
Delesseria sanguinea (Hud.) Lamouroux 1813	Ceramiales	Delesseriaceae	Strande	14.07.2009
Dumontia contorta (Gmelin) Ruprecht 1850	Gigartinales	Dumontiaceae	Strande	01.06.2010
Polysiphonia elongata (Hudson) Sprengel 1827	Ceramiales	Rhodomelaceae	Strande	21.10.2009
Polysiphonia nigra (Hudson) Batters 1902	Ceramiales	Rhodomelaceae	Strande	21.10.2009
Rhodomela confervoides (Hudson) Silva 1952	Ceramiales	Rhodomelaceae	Falkenstein	20.11.2010

Table 2 Antibacterial activity and growth stimulation effect of the dichloromethane extracts of macroalga against a standard set of bacteria and a set of macroalga-associated bacteria

Macroalga	**Standard Set**					**Macroalga-associated Set**				
						Surface-associated			**Pathogenic**	
	B.s.	***S.l.***	***E.a.***	***E.c.***	***P.a.***	***B.a.***	***P.l.***	***P.m.***	***A.b.***	***P.e.***
Chlorophyta										
C. linum	**0**	**(+)**	**+**	**0**	**0**	**(+)**	**(+)**	**+**	**++**	**+**
C. rupestris	**(-)**	**(+)**	**(+)**	**0**	**0**	**(+)**	**0**	**+**	**+**	**(+)**
Heterokontophyta										
F. serratus	**-**	**0**	**(+)**	**0**	**0**	**(+)**	**0**	**+**	**0**	**0**
F. vesiculosus	**-**	**(-)**	**+**	**0**	**0**	**(+)**	**0**	**+**	**0**	**+**
H. tomentosus	**-**	**0**	**+**	**0**	**0**	**(+)**	**(+)**	**+**	**(+)**	**(+)**
S. latissima	**--**	**0**	**(+)**	**0**	**0**	**(+)**	**0**	**+**	**(+)**	**(+)**
Rhodophyta										
B. hamifera	**(-)**	**(-)**	**+**	**0**	**0**	**(+)**	**(+)**	**0**	**(+)**	**0**
C. corymbosum	**-**	**0**	**+**	**0**	**0**	**(+)**	**0**	**+**	**(+)**	**(+)**
C. tenuicorne	**(-)**	**0**	**(+)**	**0**	**-**	**+**	**0**	**+**	**0**	**(+)**
C. virgatum	**-**	**0**	**(+)**	**0**	**0**	**(+)**	**0**	**+**	**++**	**(+)**
D. baillouviana	**-**	**0**	**+**	**0**	**0**	**(+)**	**(+)**	**(+)**	**++**	**++**
D. sanguinea	**0**	**0**	**(+)**	**0**	**0**	**(+)**	**0**	**+**	**++**	**++**
D. contorta	**(-)**	**(-)**	**+**	**0**	**0**	**(+)**	**(+)**	**+**	**++**	**+**
P. elongata	**(-)**	**0**	**+**	**0**	**0**	**0**	**(+)**	**+**	**+**	**0**
P. nigra	**--**	**0**	**(+)**	**0**	**0**	**(+)**	**0**	**+**	**+**	**+**
R. confervoides	**-**	**(-)**	**(+)**	**0**	**0**	**0**	**(+)**	**++**	**(+)**	**(+)**

‘Standard set’ strains: B.s.: *Bacillus subtilis*, E.a.: *Erwinia amylovora*, E.c.: *Escherichia coli*, P.a.: *Pseudomonas aeruginosa*, and S.l.: *Staphylococcus lentus*; and ‘Macroalga-associated set’ strains: A.b.: *Algicola bacteriolytica*, B.a.: *Bacillus algicola*, P.l.: *Paenibacillus lautus*, P.e.: *Pseudoalteromonas elyakovii*, and P.m.: *Pseudomonas marincola*. MTP: growth stimulation **(+)** = 20 – 49%, **+** = 50 – 79% **++** = ≥ 80%; 0 = no biological activity; growth inhibition **(-)** = 20 – 49%, **-** = 50 – 79%, **--** = ≥ 80%.

8 CHAPTER V

Potential pathogens of a Chilean macroalga

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Will be submitted as Research Paper
Goecke et al.

ABSTRACT

Durvillaea antarctica (Fucales, Phaeophyceae) is a common brown macroalgae in high-energy rocky shores of the Southern Hemisphere, and is considered of high ecological and economical significance. We reported a phenomenon affecting this bull-kelp in natural beds of central coast of Chile (Pacific Ocean): Abnormal growth characterized by evident gall development and discolorations were found on the thallus of this macroalga. Observations by light microscopy and scanning electron microscopy revealed the presence of two different morphotypes of endophytes exclusively within the galls, but its absence in healthy specimens. In algal populations at Coliumo bay, dark-brown endophytes were placed in subcortical tissue, only visible by cross section of diseased macroalgae. We described the morphological features of these endophytes which resemble to a member of Phytomyxea, microorganisms reported once 23 years ago as parasites in those macroalgae. This endophyte was observed occupying a modified host cell, and forming a thick-walled structure that contained a huge number of round spores. In samples at Valparaíso bay, none of those structures were observed. Instead, bundles of colourless hyphae emerged from the centre of the gall out to the surface. The hyphae penetrate the cortical tissue of the macroalga. In some sections, the presence of reproductive structures was observed. On the basis of morphological features these endophytes resemble members of the Oomycota, which were associated previously with diseases in different macroalgae, but not for *D. antarctica*.

INTRODUCTION

Durvillaea antarctica (Chamisso) Hariot is a large brown seaweed which belongs to the order Fucales (Phaeophyceae) (Cho et al. 2006). It has a subantarctic distribution limited only to the Southern Hemisphere, specifically South America, New Zealand and subantarctic islands (Ramírez and Santelices 1991; Hoffmann and Santelices 1997). There, this genus plays an important role in the occupation of habitat and the structuring of coastal communities. *Durvillaea* species frequently dominate the intertidal and shallow subtidal flora in regions with a stable, rocky substratum and exposed to wave force (Cheshire et al. 1995). There, it is the dominant primary producer and repository of organic material and energy (Santelices et al. 1980). Together with *Lessonia nigrescens* Bory (Phaeophyceae) it forms conspicuous belt in high-energy rocky shores where its long, floating fronds can reach a length of more than 15 m (Lawrence 1986; Westermeier et al. 1994). Like other large brown algae in temperate coasts of the world, they modify the microenvironments providing much of the vertical structure inhabited by smaller species from all domains of life (Taylor and Schiel 2000; Jaramillo et al. 2006). Its holdfast by itself constitutes temporal or permanent habitat for a rather large number of different species of invertebrates, giving them shelter, conforming spawning and habitat substrates, and minimizing wave and predation pressure (Vásquez and Buschmann 1997).

Kelp species (common name for large brown seaweeds) have economic and social impacts since many local inhabitants base their economy on these resources. The stipe and dried fronds of *Durvillaea*, locally known as "*cochayuyo*", are utilized in Chile for human consumption in traditional food initially by the *Mapuches* culture (prior to pre-Spanish settlement), and later on by modern intertidal subsistence food-gatherers and artisanal fishers who sold it in local markets (Castilla et al. 2007). It is considered as a good source of fibres and polysaccharides including hydrocolloids as alginic acid (Miller 1996; Kelly and Brown 2000; Ortiz et al. 2006). During recent years, *Durvillaea antarctica* have been heavily exploited and exported as raw material for the extraction of those alginates which have widely applications on food and pharmaceutical industries (Bustamante and Castilla 1990; Schiel and Nelson 1990). Chile is an important producer of brown seaweeds representing 10% of world supply (Vásquez 2008). Specifically referred to *D*. *antarctica*, the landings of this kelp in Chile have been *ca*. 2600 (±900) wet metric tons (Mg) per year (Castilla et al. 2007) in the past eight years.

This global demand of algal products in the last decades (i.e. cosmetic, food, and pharmaceutical industries) has notoriously increased and expanded, increasing the need to understand the nature and severity of diseases in seaweed mariculture too (Apt 1984). Algal diseases are more under the spotlight than ever before (Gachon et al. 2010), looking forward to the development of prevention strategies to lowering the frequency risk, providing adequate or improved protection to the algae (Park et al. 2006).

Algae are frequently attacked by various pathogens including virus (Müller et al. 1998), oomycetes, chytrids and hyphochytrids (Raghukumar 2002; Li et al. 2010; Rasconi et al. 2011), phytomyxids (plasmodiophoromycetes) (Aguilera et al. 1988; Maier et al. 2000), fungi (Kohlmeyer 1979), protozoa (Polne-Fuller and Gibor 1987), invertebrates (Park et al. 2008), algae (Heesch et al. 2008; Gauna et al. 2009; Thomas et al. 2009), and bacteria (for review see Goecke et al. 2010, Paper I). Those infections may cause obvious changes in macroalgal morphology, galls, appearance of holes, discolorations, or even not produce any visual changes at all (Goecke et al. 2010).

Galls and tumour-like growths can be found on numerous macroalgae. A variety of prokaryotic and eukaryotic organisms (i.e. virus, bacteria, fungi, microalgae, nematodes, copepods, but also industrial pollutants and unknown causes) are associated with them

(Apt 1988; Correa et al. 1993). In brown algae, galls were commonly associated with fungal infections caused by species of *Haloguignardia* spp. (Sphaeriales, Ascomycetes) and *Massarina cystophoreaea* (Pleosporales, Ascomycetes), which induce those malformations in their hosts i.e. *Cystoseira*, *Halidrys*, *Sargassum* and *Cystophora* (Kohlmeyer 1979; Apt 1988). Along the Pacific coasts of South America, in only few macroalgae have been described the occurrence of gall-like structures and thallus deformation associated with endophytic organisms (Thomas et al. 2009) (Table 1).

Table 1 Macroalgae that showed gall-formations in the Chilean coast and the causative agent associated to them. B = brown alga, R = red alga, and A = filamentous algae, C = cyanobacteria, P = Phytomyxea

Macroalgae	Causative agent	Locality (in Chile)	Reference
Mazzaella laminarioides (R)	*Pleurocapsa* sp. (C)	Matanzas, Constitución	Correa et al. 1993
Durvillaea antarctica (B)	Plasmodiophoromycete (P)	Valdivia, Pudá	Aguilera et al. 1988
Ectocarpus siliculosus (B)	*Maullinia ectocarpii* (P)	Puerto Montt	Maier et al. 2000
Lessonia nigrescens (B)	*Laminariocolax* sp. (A)	Pan de Azúcar, Maitencillo	Thomas et al. 2009
Macrocystis pyrifera (B)	*Laminariocolax macrocystis* (A)	Valdivia	Burkhard & Peters 1998

The genus *Durvillaea* has been associated with only few notorious pathogens, parasites or obligate epiphytes, including filamentous algae and plasmodiophorids. The most conspicuous, *Herpodiscus durvillaeae* (Lindauer) South, is an obligate parasitic alga endemic from New Zealand (South 1974). This brown filamentous alga produces velvety red-brown patches on the host frond surface (Peters 1991; Heesch et al. 2008). It grows epi-endophytically leading to an erosion of the surface of the host, which may result in eventual loss of its phylloid (South 1974). Also in New Zealand, a yet undescribed endophytic ectocarpalean alga was associated with galls or pale spots on *Durvillaea antarctica* and *D. willana* (S. Heesch, *personal comunication*). And, a fungus identified as *Gliocladium* sp. was cultured from thallus samples of the macroalga (Lang et al. 2006) and an obligate epiphytic alga (*Porphyra subtumens*) have been described living associated with bull-kelp too (Nelson and Knight 1996). However, in both cases, no direct evidence for the pathogenicity has been shown.

In Chile, in the area of Valdivia and Concepción (Pudá) (Fig. 1), using light microscopy observations Aguilera et al. (1988) found in subcortical cells of the thallus of *Durvillaea antarctica* an endophytic parasite and associated it with external gall structures which may be of 11 cm² surface area. The authors assigned the unnamed parasite by morphological characteristics into the Plasmodiophoromycetes. No other outbreaks of this phenomena or verification of parasites have been described in Chilean specimens of *Durvillaea antarctica* yet. The local and national economic importance of this resource altogether with the mentioned ecological impact that have on other organisms (i.e. fish and crustacean), some of them also economical resources, enhance the relevance of notice and investigation of unusual gall formations on *Durvillaea antarctica*. The aim of this study is to describe an outbreak occurring in the central coast of Chile, which produced lesions, galls and discolorations on the fronds of this important algal resource.

MATERIALS AND METHODS

Field sampling

Samples of *D. antarctica* (n = 2) were taken from distinct sites of Necochea beach, Coliumo bay (36º31’36’’S; 72º57’24’’W), and Las Cujas beach, Valparaíso bay (32º56’67’’S; 71º46’67’’W), central coast of Chile, Pacific Ocean (Fig. 1). Pieces of different sections of diseased and healthy thalli of the algae were collected from the intertidal zone in summer (January) 2010; and carefully cut with a knife and transferred into sterile plastic bags. In the laboratory, the macroalgae were rinsed three times with sterile seawater to remove associated debris, planktonic and loosely attached microorganisms. Different sections of 1 cm² of the thallus were prepared by fixation with 2% formaldehyde in seawater for microscopy, and in 95% ethanol for the posterior molecular analysis.

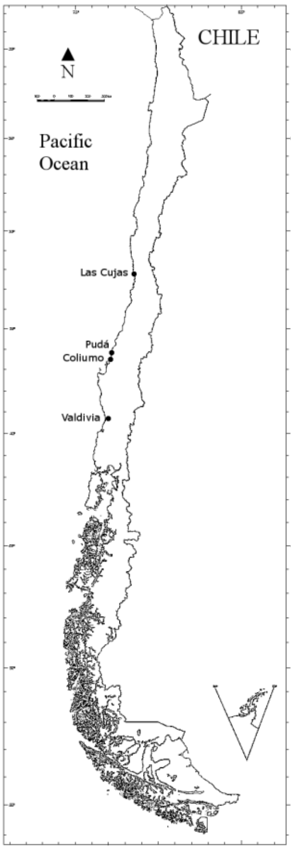


Fig. 1 *Durvillaea antarctica* presenting gall-formations were found in Necochea beach at Coliumo bay, and Las Cujas beach at Valparaíso bay, in the Central coast of Chile, Pacific Ocean (modified from SERNAGEOMIN 2003). Previous registers of gall formation on the species are also shown (Pudá and Valdivia) as reported by Aguilera et al. (1988).

Light microscopy and characterization of the microorganisms

For microscopic study of fresh material to observe cellular anomalies, pigmentation or damage in algal tissue, small pieces of 1 cm² of the thallus were cut with a razor blade (cross-sections of approx. 40µm thick), placed on slides and stained with a mixture of 1.0% aniline blue with 30% Karo Syrup acidified with HCl or similarly mounted but not stained (Womersley 1984). For the photomicrographs, we used a Olympus (CX21) microscope equipped with a camera. The images were compared to specialized literature on morphology, reproduction and cytology of *D. antarctica* (i.e. Herriott 1923; Naylor 1949; Roberts 1979; Collantes et al. 2002).

Scanning electron microscopy (SEM)

Samples of healthy and diseased macroalgae were investigated by scanning electron microscopy to observe differences on the surface and in cross-sections of the macroalgae according to the methodology described by Heindl *et al*. (2010). Briefly, after dehydration in a gradient ethanol series (30%, 50%, 70%, 90%, and 100%; v/v) the samples were critical point dried with carbon dioxide (Balzers CPD030) and sputter coated with gold–palladium (Balzers Union SCD004). The specimens were examined in triplicate per sample with a scanning electron microscope (Zeiss DSM960). Pictures were taken with a Contax SLR camera.

RESULTS

Abnormal growth and gall development over the surface of the thallus of *Durvillaea antarctica* was observed in wild populations at two different places (Valparaíso and Coliumo bay) from the Central coast of Chile (Fig. 1) and compared to healthy individuals.

Healthy individuals, both sampling sites

Healthy fronds of the macroalgae were characterized by firm, elastic, smooth, and shiny thalli, in which the color varied normally from brown till dark-olive tones (Fig. 2 a). In cross sections a meristoderm, a cortex and a medulla was observed (Fig. 2 b). As described by Naylor (1949), meristoderm consisted of a layer of 5-6 small, polyhedral, brick-shaped cells (Fig. 2 c-d). The cortical zone was formed by regular radial rows of 8-15 elongate cells, and the medullary zone of irregularly interwoven hyphae (Fig. 2 b, d), which originated air-filled cavities separated by septa (as described by Hoffmann and Santelices 1997; Collantes et al. 2002). Gametes in antheridia and oogonia (in a male or female fronds respectively), differentiated inside ovoid conceptacles that develop in the cortical zone with a small ostiole that opens to the surface (Fig. 2 d) as described by Hoffmann and Santelices (1997). In transversal sections of a reproductive frond, one layer of conceptacles was observed (Fig. 2 b, d).

Diseased individuals, macroscopic appearance

Diseased fronds instead were recognized by the presence of yellowish protruded masses of scattered tissue, of irregular form tending to be circular or elliptic, with a diameter from 0.5 - 4 cm (Fig. 3 a; 4 a). Although both macroalgal populations produced galls, cross-sections under light microscopy showed two kinds of endophytes associated with them (Fig. 3; 4).

Diseased individuals from Coliumo bay samples

In *D*. *antarctica* samples taken at Necochea beach (Coliumo bay), the endophytes were observed only in the yellowish brown subcortical tissue from the alga (Fig. 3). The endophytes were dark-brown coloured and presented irregular forms between 20-40 µm in diameter (Fig. 3 b-d). Cross-section under light microscopy showed a detail of the microorganisms structures settled in those lesions (Fig. 3 d1). It developed into a compacted polyhedral structure that corresponded to resting esporangia (Fig. 3 d2). Those resting sporangia of these endophytes developed thick-walled structures that contain numerous endospores of 2.5 µm of diameter occupying the cell (3 e). The spores were subspherical to spherical and aggregated all together with no special form or distribution in the cystosori, which were released after disruption of the host cell (Fig. 3 e, f). Such structures are not described as common for Fucales and specially did not correspond to the reproduction process in *Durvillaea* species (as Fig. 2 b, d; and see Herriott 1923; Collantes et al. 2002). Morphological features of these endophytes resemble those of members of Phytomyxea, microorganisms suggested previously as parasites in those macroalgae. In a detailed scanning electron microphotograph of the endospores, no flagella were observed (Fig. 3 f). Similar spores have been described previously for *Plasmodiophora brassicae*, although no spines cover the surface of the spores (Kageyama and Asano 2009). Form, size, (pigmentation), and aggregation mode of the cystosori are important for species delimitation in Plasmodiophoromycetes (Neuhauser et al. 2010; 2011b).

Diseased individuals from Valparaíso bay samples

Transversal sections in galls of samples of *Durvillaea antarctica* at Las Cujas beach (Fig. 4 a) showed none of the previously described structures associated with the Coliumo bay endophytes (Fig. 4). Instead, bundles of colourless, irregularly septate hyphae were observed associated with the affected algal tissue (Fig. 4 b-f). This endophyte penetrates the cortex cell layers growing around algal cells (Fig. 4 d), and emerged from the centre of the gall out to the surface by ruptures the outer cell layers of the gall (Fig. 4 b-c). In a different manner as compared to the Coliumo’s endophyte, the distribution in the algal cortex with a connection to the surface may suggest a relationship with conceptacle cavities in the host, but this assumption needs to be confirmed (Fig. 4 b-c). The parasite seems to have started to invade the surroundings of the gall opening in the host surface (Fig. 4 f1)

The irregular shaped hyphae (ca. 2 µm) presented papillae and cross sections (Fig. 4 d). In some sections of the surface of the macroalga the presence of reproductive structures was visible. Hyphas developed a terminal small sized spherical oogonium (between 4-10 µm), but intercalary oogonium were observed too (Fig. 4 f3-4). The morphological features of these endophytes resemble those of members of Oomycota, microorganisms associated previously with diseases in macroalgae.

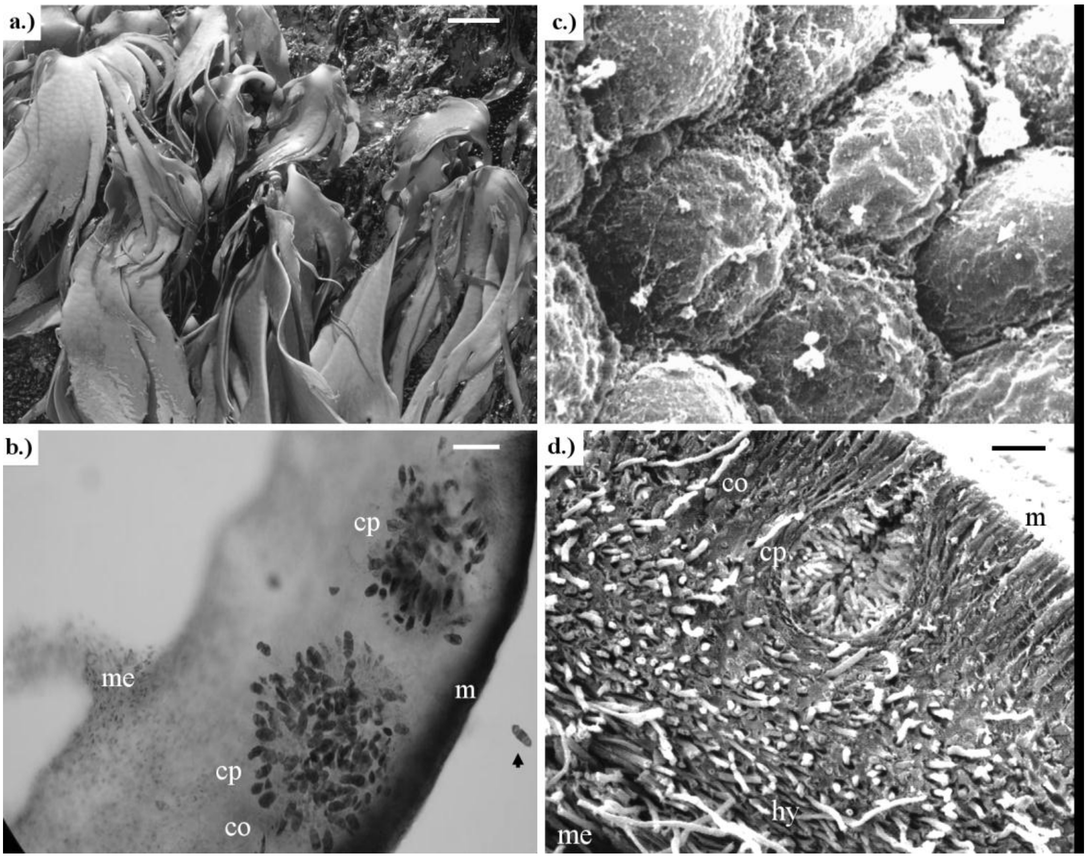


Fig. 2 a) Healthy populations of *Durvillaea antarctica* in the natural environment in Chile; **b)** Light microscopic microphotograph of a cross-section of a dioecious frond of *D. antarctica* (stained with aniline blue) showing two female conceptacle (cp) with one free oogonium (black arrow), meristoderm (m), cortical (co) and medullary (me) zones in a normal frond (picture: A. Núñez); **c)** Scanning electron microphotograph (SEM) with details of the surface of the thallus, and cells disposition in the algal surface (arrow shows one cell); **d)** Detail of a cross-section of a normal thallus using SEM showing early stages in conceptacle development (cp), meristoderm (m), cortical (co) and medullar (me) tissue with normal swift hyphae (hy). Scale bar: a) 10 cm; b) 100µm; c) 2µm; and d) 50µm.

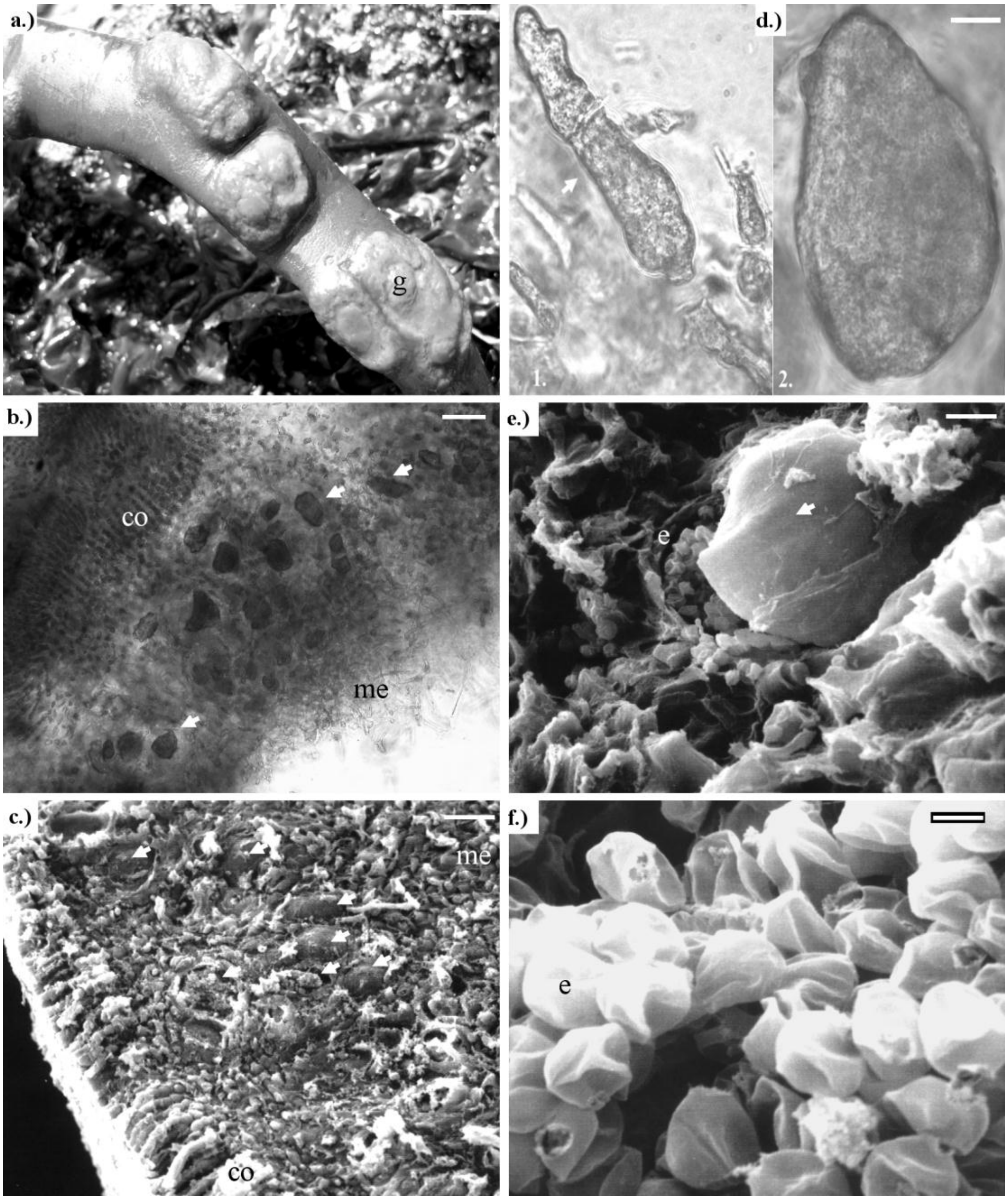


Fig. 3 Macroalgal samples of Coliumo bay, central Chile. **a)** Photography of gall-formations (g) over the thallus of *D*. *antarctica* in Coliumo bay, Chile; **b)** Cross-section under light microscopy showing endophytes in the yellowish brown subcortical tissue from the alga. The parasites were dark-brown coloured (see arrows) and presented irregular forms between 20-40µm in diameter; **c)** Cross-section under scanning electron microscopy (SEM) showing the formation of the cortex (co), medulla (me), and the microorganisms settled in these lesions (arrows); **d)** (1) Microphotograph with the detail of zoosporangial plasmodiums (arrow) of the endophyte within an abnormally growing host-cell, (2) detail of a thick-walled resting sporangia; **e)** SEM microphotograph of a thick-walled resting sporangia of the endophyte releasing numerous endospores (e) by a localized disruption of the host cell; **f)** SEM microphotograph with detail of the resting endospores at the cystosori. Scale bar: a) 1 cm; b-c) 50µm; d-e) 10µm; and f) 2µm.

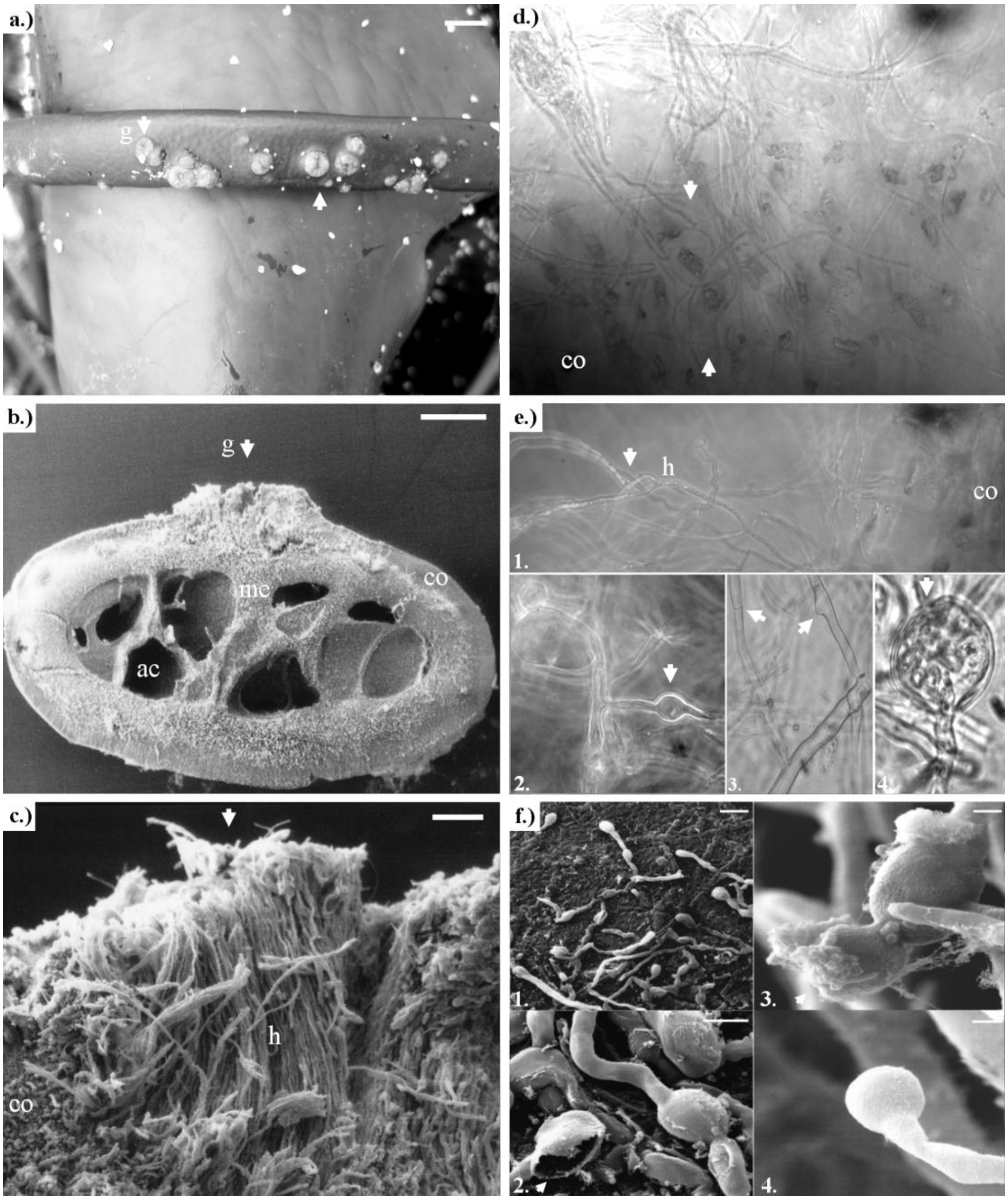


Fig. 4 Macroalgal samples of Valparaíso bay, central Chile. **a)** Galls over the thallus of *Durvillaea antarctica*; **b)** Cross section of the thallus under SEM with detail of the gall, cortex, medulla and normal air-filled cavities (ac); Cross section of the algal thallus under light microscopy, meristoderm (m), cortex (co), medulla (me); **c)** Cross section under SEM with details the endophyte forming a bundle of hyphae in the centre of the gall with a rupture of the outer cortex; **d)** Detail of a cross section in the apical zone of the cortex, with the presence of hyphae (arrows) penetrating the cortex and growing between algal cells; **e)** Detail of the endophyte's morphology, special structures are shown with arrows: 1) irregular shaped hyphae with vacuolated material (arrow), 2) empty zoosporangium, 3) detail of the vegetative hyphae with papillae and cross walls (arrows), 4) spherical oogonium; **f)** Detail of the endophyte's morphology using SEM. 1) host surface in the surroundings of the gall opening being invaded by the parasite, 2) disrupted terminal oogonium (arrow), 3) intercalary oogonium, 4) small sized spherical oogonium. Scale bar: a) 0.5 cm; b) 1mm; c) 100µm; d) 40x; e) e1: 40x, e2-4: 100x; and f) f1: 20µm, f2: 5µm, f3: 2µm, f4: 2µm.

DISCUSSION

Microbial pathogens may play an important role as a direct and indirect source of algal mortality and could modify the competitive abilities of diseased hosts in the field, reducing the host's growth and reproduction due the allocation of resources for the defense and healing mechanisms, and causing largest losses in seaweed aquaculture (Ramaiah 2006). Recent reviews pointed the importance of reassess the largely unexplored influence of parasites in algal diseases, suggesting that they occur more often in natural populations as we previously though, which are increasingly being considered of equal importance with predators for ecosystem functioning (see Gachon et al. 2010; Goecke et al. 2010; Li et al. 2010; Neuhauser et al. 2010a, Rasconi et al. 2011).

We reported a disease affecting bull-kelp in natural beds of Central coast of Chile (Pacific Ocean): Abnormal growth characterized by gall development and discolorations were found on the thallus of this macroalga. Based on previous biomechanical studies, *Durvillaea* spp. is considered as one of the strongest algae in the world (Stevens et al. 2001). The special morphology, together with high level of alginate as important component in new tissue produced within the entire blade, allows *Durvillaea* spp. to be elastic and flexible, necessary features to withstand the rigours of the extreme high hydrodynamic environment in which the macroalga lives (Kelly and Brown 2000). Thus, development of diseases that produce galls, holes and hardness of the thallus modified seriously this feature and probably affects the survival of the host (Aguilera et al. 1988). Observations by light microscopy and scanning electron microscopy revealed the presence of two different morphotypes of endophytes exclusively within the galls (Fig. 3 & 4).

Coliumo bay samples

In Coliumo bay, we found an endophytic microorganism associated with galls in the cortex of *D*. *antarctica* (Fig. 3). The fact that no rhizoids, hyphae, exit papillae or exit tubes were visible, helped to differentiate the observed endophyte from closely related groups as Chytridomycetes (Letcher et al. 2008) or Oomycetes (Sekimoto et al. 2008) (Fig. 3 a-f). Based on morphological characters, we were able to assign them into the class Phytomyxea of the phylum Cercozoa (known as Plasmodiophoromycetes). The endophyte matched to descriptions given by Karling (1942) and Sparrow (1969) for the genera *Plasmodiophora*, *Ligniera* or *Phagomyxa*. Similar structures, size and reproduction system were observed in infestations in other hosts elsewhere (see Tewari and Baines 1983; Schnepf et al. 2000; Kanyuka et al. 2003; Kageyama and Asano 2009).

23 years ago, the presence of a similar endophytic parasite associated to galls in *D. antarctica* was described in the south of Chile by Aguilera et al. (1988) (Fig. 1). The authors observed similar structures localized in subcortical cells of the macroalga, and placed them into the class Phytomyxea. This study was the only one reporting these parasites on this taxon. Unfortunately, it was based only on light microscopic observations, and the structure and/or development of the parasite are insufficiently known. There was a lack of descriptions of special morphological characteristics (i.e. form and distribution of resting spores), that has lead years later to question this designation into plasmodiophoroids (see Maier et al. 2000). However, we found macroalgae with galls in the same geographic region of this prior study. Applying scanning electron microscopy we were able to confirm the affiliation to the Phytomyxea. Nevertheless, molecular analyses of the samples are necessary to confirm any genera.

Phytomyxea is an enigmatic group of obligate biotroph parasites, from which only few species have been studied intensively during the past decades, restricting the investigation to species that cause notorious plant diseases or transmit terrestrial plant virus of economic importance (Neuhauser et al. 2010). Therefore information about distribution in the ocean, abundance, biodiversity and ecological roles of these organisms is either incomplete or lacking (Neuhauser et al. 2011a). In brown algae, *Phagomyxa algarum* was described as parasitizing *Bachelotia antillarum* (formerly *Pylaiella fulvescens*) and *Hincksia mitchelliae* (as *Ectocarpus mitchelliae*) in North Carolina, USA (Karling 1944). *Maullinia ectocarpii* was identified affecting *Ectocarpus siliculosus* in the south of Chile (Maier et al. 2000). In fact, of the 41 species reported till date for Phytomyxea, at least 7 of them have been associated to algae, but many of them have been just reported once, with no further research (Neuhauser et al. 2011a; b).

Valparaíso bay samples

In a northern population of *D. antarctica* growing in the Central coast of Chile gall formation was also noticed (Fig. 1 & 4). In this case, the presence of a different endophytic microorganism was observed in associated with those malformations. The development of hyphae and the presence of particular reproductive structures allowed us to characterise this endophyte into Oomycota (known as oomycetes). This is the first report establishing that gall development in *D. antarctica* may be caused by a oomycetes infection. Once again, molecular analyses of the samples are necessary to confirm any genera.

Worldwide a total of 15 species of oomycetes have been reported to infect marine algae. The majority of these parasites are obligate pathogens in algae or diatoms that may exhibit symptoms such as changes in colour, rot lesions and abnormal growth (reviewed by Wei et al. 2010). *Eurychasma dicksonii*, *Petersenia lobata* and *Sirolpidium andreei* have been reported to affect in nature 6 brown algal host species, especially filamentous algae from the order Ectocarpales, but in infection experiments, a much wider spectrum of species (including *Lessonia trabeculata* and *Macrocystis pyrifera* from Chile) have shown susceptibility to these pathogens (Müller et al. 1999). The red rot disease, caused by the oomycete *Pythium porphyrae*, is one of the most destructive diseases of *Porphyra* and can seriously reduce both yield and quality in *Porphyra* farms every year (Park et al. 2006).

This is the first report for endophytic oomycete, and the second for phytomyxids associated with malformations in *Durvillaea*. Although the cause for development of galls in *Durvillaea antarctica* has not been unequivocally established, because the necessary Koch postulates are not yet fulfilled, the observation strongly suggests that these organisms or a factor related to them (i.e. viruses) triggered such growths. Important evidence comes from the repeated observation of the suspected agent exclusively within the affected area (gall) and not in healthy tissue/plants; that no other organisms was visible in the affected area, and finally, by the presence of reproduction structures produced by the endophyte, as suggested by Correa et al. (1993) for gall-formations in *Mazzaella laminarioides* with the cyanobacterium *Pleurocapsa* sp.

Microbial parasites typically are characterized by their small size, short generation time, and high rates of reproduction, with a simple life cycle occurring generally within a single host (Rasconi et al. 2011). Unfortunately, Oomycota and specially Phytomyxea have developed complex life cycles involving in the latter case two free swimming zoosporic stages, two plasmodial stages that are linked to the host, a thin walled zoosporangial stage, and thick-walled resting spores (Neuhauser et al. 2011a). Therefore, detection, isolation and cultivation of those pathogens often remain problematic (Gachon et al. 2010). Only few previous studies have been carried out on holocarpic endoparasites because they are intracellular, obligate biotrophs, and clonal cultures are not available (Sekimoto et al. 2008). To identify the pathogen is the first natural step of many. So far, very little is still known concerning the ecology of the microbial pathogens of macroalgae (Andrews 1976; Jaffray and Coyne 1996; Neuhauser et al. 2011a; b). Which are the mechanisms of pathogenesis and resistance, and a quantification of diseased individuals in the area is still missing. There are no studies related early stages of *Durvillaea* and those parasites. In other order of kelps (Laminariales) heavy parasite attacks on the microscopic stages of host have been already proposed as a regulatory factor for the population dynamics of macroalgae (Müller et al. 1999).

The Asian experience with extensive farming of brown, red and green seaweeds has shown that all are susceptible to disease (Craigie and Correa 1996). The development and expansion of the seaweed farming stresses the need to understand the relationship between seaweeds and their pathogens in both wild and cultivated populations (Correa 1996). Together with microscopy, cultivation, infecting experiments and the ongoing development of molecular and genome-enable approaches offers a huge potential for improving the description of algal parasites, their physiology and wider impacts (Gachon et al. 2010). Macroalgae are key organisms in tidal environments, but still we know too little about their interactions with parasites and pathogens, which have also demonstrated key roles for ecosystem functioning.

9 GENERAL DISCUSSION

Algae are a diverse group of autotrophic organisms, being the major primary producers in the aquatic ecosystem. They are key organisms which range in size from microscopic single cells that grow as plankton to giant seaweeds over 50 m long that form dense forests in coastal waters (Thomas 2002), providing food and shelter for a wide range of organisms from all domains of life, including millions of microorganisms (Chapter I). Recently it has been demonstrated by molecular methods that different species of marine macroalgae in the same habitat are associated with different microbial communities (Lachnit *et al.* 2009; Nylund *et al.* 2010). We confirmed these molecular data by means of a cultivation-based study on two co-occurring macroalgae from the Baltic Sea (Chapter II). The ecological explanation for the specific association is considered to depict a very unique microenvironment formed by each algal species, representing different niches in the same habitat. Even simple physical factors, as the form of the thallus and microtopography of the surface modified physico-chemical conditions such as light intensity, micro-currents or sediment deposition, (Supporting information S11), which were shown to influence the associations with organisms (Dean & Connell 1987). More important probably, each algal species has unique metabolic properties and performs specific physiological reactions, e.g. with its own secondary metabolites, with different reserve materials and cell wall structures. Thereby the algae produce a particular chemical microenvironment (the ‘phycosphere’) in their immediate surroundings, where uptake and release of nutrients and metabolic products, degradation of senescent material, and accumulation of bioactive molecules for defense or any other biological interaction take place (Grossart & Simon 2007, Lachnit *et al*. 2010).

During evolution, macroalgae have developed different strategies to deal with biofouling and pressure due to microbial attack, which involve both chemical and physical reactions, i.e. fast growth, continuous sloughing of outer layers, tough external coating or thick walls, and formation of mucus (Wahl 1989). Chemical defenses typically include the production of secondary metabolites that are toxic, distasteful or both to potential consumers and pathogens.

Chemical and physical defenses are not mutually exclusive and a variety of organisms utilize both of them in combination (Amsler *et al.* 1999). Actually, many algal substances produce inhibitory effects on microorganisms (Goecke *et al*. 2010, Paper I). A number of macroalgae of this present study displayed antibacterial activity against at least one test strain (Chapter IV). This production of antibiotic substances is expected to be of ecological advantage for the producing species as compared to non-producers in the same habitat, as these substances should influence biofouling charge and display defense against microbial pathogens (Lane & Kubanek 2008). Nevertheless, the production of secondary metabolites is costly in terms of energy (Wahl 1989) and does not lead to a general and uniform effect over all microorganisms (as shown in Chapter II & IV).

The association with members of the microbial community may also represent an ecologically advantage, not only against competitors (see Chisholm *et al*. 1996), but also against microbial epibiosis. Heterotrophic bacteria play a key role in regulating accumulation, export, remineralisation and transformation of the largest part of organic matter in those aquatic systems (Mudryk & Skórczewski 2006). As bacteria breakdown the organic matter, nutrients such as nitrogen and phosphorous are released back into the water and can be taken up again by the seaweeds for new growth (Thomas 2002). Due the attractive microhabitat produced by an algal species for microbial settlement, the competition between colonizers is intense: Space and nutrient limitation as well as a highly competitive environment force surface dwelling microorganisms to evolve particular adaptive responses as well as antagonistic strategies to prevent colonization or growth of potential competitors (Egan *et al.* 2008). In doing so, the development of antimicrobial metabolites is widespread among alga-associated bacteria (Wiese *et al*. 2009b). In the present work, we observed that the associated bacteria have a high potential to produce new antibiotic substances (Chapter II), a quality which the macroalgae may use for their own benefit.

Beneficial relationships among algae and bacteria have been observed already (Croft *et al*. 2005, Amin *et al*. 2009, Wagner-Dobler *et al*. 2011). The development of mutualistic interactions may be a logic evolutionary solution. The repeated isolation of certain strains from algae in this study indirectly supports this assumption (Chapter I & II). Specificity and consistency over time usually implies commensal or mutual benefits (Thiel 2006). Although it has been poorly studied, recent technological developments are increasingly showing evidence of symbiotic relationships between bacteria and macroalgae, an exciting topic for further researches.

The consistency of associations over time is mentioned because environmental conditions affect the metabolism, physiology and even survival of both, host and microbiota. Therefore, it is reasonable to expect seasonal effects on the composition of epiphytic communities, which we have observed microscopically on the surface of some algae (S11 in supporting information) and also could be recognized based on season differences in bacterial isolates (Chapter II). Seasonal dynamics of bacterioplankton and epiphytic bacterial communities in the Baltic Sea including *F. vesiculosus* have been previously observed using molecular methods (Andersson *et al*. 2010, Lachnit *et al*. 2011). The present data suggest a three dimensional factorial regulation of the epiphytic bacterial communities on algae. Since symbiotic bacteria, pathogens and fouling bacteria (present in one season) first select, then settle, and finally attach to the host, macroalgae may prevent damage by also producing secondary metabolites that inhibit one or all of these steps. Such metabolites represent the chemical first line of defence against the microbial challenge. If the bacterial attachment is not successfully inhibited, other secondary metabolites (i.e. quorum sensing, reviewed in Paper I, S01) may inhibit the growth, survival, virulence, or reproduction of possibly invading organisms. Such compounds of the second line of defense may be produced by the macroalgae or by epiphytic and endophytic microbes associated with them (Egan *et al*. 2000, Rao *et al*. 2007, Lane & Kubanek 2008). The selection of these 'associated' microorganisms might also be chemically mediated (Lachnit *et al*. 2010), although only few experiments have tested this 'selection' in nature (see Sneed & Pohnert 2011a). By using ecologically relevant strains in the bioactivity tests, we observed different effects of macroalgal extracts on the growth of these bacteria including stimulatory effects (Chapter IV), but higher inhibitory effects by using the bacterial extracts (Chapter II). A mutualistic relationship can be postulated in which the bacterial community protects the host from biofouling, while the host surface may provide nutrients and physical protection to the bacteria (Penesyan *et al*. 2010).

Interactions between bacteria and algae are thought to be important in controlling the dynamics of both communities and yet are only beginning to be understood at the species level (Jasti *et al.* 2005). Many questions are still unresolved concerning specificity and mutualism. One important aspect of our fragmentary knowledge concerns the composition of bacterial communities associated with distinct algal populations (Grossart *et al.* 2005). More intense investigations of the large variety of interactions between hosts and microbes and between different microbes should reveal the different communication pathways, which include the production of defensive or deterrent compounds, attractants and other signalling substances (Goecke *et al*. 2010).

During the last 3 years, there was a special enhancement of research focused on association between macroalgae and bacteria (covered in Papers I, II, III). The present work was carried out in order to contribute towards our understanding of alga-bacteria relationships. It covered potential effects from the host, season, and epibiota in regulating the associated microbial communities. It also covered pathogenic relationships and possible specific associations. No doubt, we are only starting to discover the complexity of relationships between algae and bacteria in nature. The role of the associated bacteria as defense of the host against pathogens is almost completely unknown, starting with the fact that diseases of algae are mostly overlooked (Chapter V). There are still many gaps in our understanding of the ecological role and importance of diseases, pathogens and parasites in aquatic environments (Chapter III, V). Because algae, as main primary producers, habitat-forming organisms and economic resources, are key elements of the aquatic environment, algal diseases have profound ecological and economic impacts. Algae itself constitute food for many humans and are treated in industrial, pharmaceutical, cosmetic and food industry for a wide range of applications involving multimillion-dollar transactions.

To sum up, there is a strong need to integrate aspects of ecology, cell biology, and chemistry in further studies (Steinberg & de Nys 2002) in order to understand the production and the distribution of the bioactive molecules *in situ* as well as their ecological impact on the macroalgal–bacterial interactions (Goecke *et al*. 2010). Different macroalgae in the same habitat may all exert the same ecological role as primary producers but in terms of interactions every single species may represent completely different microenvironments occupied by rich microbial communities.

10 EXPERIMENTAL SECTIONS

10.1 NUTRIENT MEDIA COMPOSITION (all amounts in g L^{-1})

GPY medium	(Rolf Schmaljohann, *pers. comm.*)	
0.5 g	peptone from soymeal	(Merck, Nr. 107212.0500)
0.1 g	yeast extract	(Difco, Nr. 212720)
15 g	Bacto agar	(Difco, Nr. 214030)
1 g	glucose	(Merck, Nr. 108342.2500)
pH 7.2	in sterile Baltic Sea water	

186/3 medium		
3 g	yeast extract	(Difco, Nr. 212720)
3 g	malt extract	(Bacto TM, Nr. 218630)
5 g	peptone from soymeal	(Merck, Nr. 107212.0500)
10 g	glucose	(Merck, Nr. 108342.2500)
	in distilled water	

HWO medium	(Muscholl-Silberhorn *et al*. 2008)	
0.1 g	yeast extract	(Difco, Nr. 212720)
15 g	Bacto agar	(Difco, Nr. 214030)
	in sterile Baltic Sea water	

M1 medium		
5 g	peptone from soymeal	(Merck, Nr. 107212.0500)
3 g	meat extract	(Merck, Nr. 1.03979)
pH 7	in distilled water	

SCA medium	(Antje Labes, *pers. comm.*)	
10 g	Bacto Trypton	(BD, Nr. 211705)
5 g	yeast extract	(Difco, Nr. 212720)
20 g	NaCl	(Roth, Nr. 9265.2)
1 g	MgSO$_4$ xH$_2$O	(Merck, Nr. 1.05886.1)
6 g	Tris	(Roth, Nr. 4855.2)
5 mL	glycerol	(Merck, Nr. 1.04092.2500)
15 g	Bacto agar	(Difco, Nr. 214030)
pH 7.5	in distilled water	

TM medium	(Tropic Marine Medium modified from Zobell 1941)	
1 g	peptone from soymeal	(Merck, Nr. 107212.0500)
5 g	yeast extract	(Difco, Nr. 212720)
15 g	Bacto agar	(Difco, Nr. 214030)
30 g	Tropic Marin Salt in distilled water	

TSB3 medium		
3 g	Difco tryptic soy broth	(Otto Nordewald, Nr. 211772)
	in distilled water	

TSB12 medium	(Schneemann *et al*. 2010a)	
12 g	Difco tryptic soy broth	(Otto Nordewald, Nr. 211772)
10 g	NaCl	(Roth, Nr. 9265.2)
15 g	Bacto agar	(Difco, Nr. 214040)
pH 7.2	in distilled water	

10.2 MOLECULAR METHODS

To obtain different polymerase chain reaction products we used PuReTaq™ Ready-To-Go™ PCR Beads (Amersham Biosciences, Munich, Germany) and Taq DNA Polymerase with ThermoPol Buffer Kit (New England BioLabs, Ipswich; MA, USA), in a T1 thermocycler (Whatman Biometra®, Göttingen, Germany). Cycler conditions are shown below. Results of amplification were checked on a 2% agarose gel after each PCR (Staufenberger *et al.* 2008).

At the final volume of the beads (25 µl) each bead yields a reaction containing ~ 2.5 units of PuReTaq DNA polymerase, 10 mM Tris-HCl, (pH 9.0 at room temperature), 50 mM KCl, 1.5 mM MgCl2, 200 µM of each dNTP, stabilizers, and BSA.

Final volume: 25 µl/Assay

Addition	Volume
Primer 1	1 µl
Primer 2	1 µl
DNA-free Water	22 µl
Portionieren à	**24 µl**
Template-DNA	**1 µl**

For 'primer 1' we used "Eub27F" and for 'primer 2' „Univ1492R" (or „1387R" in cases when the previously primer did not give satisfactory results). The 16S rDNA-fragment has a lenght of 1501 bp (from base 9 to 1510).

Cycler conditions (final time 1,5 h) :

Denaturation (1 cycle)	93 °C	2 min
Amplification (30 cycles)	55 °C	30 sec
	72 °C	30 sec
	92 °C	30 sec
Last elongation (1 cycle)	42 °C	1 min
	72 °C	5 min
Cooling	15 °C	till 100 h possible

Primers:

1) PCR-Bacteria (universal 16S rRNA)

Eub27f	5'-GAGTTTGATCCTGGCTCAG-3'	Biomers.net
3'-1387	5'-CGGGCGGTGTGTACAAGG-3'	Biomers.net
Ûniv1492r	5'-GGTTACCTTGTTACGACTT-3'	MWG-Biotech AG

2) Secuencing-Bacteria

5'-534r	5'-CCAGCAGCCGCGGTAAT-3'	MWG-Biotech AG
5'-342f	5'-TACGGGAGGCAGCAG-3'	MWG-Biotech AG
5'-790f	5'-GATACCCTGGTAGTCC-3'	MWG-Biotech AG

10.3 Laboratory Equipment

Analytical Balances: Sartorius CP64
Sartorius CP423S

Centrifuges: Heraeus Multifuge 1 L-R Benchtop Centrifuges
Heraeus Biofuge Pico

Clean bench Secuflow

Contax SLR camera

Critical Point Dryer Balzers CPD030 / Balzers. Union SCD004 Sputtering Machine

Freeze Dryer CHRIST Alpha 2-4 LSC

Homogenizer: IKA T-25 Ultra-Turrax

Incubation shaker INFORS HT Ecotron

Microplate reader: Tecan Infinite M200 multimode reader / Software: i-control (tm)
SealPlate (adhesive sealing film), Carl Roth, Karlsruhe
Microplate, Greiner bio-one, Germany

Microscope Zeiss Axioskop 40

Power supply: Power-Pac Basic

Scanning Electron Microscope Zeiss DSM960

Shakers: Multi-Flask VKS 75A/B control Edmund Bühler GmbH

HPLC LaChrom Elite VWR-Hitachi Organizer

Spectrophotometer: Spekol 1500 Analytik Jena

Thermal Mixer TMix 220 Analytik Jena

Thermocycler Biometra

Vacuum Concentrator CHRIST SpeedDry 2-33IR / Freeze Drying Systems CHRIST CT04-50

10.4 CHEMICALS

Acetonitril	HPLC Gradient Grade LGC Promochem, Wesel
Agar	BactoTM, VWR, Hannover
Agarose NEEO	Carl Roth, Karlsruhe
Formic acid, p.a.	Fluka, Sigma-Aldrich, St. Louis, USA
Aniline blue W.S.	Harleco, Gibbstown, USA
CaCO3	Carl Roth, Karlsruhe
Chloramphenicol	Sigma-Aldrich, München
D(+)-Glucose-Monohydrat	Merck, Darmstadt
Dichlormethan	Carl Roth, Karlsruhe
DNA-free Water	Sigma-Aldrich, St. Louis, USA
DNA Molecular Weight Marker X	Roche, Mannheim
Ethanol 99 %	vergällt Walter-CMP, Hamburg
Formaldehyde	nd
Glucose monohydrate	Merck, Darmstadt
Glycerol	Carl Roth, Karlsruhe
HCl	Carl Roth, Karlsruhe
Yeast extract	BactoTM, VWR, Hannover
Hexane	Carl Roth, Karlsruhe
Isopropanol	Baker, Griesheim
Magnesium sulphate	Carl Roth, Karlsruhe
Malt extrakt	BactoTM, VWR, Hannover
Marine Broth	DifcoTM, VWR, Hannover
Methanol	HPLC Gradient Grade Carl Roth, Karlsruhe
NaOH	Carl Roth, Karlsruhe
Peptone BactoTM,	VWR, Hannover
Peptone from soymeal	Merck, Darmstadt
Resazurin	Redoxindikator Riedel-de Häen, Sigma-Aldrich
Sodium cloride	p.a. Merck, Darmstadt
Sodium hydroxide	p.a. Carl Roth, Karlsruhe
Soymeal	Difco, VWR, Hannover
Streptomycin	Invitrogen, Carlsbad, USA
SYBR® Safe	DNA gel strain Invitrogen, Carlsbad, USA
Tris	Carl Roth, Karlsruhe

11 REFERENCES

Abraham W-R, Strömpl C, Meyer H, Lindholst S, Moore ERB, Christ R, Vancanneyt M, Tindall BJ, Bennasar A, Smit J, Tesar M (1999) Phylogeny and polyphasic taxonomy of *Caulobacter* species. Proposal of *Maricaulis* gen. nov. with *Maricaulis maris* (Poindexter) comb. nov. as the type species, and emended description of the genera *Brevundimonas* and *Caulobacter*. Int J Syst Bact 49:1053-1073

Aguilera M, Rivera PJ, Westermeier R (1988) Presencia de hongos Plasmodiophorales en plantas de *Durvillaea antarctica* (Cham.) Hariot (Phaeophyta, Durvillaeaceae) del sur de Chile. Gayana Bot 45:337-343

Akagawa-Matsushita M, Matsuo M, Koga Y, Yamasat K (1992) *Alteromonas atlantica sp.* nov. and *Alteromonas carrageenovora* sp. nov., bacteria that decompose algal polysaccharides. Int J Syst Bacteriol 42:621-627

Alavi M, Miller T, Erlandson K, Schneider R, Belas R (2001) Bacterial community associated with *Pfiesteria*-like dinoflagellate cultures. Environ Microbiol 3:380-396

Alonso C, Warnecke F, Amann R, Pernthaler J (2007) High local and global diversity of *Flavobacteria* in marine plankton. Environ Microbiol 9:1253-1266

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403-410

Amin SA, Green DH, Hart MC, Küpper FC, Sunda WG, Carrano CJ (2009) Photolysis of iron-siderophore chelates promotes bacterial-algal mutualism. PNAS 106:17071-17076

Amsler CD, McClintock JB, Baker BJ (1999) An Antarctic feeding triangle: Defensive interactions between macroalgae, sea urchins, and sea anemones. Mar Ecol Prog Ser 183:105-114

Andersson AF, Riemann L, Bertilsson S (2010) Pyrosequencing reveals contrasting seasonal dynamics of taxa within Baltic Sea bacterioplankton communities. ISME J 4:171-181

Andrews JH (1976) The pathology of marine algae. Biol Rev 51:211-253

Apt K (1984) Effect of the symbiotic red alga *Hypneocolax stellaris* on its host *Hypnea musciformis* (Hypneaceae, Gigartinales). J Phycol 20:148-150

Apt K (1988) Galls and tumor-like growths on marine macroalgae. Dis Aquat Organ 4:211-217

Ara J (2001) Studies on bioactivity in some seaweeds from Karachi coast. PhD thesis, University of Karachi, Pakistan

Armstrong E, Rogerson A, Leftley JW (2000) The abundance of heterotrophic protests associated with intertidal seaweeds. Estuar Coast Shelf Sci 50:415-424

Armstrong E, Yan L, Boyd KG, Wright PC, Burgess JG (2001) The symbiotic role of marine microbes on living surfaces. Hydrobiologia 461:37-40

Arunkumar K, Sivakumar SR, Rengasamy R (2010) Review on bioactive potential in seaweeds (marine macroalgae): a special emphasis on bioactivity of seaweeds against plant pathogens. Asian J Plant Sci 9:227-240

Bae SS, Kwon KK, Yang SH, Lee H-S, Kim S-J, Lee J-H (2007) *Flagellimonas eckloniae* gen. nov., sp. nov., a mesophilic marine bacterium of the family Flavobacteriaceae, isolated from the rhizosphere of *Ecklonia kurome*. Int J Syst Evol Microbiol 57:1050-1054

Bansemir A, Blume M, Schröder S, Lindequist U (2006) Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. Aquaculture 252:79-84

Barbeyron T, L'Haridon S, Corre E, Kloareg B, Potin P (2001) *Zobellia galactanovorans* gen. nov., sp. nov., a marine species of Flavobacteriaceae isolated from a red alga, and classification of three novel *Zobellia* species [*Cytophaga*] *uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Zobellia uliginosa* gen. nov., comb. nov. Int J Syst Evol Microbiol 51:985-997

Barbeyron T, Lerat Y, Sassi JF, Le Panse S, Helbert W, Collen PN (2011) *Persicivirga ulvanivorans* sp. nov., a marine member of the family Flavobacteriaceae that degrades ulvan from green algae. Int J Syst Evol Microbiol 61:1899-1905

Beleneva IA, Zhukova NV (2006) Bacterial communities of some brown and red algae from Peter the Great Bay, the Sea of Japan. Microbiology (Moscow) 75:410-419

Bell WH, Lang JM, Mitchell R (1974) Selective stimulation of marine bacteria by algal extracellular products. Limnol Oceanogr 19:833-839

Bengtsson MM, Sjøtun K, Øvreås L (2010) Seasonal dynamics of bacterial biofilms on kelp (*Laminaria hyperborea*). Aquat Microb Ecol 60:71-83

Bengtsson MM (2011) Bacterial biofilms on the kelp *Laminaria hyperborea*. Dissertation for the degree philosophiae doctor (PhD) at the University of Bergen, 140 pp.

Bengtsson MM, Sjøtun K, Storesund JE, Øvreås L (2011) Utilization of kelp-derived carbon sources by kelp surface-associated bacteria. Aquat Microb Ecol 62:191-199

Berger R, Malm T, Kautsky L (2001) Two reproductive strategies in Baltic *Fucus vesiculosus* (Phaeophyceae). Eur J Phycol 36:265-273

Berland BR, Bonin DJ, Maestrini SY (1972) Are some bacteria toxic for marine algae? Mar Biol 12:189-193

Berry A, Janssens D, Humbelin M, Jore JPM, Hoste B, Cleenwerck I, Vancanneyt M, Bretzel W, Mayer AF, Lopez-Ulibarri R, Shanmugam B, Swings J, Pasamontes L (2003) *Paracoccus zeaxanthinifaciens* sp. nov., a zeaxanthin-producing bacterium. Int J Syst Evol Microbiol 53:231-238

Biebl H, Allgaier M, Lunsdorf H, Pukall R, Tindall BJ, Wagner-Dobler I (2005a) *Roseovarius mucosus* sp. nov., a member of the Roseobacter clade with trace amounts of bacteriochlorophyll a." Int J Syst Evol Microbiol 55:2377-2383

Biebl H, Allgaier M, Tindall BJ, Koblizek M, Lunsdorf H, Pukall R, Wagner-Dobler I (2005b) *Dinoroseobacter shibae* gen. nov., sp. nov., a new aerobic phototrophic bacterium isolated from dinoflagellates. Int J Syst Evol Microbiol 55:1089-1096

Biebl H, Tindall BJ, Pukall R, Lunsdorf H, Allgaier M, Wagner-Dobler I (2006) *Hoeflea phototrophica* sp. nov., a novel marine aerobic alphaproteobacterium that forms bacteriochlorophyll a. Int J Syst Evol Microbiol 56:821-826

Biebl H, Pukall R, Lunsdorf H, Schulz S, Allgaier M, Tindall BJ, Wagner-Dobler I (2007) Description of *Labrenzia alexandrii* gen. nov., sp. nov., a novel alphaproteobacterium containing bacteriochlorophyll a, and a proposal for reclassification of *Stappia aggregata* as *Labrenzia aggregata* comb. nov., of *Stappia marina* as *Labrenzia marina* comb. nov. and of *Stappia alba* as *Labrenzia alba* comb. nov., and emended descriptions of the genera *Pannonibacter*, *Stappia* and *Roseibium*, and of the species *Roseibium denhamense* and *Roseibium hamelinense*. Int J Syst Evol Microbiol 57:1095-1107

Biegala IC, Kennaway G, Alverca E, Lennon JF, Vaulot D, Simon N (2002) Identification of bacteria associated with dinoflagellates (Dinophyceae) *Alexandrium* spp. using tyramide signal amplification–fluorescent in situ hybridization and confocal microscopy. J Phycol 38:404-411

Bolch CJS, Subramanianand TA, Green DH (2011) The toxic dinoflagellate *Gymnodinium catenatum* (Dinophyceae) requires marine bacteria for growth. J Phycol 47:1009-1022

Bolinches J, Lemos ML, Barja JL (1988) Population dynamics of heterotrophic bacterial communities associated with *Fucus vesiculosus* and *Ulva rigida* in an estuary. Microb Ecol 15:345-357

Bouarab K, Potin P, Weinberger F, Correa J, Kloareg B (2001) The *Chondrus crispus-Acrochaete operculata* host-pathogen association, a novel model in glycobiology and applied phycopathology. J Appl Phycol 13:185-193

Bowman JP (2000) Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. Int J Syst Evol Microbiol 50:1861-1868

Boyd KG, Adams DR, Burgess JG (1999) Antibacterial and repellent activities of marine bacteria associated with algal surfaces. Biofouling 14:227-236

Braune W (2008) Meeresalgen. Ein Farbbildführer zu den verbreiteten benthischen Grün- Braun- und Rotalgen der Weltmeere, 596 pp., 266 pls. Ruggell: ARG Gantner Verlag.

Brawley SH, Johnson LE, Pearson GA, Speransky V, LI R, Serrao E (1999) Gamete release at low tide in Fucoid algae: Maladaptive or advantageous? Am Zool 39: 218-229

Brock TD (1966) The habitat of *Leucothrix mucor*, a widespread marine organism. Limnol Oceanogr 11:303-307

Brock TD, Clyne J (1984) Significance of algal excretory products for growth of epilimnetic bacteria. Appl Environ Microbiol 47:731-734

Bruckner CG, Bahulikar R, Rahalkar M, Schink B, Kroth PG (2008) Bacteria associated with benthic diatoms from lake Constance: Phylogeny and influences on diatom growth and secretion of extracellular polymeric substances. Appl Environ Microbiol 74:7740-7749

Buchan A, Gonzalez JM, Moran MA (2005) Overview of the marine *Roseobacter* lineage. Appl Environ Microbiol 71:5665-5677

Burgess JG, Boyd KG, Armstrong E, Jiang Z, Yan L, Berggren M, May UT, Granmo A, Adams DR (2003) The development of a marine natural product–based antifouling paint. Biofouling 19:197-205

Burke C, Thomas T, Lewis M, Steinberg P, Kjelleberg S (2011) Composition, uniqueness and variability of the epiphytic bacterial community of the green alga *Ulva australis*. ISME J 5:590-600

Burkhard E, Peters A (1998) Molecular evidence from nrDNA ITS sequences that *Laminariocolax* (Phaeophyceae, Ectocarpales *sensu lato*) is a worldwide clade of closely related kelp endophytes. J Phycol 34:682-691

Buschmann A, Correa JA, Beltran J, Retamales CA (1997) Determinants of disease expression and survival of infected individual fronds in wild populations of *Mazzaella laminarioides* (Rhodophyta) in central and southern Chile. Mar Ecol Prog Ser 154:269-280

Bustamante RH, Castilla JC (1990) Impact of human exploitation on populations of the intertidal Southern bull-kelp *Durvillaea antarctica* (Phaeophyta, Durvilleales) in Central Chile. Biol Conserv 52:205-220

Callow ME, Coughlan SJ, Evans LV (1978) Fertilization in brown algae. I. SEM and other observations on *Fucus serratus*. J Cell Sci 32: 45-54

Castilla JC, Campo MA, Bustamante RH (2007) Recovery of *Durvillaea antarctica* (Durvilleales) inside and outside Las Cruces marine reserve, Chile. Ecol Appl 17:1511-1522

Cheshire AC, Conran JG, Hallam ND (1995) A cladistic analysis of the evolution and biogeography of *Durvillaea* (Phaeophyta). J Phycol 31:644-655

Chisholm JRM, Dauga C, Ageron E, Grimont PAD, Jaubert JM (1996) 'Roots' in mixotrophic algae. Nature 381:382

Cho GY, F Rousseau, B De Reviers, Boo SM (2006) Phylogenetic relationships within the Fucales (Phaeophyceae) assessed by the photosystem I coding *psa*A sequences. Phycologia 45:512-519

Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Klam-Syed-Mohideen AS, McCarell DM, Marsh T, Garrity GM, Tiedje JM (2009) The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res 37:D141-145 (Web Server issue)

Collantes G, Merino A, Lagos V (2002) Fenología de la gametogénesis, madurez de conceptáculos, fertilidad y embriogénesis en *Durvillaea antarctica* (Chamisso) Hariot (Phaeophyta, Durvillaeales). Rev Biol Mar Oceanogr 37:83-112

Collins LA, Franzblau SG (1997) Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. Antimicrob Agents Chemother 42:1004-1009

Corre A, Prieur D (1990) Density and morphology of epiphyte bacteria on the kelp *Laminaria digitata*. Bot Mar 33:515-523

Correa JA (1996) Diseases in seaweeds: an introduction. Hydrobiologia 326/327:87-88

Correa JA, Flores V, Sánchez P (1993) Deformative disease in *Iridaea laminarioides* (Rhodophyta): gall development associated with an endophytic cianobacterium. J Phycol 29:853-860

Cottrell MT, Kirchman DL (2000a) Natural assemblages of marine Proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. Appl Environ Microbiol 66:1692-1697

Cottrell MT, Kirchman DL (2000b) Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence in situ hybridization. Appl Environ Microbiol 66:5116-5122

Coveney MF, Wetzel RG (1989) Bacterial metabolism of algal extracellular carbon. Hydrobiologia 173:141-149

Craigie JS, Correa JA, Gordon M (1992) Cuticles from *Chondrus crispus*. J Phycol 28:777-786

Craigie JS, Correa JA (1996) Etiology of infectious diseases in cultivated *Chondrus crispus* (Gigartinales, Rhodophyta). Hydrobiologia 326/327:97-104

Croft MT, Lawrence AD, Raux-Deery E, Warren MJ, Smith AG (2005) Algae acquire vitamin B12 through a symbiotic relationship with bacteria. Nature 438:90-93

Croft MT, Warren MJ, Smith AG (2006) Algae need their vitamins. Eukaryot Cell 5:1175-1183

Dean RL, Connell JH (1987) Marine invertebrates in algal succession. III. Mechanisms linking habitat complexity with diversity. J Exp Mar Biol Ecol 109:249-273

Dimitrieva GY, Dimitriev SM (1996) Symbiotic microflora of the brown algae from the genus *Laminaria* as a bioindicator of the ecological state of coastal *Laminaria* biocoenoses. Russ J Mar Biol 22:276-281

Dimitrieva GY, Crawford RL, Yuksel GU (2006) The nature of plant growth-promoting effects of a pseudoalteromonad associated with the marine algae *Laminaria japonica* and linked to catalase excretion. J Appl Microbiol 100:1159-1169

Ding H, Ma J (2005) Simultaneous infection by red rot and chytrid diseases in *Porphyra yezoensis* Ueda. J Appl Phycol 17:51-56

Dobretsov SV, Qian PY (2002) Effect of bacteria associated with the green alga *Ulva reticulata* on marine micro- and macrofouling. Biofouling 18:217-228

Dobretsov S, Dahms HU, Qian PY (2006a) Inhibition of biofouling by marine microorganisms and their metabolites. Biofouling 22:43-54

Dobretsov S, Dahms HU, Harder T, Qian PY (2006b) Allelochemical defense against epibiosis in the macroalga *Caulerpa racemosa* var. *turbinata*. Mar Ecol Prog Ser 318:165-175

Du ZJ, Lv GQ, Rooney AP, Miao TT, Xu QQ, Chen GJ (2011) *Agarivorans gilvus* sp. nov. isolated from seaweed. Int J Syst Evol Microbiol 61:493-496

Dubber D, Harder T (2008) Extracts of *Ceramium rubrum*, *Mastocarpus stellatus* and *Laminaria digitata* inhibit growth of marine and fish pathogenic bacteria at ecologically realistic concentrations. Aquaculture 274:196-200

Egan S, Thomas T, Holmstrom C, Kjelleberg S (2000) Phylogenetic relationship and antifouling activity of bacterial epiphytes from the marine alga *Ulva lactuca*. Environ Microbiol 2:343-347

Egan S, Holmström C, Kjelleberg S (2001) *Pseudoalteromonas ulvae* sp. nov., a bacterium with antifouling activities isolated from the surface of a marine alga. Int J Syst Evol Microbiol 51:1499-1504

Egan S, Thomas T, Kjelleberg S (2008) Unlocking the diversity and biotechnological potential of marine surface associated microbial communities. Curr Opin Microbiol 11:219-225

Engel S, Jensen PR, Fenical W (2002) Chemical ecology of marine microbial defense. J Chem Ecol 28:1971-1985

Engel S, Puglisi MP, Jensen PR, Fenical W (2006) Antimicrobial activities of extracts from tropical Atlantic marine plants against marine pathogens and saprophytes. Mar Biol 149:991-1002

Englebert ET, McDermott C, Kleinheinz GT (2008) Effects of the nuisance algae, *Cladophora*, on *Escherichia coli* at recreational beaches in Wisconsin. Sci Total Environ 404:10-17

Euzeby JP (1997) List of bacterial names with standing in nomenclature: a folder available on the internet. Int J Syst Bacteriol 47:590-592

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783-791

Felsenstein J (1989) PHYLIP – Phylogeny Interface Package (Version 3.2). Cladistics 5:164-166

Freile-Pelegrin Y, Morales JL (2004) Antibacterial activity in marine algae from the coast of Yucatan, Mexico. Bot Mar 47:140-146

Fu XT, Kim SM (2010) Agarase: Review of major sources, categories, purification method, enzyme characteristics and applications. Mar Drugs 8:200-218

Fujita Y, Zenitani B, Nakao Y, Matsubara T (1972) Bacteriological studies on diseases of cultured laver. II. Bacteria associated with diseased laver. B Jpn Soc Sci Fish 38:565-569

Fukui Y, Abe M, Kobayashi M, Ishihara K, Oikawa H, Yano Y, Satomi M (pub ahead) Description of *Maritalea porphyrae* sp. nov. isolated from red algae (*Porphyra yezoensis*) and transfer of *Zhangella mobilis* to *Maritalea mobilis* comb. nov. Int J Syst Evol Microbiol (pub ahead) doi:10.1099/ijs.0.028704-0

Fukunaga Y, Kurahashi M, Sakiyama Y, Ohuchi M, Yokota A, Harayama S (2009) *Phycisphaera mikrensis* gen. nov., sp. nov., isolated from a marine alga, and proposal of Phycisphaeraceae fam. nov., Phycisphaerales ord. nov. and Phycisphaerae classis nov. in the phylum Planctomycetes. J Gen Appl Microbiol 55:267-275

Gachon CMM, Sime-Ngando T, Strittmater M, Chambouvet A, Kim GH (2010) Algal diseases spotlight on a black box. Trends Plant Sci 15:633-640

Gärdes A, Iversen MH, Grossart HP, Passow U, Ullrich MS (2010) Diatom-associated bacteria are required for aggregation of *Thalassiosira weissflogii*. ISME J 5:436-445

Gauna MC, Parodi ER, Caceres EJ (2009) Epi-endophytic symbiosis between *Laminariocolax aecidioides* (Ectocarpales, Phaeophyceae) and *Undaria pinnatifida* (Laminariales, Phaeophyceae) growing on Argentinian coasts. J Appl Phycol 21:11-18

Glombitza KW (1969) Antibakterielle Inhaltsstoffe in Algen. Helgol wiss Meeresuntersuch 19:376-384

Goecke F, Labes A, Wiese J, Imhoff JF (2010) Chemical interactions between marine macroalgae and bacteria. Mar Ecol Prog Ser 409:267-300

González JM, Moran MA (1997) Numerical dominance of a group of marine bacteria in the a-subclass of the class Proteobacteria in coastal seawater. Appl Environ Microbiol 63:4237-4242

Green DH, Llewellyn LE, Negri AP, Blackburn SI, Bolch CJ (2004) Phylogenetic and functional diversity of the cultivable bacterial community associated with the paralytic shellfish poisoning dinoflagellate *Gymnodinium catenatum*. FEMS Microbiol Ecol 47:345-357

Green DH, Bowman JP, Smith EA, Gutierrez T, Bolch CJ (2006) *Marinobacter algicola* sp. nov., isolated from laboratory cultures of paralytic shellfish toxin-producing dinoflagellates. Int J Syst Evol Microbiol 56:523-527

Gromov BV, Mamkaeva KA (1980) Proposal of a new genus *Vampirovibrio* for chlorellavorus bacteria previously assignated to *Bdellovibrio*. Mikrobiologia 49:165-167

Grossart HP, Levold F, Allgaier M, Simon M, Brinkhoff T (2005) Marine diatom species harbour distinct bacterial communities. Environ Microbiol 7:860-873

Grossart HP, Simon M (2007) Interactions of planktonic algae and bacteria: Effects on algal growth and organic matter dynamics. Aquat Microb Ecol 47:163-176

Guiry MD, Guiry GM (2011) AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 31 January 2011

Hahnke S, Tindall BJ, Schumann P, Sperling M, Brinkhoff T, Simon M (pub ahead) *Planktotalea frisia*, gen. nov., sp. nov., isolated from the southern North Sea. Int J Syst Evol Microbiol (pub ahead) doi:10.1099/ijs.0.033563-0

Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95-98

Harder T (2009) Marine epibiosis: concepts, ecological consequences and host defence. In: Flemming HC, Murthy PS, Venkatesan R, Cooksey K (eds) pp. 219-231. Marine and industrial biofouling. Springer-Berlin Heidelberg

Heesch S, Peters AF, Broom JE, Hurd CL (2008) Affiliation of the parasite *Herpodiscus durvillaeae* (Phaeophyceae) with the Sphacelariales based on DNA sequence comparisons and morphological observations. Eur J Phycol 43:283-295

Heindl H, Wiese J, Thiel V, Imhoff JF (2010) Phylogenetic diversity and antimicrobial activities of bryozoan-associated bacteria isolated from Mediterranean and Baltic Sea habitats. Syst Appl Microbiol 33:94-104

Hellio C, Bremer G, Pons AM, Le Gal Y, Bourgougnon N (2000) Inhibition of the development of microorganisms (bacteria and fungi) by extracts of marine algae from Brittany (France). Appl Microb Biotechnol 54:543-549

Hellio C, De La Broise D, Dufossé L, Le Gal Y, Bourgougnon N (2001) Inhibition of marine bacteria by extracts of macroalgae: Potential use for environmentally friendly antifouling paints. Mar Environ Res 52:231-247

Hellio C, Marechal JP, Véron B, Bremer G, Clare AS, Le Gal Y (2004) Seasonal variation of antifouling activities of marine algae from the Brittany Coast, France. Mar Biotechnol 6:67-82

Hengst MB, Andrade S, Gonzalez B, Correa JA (2010) Changes in epiphytic bacterial communities of intertidal seaweeds modulated by host, temporality, and copper enrichment. Microb Ecol 60:282-290

Herriott EM (1923) Some morphological notes on the New Zealand giant kelp, *Durvillaea antarctica* (Chamisso). Trans Proc New Zealand Inst 54:549-564

Hoffmann A, Santelices B (1997) Flora Marina de Chile Central. Ediciones Universidad Católica de Chile

Hold GL, Smith EA, Rappe MS, Maas EW, Moore ERB, Stroempl C, Stephen JR, Prosser JI, Birkbeck TH, Gallacher S (2001) Characterisation of bacterial communities associated with toxic and non-toxic dinoflagellates: *Alexandrium* spp. and *Scrippsiella trochoidea*. FEMS Microbiol Ecol 37:161-173

Hollants J, Leroux O, Leliaert F, Decleyre H, de Clerck O, Willems A (2011) Who is in there? Exploration of endophytic bacteria within the siphonous green seaweed *Bryopsis* (Bryopsidales, Chlorophyta). Plos One 6: e26458. doi:10.1371/journal.pone.0026458

Holmström C, Kjelleberg S (1999) Marine *Pseudoalteromonas* species are associated with higher organisms and produce biologically active extracellular agents. FEMS Microbiol Ecol 30:285-293

Holmström C, Egan S, Franks A, McCloy S, Kjelleberg S (2002) Antifouling activities expressed by marine surface associated *Pseudoalteromonas* species. FEMS Microbiol Ecol 41:47-58

Hosoya S, Arunpairojana V, Suwannachart C, Kanjana-Opas A, Yokota A (2006) *Aureispira marina* gen. nov., sp. nov., a gliding, arachidonic acid-containing bacterium isolated from the southern coastline of Thailand. Int J Syst Evol Microbiol 56:2931-2935

Howard GT, Elliott LP (1988) Effects of cellulolytic ruminal bacteria and of cell extracts on germination of *Euonymus americanus* L. seeds. Appl Environ Microbiol 54:218-224

Hughes CC, Fenical W (2011) Antibacterials from the sea. Chem Eur J 16:12512-12525

Hwang CY, Bae GD, Yih W, Cho BC (2009a) *Marivita cryptomonadis* gen. nov., sp. nov. and *Marivita litorea* sp. nov., of the family Rhodobacteraceae, isolated from marine habitats. Int J Syst Evol Microbiol 59:1568-1575

Hwang CY, Zhang GI, Kang S-H, Kim HJ, Cho BC (2009b) *Pseudomonas pelagia* sp. nov., isolated from a culture of the Antarctic green alga *Pyramimonas gelidicola*. Int J Syst Evol Microbiol 59:3019-3024

Ishikawa M, Ishizaki S, Yamamoto Y, Yamasato K (2003) *Paraliobacillus ryukyuensis* gen. nov., sp. nov., a new Gram positive, slightly halophilic, extremely halotolerant, facultative anaerobe isolated from a decomposing marine alga. J Gen Appl Microbiol 48:269-279

Ishikawa M, Nakajima K, Itamiya Y, Furukawa S, Yamamoto Y, Yamasato K (2005) *Halolactibacillus halophilus* gen. nov., sp. nov. and *Halolactibacillus miurensis* sp. nov., halophilic and alkaliphilic marine lactic acid bacteria constituting a phylogenetic lineage in *Bacillus* rRNA group 1. Int J Syst Evol Microbiol 55:2427-2439

Ishikawa M, Tanasupawat S, Nakajima K, Kanamori H, Ishizaki S, Kodama K, Okamoto-Kainuma A, Koizumi Y, Yamamoto Y, Yamasato K (2009) *Alkalibacterium thalassium* sp. nov., *Alkalibacterium pelagium* sp. nov., *Alkalibacterium putridalgicola* sp. nov. and *Alkalibacterium kapii* sp. nov., slightly halophilic and alkaliphilic marine lactic acid bacteria isolated from marine organisms and salted foods collected in Japan and Thailand. Int J Syst Evol Microbiol 59:1215-1226

Ishikawa M, Nakajima K, Ishizaki S, Kodama K, Okamoto-Kainuma A, Koizumi Y, Yamamoto Y, Yamasato K (pub ahead) *Alkalibacterium subtropicum* sp. nov., a slightly halophilic and alkaliphilic marine lactic acid bacterium isolated from decaying marine algae collected at a subtropical area of Japan. Int J Syst Evol Microbiol (pub ahead) doi:10.1099/ijs.0.027953-0

Ivanova EP, Nedashkovskaya OI, Chun J, Lysenko AM, Frolova GM, Svetashev VI, Vysotskii MV, Mikhailov VV, Huq A, Colwell RR (2001) *Arenibacter* gen. nov., new genus of the family Flavobacteriaceae and description of a new species, *Arenibacter latericius* sp. nov. Int J Syst Evol Microbiol 51:1987-1995

Ivanova EP, Bakunina IY, Sawabe T, Hayashi K, Alexeeva YV, Zhukova NV, Nicolau DV, Zvaygintseva TN, Mikhailov VV (2002) Two species of culturable bacteria associated with degradation of brown algae *Fucus evanescens*. Microb Ecol 43:242-249

Ivanova EP, Alexeeva YA, Zhukova NV, Gorshkova NM, Buljan V, Nicolau DV, Mikhailov VV, Christen R (2004a) *Bacillus algicola* sp. nov., a novel filamentous organism isolated from brown alga *Fucus evanescens*. Syst Appl Microbiol 27:301-307

Ivanova EP, Alexeeva YV, Flavier S, Wright JP, Zhukova NV, Gorshkova NM, Mikhailov VV, Nicolau DV, Christen R (2004b) *Formosa algae* gen. nov., sp. nov., a novel member of the family Flavobacteriaceae. Int J Syst Evol Microbiol 54:705-711

Ivanova EP, Alexeeva YV, Flavier S, Wright JP, Zhukova NV, Gorshkova NM, Mikhailov VV, Nicolau DV, Christen R (2004c) *Formosa algae* gen. nov., sp. nov., a novel member of the family Flavobacteriaceae. Int J Syst Evol Microbiol 54:705-711

Ivanova EP, Christen R, Sawabe T, Alexeeva YV, Lysenko AM, Chelomin VP, Mikhailov VV (2005) Presence of ecophysiologically diverse populations within *Cobetia marina* strains isolated from marine invertebrate, algae and the environments. Microbes Environ 20:200-207

Jaffray AE, Coyne VE (1996) Development of an in situ assay to detect bacterial pathogens of the red alga *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine et Farnham. J Appl Phycol 8:409-414

Jang GI, Hwang CY, Cho BC (pub ahead) *Nitratireductor aquimarinus* sp. nov., isolated from a culture of the diatom *Skeletonema costatum*, and emended description of the genus *Nitratireductor*. Int J Syst Evol Microbiol (pub ahead) doi:10.1099/ijs.0.028373-0

Jaramillo E, De la Huz R, Duarte C, Contreras H (2006) Algal wrack deposits and macroinfaunal arthropods on sandy beaches of the Chilean coast. Rev Chil Hist Nat 79:337-351

Jasti S, Sieracki ME, Poulton NJ, Giewat MW, Rooney-Varga JN (2005) Phylogenetic Diversity and specificity of bacteria closely associated with *Alexandrium* spp. and other phytoplankton. Appl Environ Microbiol 71:3483-3494

Jiang J, Ma Y, Zhang Z, Xu H (1997) The histopathological study on ‘green decay diseases’ of *Undaria pinnatifida* in Dalian. J Dalian Fish Univ 12:7-12

Johansen JE, Nielsen P, Sjøholm C (1999) Description of *Cellulophaga baltica* gen. nov., sp. nov. and *Cellulophaga fucicola* gen. nov., sp. nov. and reclassification of [*Cytophaga*] *Iytica* to *Cellulophaga lytica* gen. nov., comb. nov. Int J Syst Bacteriol 49:1231-1240

Jormalainen V, Honkanen T (2008) Macroalgal chemical defenses and their roles in structuring temperate marine communities. Pp. 57-89. In: Amsler CD (ed.) (2008) Algal chemical ecology, 313 pp. Springer-Verlag Berlin Heidelberg

Kaeppel EC, Gärdes A, Seebah S, Grossart HP, Ullrich MS (pub ahead) *Marinobacter adhaerens* sp. nov., prominent in aggregate formation with the diatom *Thalassiosira weissflogii*. Int J Syst Evol Microbiol (pub ahead) doi:10.1099/ijs.0.030189-0
Kageyama K, Asano T (2009) Life cycle of *Plasmodiophora brassicae*. J Plant Growth Regul 28:203-211

Kain JM, Bates MJ (1993) The reproductive phenology of *Delesseria sanguinea* and *Odonthalia dentata* off the Isle of Man. Eur J Phycol 28:173-182

Kanagasabhapathy M, Sasaki H, Haldar S, Yamasaki S, Nagata S (2006) Antibacterial activities of marine epibiotic bacteria isolated from brown algae of Japan. Ann Microbiol 56:167-173

Kanagasabhapathy M, Sasaki H, Nagata S (2008) Phylogenetic identification of epibiotic bacteria possessing antimicrobial activities isolated from red algal species of Japan. World J Microbiol Biotechnol 24:2315-2321

Kang HS, Yang HL, Lee SD (2009) *Nitratireductor kimnyeongensis* sp. nov., isolated from seaweed. Int J Syst Evol Microbiol 59:1036-1039

Kang HS, Lee SD (2010) *Mesonia phycicola* sp. nov., isolated from seaweed, and emended description of the genus *Mesonia*. Int J Syst Evol Microbiol 60:591-594

Kanyuka K, Ward E, Adams MJ (2003) *Polymyxa graminis* and the cereal viruses it transmits: A research challenge. Mol Plant Path 4:393-406

Karling JS (1942) Simple holocarpic biflagellate phycomycetes. Columbia University.

Karling JS (1944) *Phagomyxa algarum* n. gen., n. sp., an unusual parasite with plasmodiophoralean and proteomyxean characteristic. Am J Bot 31:38-52

Katsuta A, Adachi K, Matsuda S, Shizuri Y, Kasai H (2005) *Ferrimonas marina* sp. nov. Int J Syst Evol Microbiol 55:1851-1855

Kelly JB, Brown MT (2000) Variations in the alginate content and composition of *Durvillaea antarctica* and *D. williana* from southern New Zealand. J Appl Phycol 12:317-324

Kersters K, de Vos P, Gillis M, Swings J, Vandamme P, Stackebrandt E (2006) Introduction to the Proteobacteria. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E. (ed.) 2006. The prokaryotes. A handbook on the biology of bacteria. 3rd ed. Springer. 5:3-37

Kimura M (1983) The neutral theory of molecular evolution. Cambridge: Cambridge University Press

Knoll S, Zwisler W, Simon M (2001) Bacterial colonization of early stages of limnetic diatom aggregates. Aquat Microb Ecol 25:141-150

Kohlmeyer J (1979) Marine fungal pathogens among Ascomycetes and Deuteromycetes. Experientia 35:437-439

Kong MK, Chan KY (1979) A study on the bacterial flora isolated from marine algae. Bot Mar 22:83-97

Koop K, Newell RC, Lucas MI (1982) Microbial regeneration of nutrients from the decomposition of macrophyte debris on the shore. Mar Ecol Prog Ser 9:91-96

Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150-163

Lachnit T, Blümel M, Imhoff JF, Wahl M (2009) Specific epibacterial communities on macroalgae: phylogeny matters more than habitat. Aquat Biol 5:181-186

Lachnit T, Wahl M, Harder T (2010) Isolated thallus-associated compounds from the macroalga *Fucus vesiculosus* mediate bacterial surface colonization in the field similar to that on the natural alga. Biofouling 26:247-255

Lachnit T, Meske D, Wahl M, Harder T, Schmitz R (2011) Epibacterial community patterns on marine macroalgae are host-specific but temporally variable. Environ Microbiol 13:655-665

Lam C, Harder T (2007) Marine macroalgae affect abundance and community richness of bacterioplankton in close proximity. J Phycol 43:874-881

Lam C, Stang A, Harder T (2008) Planktonic bacteria and fungi are selectively eliminated by exposure to marine macroalgae in close proximity. FEMS Microbiol Ecol 63:283-291

Lane AL, Kubanek J (2008) Secondary metabolite defenses against pathogens and biofoulers. pp. 229-243. In: Amsler CD (ed.) (2008) Algal chemical ecology, 313 pp. Springer-Verlag Berlin Heidelberg

Lane AL, Nyadong L, Galhena AS, Shearer TL, Stout EP, Parry RM, Kwasnik M, Wang MD, Hay ME, Fernandez FM, Kubanek J (2009) Desorption electrospray ionization mass spectrometry reveals surface-mediated antifungal chemical defense of a tropical seaweed. PNAS 106:7314-7319

Lane AL, Mular L, Drenkard EJ, Shearer TL, Engel S, Fredericq S, Fairchild CR, Prudhomme J, Le Roch K, Hay ME, Aalbersberg W, Kubanek J (2010) Ecological leads for natural product discovery: novel sesquiterpene hydroquinones from the red macroalga *Peyssonnelia* sp. Tetrahedron 66:455-461

Lang G, Mitova MI, Ellis G, van der Sar S, Phipps RK, Blunt JW, Cummings NJ, Cole ALJ, Munro MHG (2006) Bioactivity profiling using HPLC/microtiter-plate analysis: application to a New Zealand marine alga-derived fungus, *Gliocladium* sp.. J Nat Prod 69:621-624

Largo DB, Fukami K, Adachi M, Nishijima T (1997) Direct enumeration of bacteria from macroalgae by epifluorescence microscopy as applied to the fleshy red algae *Kappaphycus alvarezii* and *Gracilaria* spp. (Rhodophyta). J Phycol 33:554-557

Larsson U, Hagström A (1979) Phytoplankton exudates as an energy source for the growth of pelagic bacteria. Mar Biol 52:199-206

Lawrence JM (1986) Proximate composition and standing crop of *Durvillaea antarctica* (Pheophyta) in the Bay of Morbihan, Kerguelen (South Indian Ocean). Mar Ecol Prog Ser 33:1-5

Laycock RA (1974) The detrital food chain based on seaweeds. 1. Bacteria associated with the surface of *Laminaria* fronds. Mar Biol 25:223-231

Lee DW, Lee JM, Seo JP, Schumann P, Kim SJ, Lee SD (2008a) *Phycicola gilvus* gen. nov., sp. nov., an actinobacterium isolated from living seaweed. Int J Syst Evol Microbiol 58:1318-1323

Lee DW, Lee SD (2010) *Koreibacter algae* gen. nov., sp. nov., isolated from seaweed. Int J Syst Evol Microbiol 60:1510-1515

Lee H-S, Kwon KK, Yang S-H, Bae SS, Park CH, Kim S-J, Lee J-H (2008b) Description of *Croceitalea* gen. nov. in the family Flavobacteriaceae with two species, *Croceitalea eckloniae* sp. nov. and *Croceitalea dokdonensis* sp. nov., isolated from the rhizosphere of the marine alga *Ecklonia kurome*. Int J Syst Evol Microbiol 58:2505-2510

Lee SD (2006) *Phycicoccus jejuensis* gen. nov., sp. nov., an actinomycete isolated from seaweed. Int J Syst Evol Microbiol 56:2369-2373

Lee SD (2007) *Labedella gwakjiensis* gen. nov., sp. nov., a novel actinomycete of the family Microbacteriaceae. Int J Syst Evol Microbiol 57:2498-2502

Lee SD, Kim SJ (2007) *Aeromicrobium tamlense* sp. nov., isolated from dried seaweed. Int J Syst Evol Microbiol 57:337-341

Lee SD (2008) *Agrococcus jejuensis* sp. nov., isolated from dried seaweed. Int J Syst Evol Microbiol 58:2297-2300

Lee SH, Park MS, Jung JY, Jeon CO (pub ahead) *Leuconostoc miyukkimchii* sp. nov., isolated from brown algae (*Undaria pinnatifida* L.) kimchi in Korea. Int J Syst Evol Microbiol (pub ahead) doi:10.1099/ijs.0.032367-0

Lee RE (2008) Phycology. Cambridge University Press, USA. Fourth edition. 547 pp.

Lemos ML, Toranzo AE, Barja JL (1985) Antibiotic activity of epiphytic bacteria isolated from intertidal seaweeds. Microb Ecol 11:149-163

Lewis TE, Garland CD, McMeekin TA (1985) The bacterial biota on crustose (nonarticulated) coralline algae from Tasmanian waters. Microb Ecol 11:221-230

Li W, Zhang T, Tang X, Wang B (2010) Oomycetes and fungi: Important parasites of marine algae. Acta Oceanol Sin 29:74-81

Li Z, Qu Z, Zhang X, Zhang XH (pub ahead) *Lentibacter algarum* gen. nov., sp. nov., a novel bacterium isolated from coastal water in Qingdao, China during a massive green algae bloom. Int J Syst Evol Microbiol (pub ahead) doi:10.1099/ijs.0.029868-0

Lin B, Lu G, Zheng Y, Xie W, Li S, Hu Z (pub ahead) *Aquimarina agarilytica* sp. nov., a novel agarolytic species isolated from red alga. Int J Syst Evol Microbiol (pub ahead) doi:10.1099/ijs.0.027136-0

Lin SM, Fredericq S, Hommersand MH (2001) Systematics of the Delesseriaceae (Ceramiales, Rhodophyta) base don large subunit rDNA and rbcL sequences, including the Phycodryoideae, subfam. nov. J Phycol 37:881-899

Longford SR, Tujula NA, Crocetti G, Holmes AJ, Holmström C, Kjelleberg S, Steinberg PD, Taylor MW (2007) Comparisons of diversity of bacterial communities associated with three sessile marine eukaryotes. Aquat Microb Ecol 48:217-229

Ludwig W, Rossello-Mora R, Aznar R, Klugbauer S, Spring S, Reetz K, Beimfohr C, Brockmann E, Kirchhof G, Dorn S, Bachleitner M, Klugbauer N, Springer N, Lane D, Weizenegger M, Schleifer KH (1995) Comparative sequence analysis of 23S rRNA from Proteobacteria. Syst Appl Microbiol 18:164-188

Lüning K (1990) Seaweeds, their environment, biogeography, and ecophysiology. John Wiley and Sons

Maier I, Parodi E, Westermeier R, Müller DG (2000) *Maullinia ectocarpii* gen. et sp. nov. (Plasmodiophorea), an intracellular parasite in *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae) and other filamentous brown algae. Protist 151:225-238

Maggs CA, Hommersand MH (1993) Seaweeds of the British Isles. Vol.1. Rhodophyta. Part 3a Ceramiales, 444 pp. The Natural History Museum, London

Makk J, Acs E, Marialigeti K, Kovacs G (2003) Investigations on the Danube gravel-biofilm diatom-associated bacterial communities. Biol, Bratislava 58:729-742

Martens T, Heidorn T, Pukall R, Simon M, Tindall BJ, Brinkhoff T (2006) Reclassification of *Roseobacter gallaeciensis* Ruiz-Ponte et al. 1998 as *Phaeobacter gallaeciensis* gen. nov., comb. nov., description of *Phaeobacter inhibens* sp. nov., reclassification of *Ruegeria algicola* (Lafay et al. 1995) Uchino et al. 1999 as *Marinovum algicola* gen. nov., comb. nov., and emended descriptions of the genera *Roseobacter*, *Ruegeria* and *Leisingera*. Int J Syst Evol Microbiol 56:1293-1304

Maschek JA, Baker BJ (2008) The chemistry of algal secondary metabolism. pp. 1-24. In: Amsler CD (ed.) (2008) Algal chemical ecology, 313 pp. Springer-Verlag Berlin Heidelberg

Mazure HGF, Field JG (1980) Density and ecological importance of bacteria on kelp fronds in an upwelling region. J Exp Mar Biol Ecol 43:173-182

Mc Cully ME (1966) Histological studies on the genus *Fucus.* I. Light microscopy of the mature vegetative plant. Protoplasma 62:287-305

Mc Cully ME (1968) Histological studies on the genus *Fucus.* II. Histology of the reproductive tissues. Protoplasma 66:205-230

Mearns-Spragg A, Bregu M, Boyd KG, Burgess JG (1998) Cross-species induction and enhancement of antimicrobial activity produced by epibiotic bacteria from marine algae and invertebrates, after exposure to terrestrial bacteria. Lett Appl Microbiol 27:142-146

Meusnier I, Olsen JL, Stam WT, Destombe C, Valero M (2001) Phylogenetic analyses of *Caulerpa taxifolia* (Chlorophyta) and of its associated bacterial microflora provides clues to the origin of the Mediterranean introduction. Mol Ecol 10:931-946

Michel G, Nyval-Collen P, Barbeyron T, Czjzek M, Helbert W (2006) Bioconversion of red seaweed galactans: a focus on bacterial agarases and carrageenases. Appl Microbiol Biotechnol 71:23-33

Miller IJ (1996) Alginate composition of some New Zealand brown seaweeds. Phytochemistry 41:1315-1317

Miller TR, Hnilicka K, Dziedzic A, Desplats P, Belas R (2004) Chemotaxis of *Silicibacter* sp. strain TM1040 toward dinoflagellate products. Appl Environ Microbiol 70:4692-4701

Miyashita M, Fujimura S, Nakagawa Y, Nishizawa M, Tomizuka N, Nakagawa T, Nakagawa J (2010) *Flavobacterium algicola* sp. nov., isolated from marine algae. Int J Syst Evol Microbiol 60:344-348

Molenaar FJ, Breeman AM (1997) Latitudinal trends in the growth and reproductive seasonality of *Delesseria sanguinea*, *Membranoptera alata*, and *Phycodrys rubens* (Rhodophyta). J Phycol 33:330-3343

Moran MA, Belas R, Schell MA, Gonzalez JM, Sun F, Sun S, Binder BJ, Edmonds J, Ye W, Orcutt B, Howard EC, Meile C, Palefsky W, Goesmann A, Ren Q, Paulsen I, Ulrich LE, Thompson LS, Saunders E, Buchan A (2007) Ecological genomics of marine Roseobacters. Appl Environ Microbiol 73:4559-4569

Moss BL (1950) Studies in the Genus *Fucus*. II. The anatomical structure and chemical composition of receptacles of *Fucus vesiculosus* from three contrasting habitats. Ann Bot 14:395-410

Mudryk ZJ, Skórczewski P (2006) Enzymatic activity and degradation of organic macromolecules by neustonic and planktonic bacteria in an estuarine lake. Pol J Ecol 54:3-14

Murray RE, Cooksey KE, Priscu JC (1986) Stimulation of bacterial DNA synthesis by algal exudates in attached algal-bacterial consortia. Appl Environ Microbiol 52:1177-1182

Muscholl-Silberhorn A, Thiel V, Imhoff JF (2008) Abundance and bioactivity of cultured sponge-associated bacteria from the Mediterranean Sea. Microb Ecol 55:94-106

Müller DG, Kapp M, Knippers R (1998) Viruses in marine brown algae. Adv Virus Res 50:49-67

Müller DG, Küpper FC, Küpper H (1999) Infection experiments reveal broad host ranges of *Eurychasma dicksonii* (Oomycota) and *Chytridium polysiphoniae* (Chytridiomycota), two eukaryotic parasites in marine brown algae (Phaeophyceae). Phycol Res 47:217-223

Namba A, Shigenobu Y, Kobayashi M, Kobayashi T, Oohara I (2010) A new primer for 16S rDNA analysis of microbial communities associated with *Porphyra yezoensis*. Fish Sci 76:873-878

Naylor M (1949) Observations on the anatomy of *Durvillaea antarctica* (Chamisso) Hariot. Ann Bot NS 13:285-308

Nalyor M (1953) The New Zealand species of *Durvillaea*. Trans R Soc NZ 80:277-297

Nedashkovskaya OI, Kim SB, Han SK, Lysenko AM, Rohde M, Zhukova NV, Falsen E, Frolova GM, Mikhailov VV, Bae KS (2003) *Mesonia algae* gen. nov., sp. nov., a novel marine bacterium of the family Flavobacteriaceae isolated from the green alga *Acrosiphonia sonderi* (Kutz) Kornm. Int J Syst Evol Microbiol 53:1967-1971

Nedashkovskaya OI, Kim SB, Han SK, Lysenko AM, Mikhailov VV, Bae KS (2004a) *Arenibacter certesii* sp. nov., a novel marine bacterium isolated from the green alga *Ulva fenestrata*. Int J Syst Evol Microbiol 54:1173-1176

Nedashkovskaya OI, Suzuki M, Vancanneyt M, Cleenwerck I, Lysenko AM, Mikhailov VV, Swings J (2004b) *Zobellia amurskyensis* sp. nov., *Zobellia laminariae* sp. nov. and *Zobellia russellii* sp. nov., novel marine bacteria of the family Flavobacteriaceae. Int J Syst Evol Microbiol 54:1643-1648

Nedashkovskaya OI, Kim SB, Han SK, Rhee MS, Lysenko AM, Falsen E, Frolova GM, Mikhailov VV, Bae KS (2004c) *Ulvibacter litoralis* gen. nov., sp. nov., a novel marine bacterium of the family Flavobacteriaceae isolated from the green alga *Ulva fenestrata*. Int J Syst Evol Microbiol 54:119-123

Nedashkovskaya OI, Kim SB, Han SK, Lysenko AM, Rohde M, Rhee MS, Frolova GM, Falsen E, Mikhailov VV, Bae KS (2004d) *Maribacter* gen. nov., a new member of the family Flavobacteriaceae, isolated from marine habitats, containing the species *Maribacter sedimenticola* sp. nov., *Maribacter aquivivus* sp. nov., *Maribacter orientalis* sp. nov. and *Maribacter ulvicola* sp. nov. Int J Syst Evol Microbiol 54:1017-1023

Nedashkovskaya OI, Kim SB, Han SK, Rhee M-S, Lysenko AM, Rohde M, Zhukova NV, Frolova GM, Mikhailov VV, Bae KS (2004e) *Algibacter lectus* gen. nov., sp. nov., a novel member of the family Flavobacteriaceae isolated from green algae. Int J Syst Evol Microbiol 54:1257-1261

Nedashkovskaya OI, Vancanneyt M, Van Trappen S, Vandemeulebroecke K, Lysenko AM, Rohde M, Falsen E, Frolova GM, Mikhailov VV, Swings J (2004f) Description of *Algoriphagus aquimarinus* sp. nov., *Algoriphagus chordae* sp. nov. and *Algoriphagus winogradskyi* sp. nov., from seawater and algae, transfer of *Hongiella halophila* Yi and Chun 2004 to the genus *Algoriphagus* as *Algoriphagus halophilus* comb. nov. and emended descriptions of the genera *Algoriphagus* Bowman et al. 2003 and *Hongiella* Yi and Chun 2004. Int J Syst Evol Microbiol 54:1757-1764

Nedashkovskaya OI, Kim SB, Lee KH, Bae KS, Frolova GM, Mikhailov VV, Kim IS (2005a) *Pibocella ponti* gen. nov., sp. nov., a novel marine bacterium of the family Flavobacteriaceae isolated from the green alga *Acrosiphonia sonderi*. Int J Syst Evol Microbiol 55:177-181

Nedashkovskaya OI, Kim SB, Lee DH, Lysenko AM, Shevchenko LS, Frolova GM, Mikhailov VV, Lee KH, Bae KS (2005b) *Roseivirga ehrenbergii* gen. nov., sp. nov., a novel marine bacterium of the phylum 'Bacteroidetes', isolated from the green alga *Ulva fenestrata*. Int J Syst Evol Microbiol 55:231-234

Nedashkovskaya OI, Kim SB, Han SK, Snauwaert C, Vancanneyt M, Swings J, Kim K-O, Lysenko AM, Rohde M, Frolova GM, Mikhailov VV, Bae KS (2005c) *Winogradskyella thalassocola* gen. nov., sp. nov., *Winogradskyella epiphytica* sp. nov. and *Winogradskyella eximia* sp. nov., marine bacteria of the family Flavobacteriaceae. Int J Syst Evol Microbiol 55:49-55

Nedashkovskaya OI, Kim SB, Vancanneyt M, Snauwaert C, Lysenko AM, Rohde M, Frolova GM, Zhukova NV, Mikhailov VV, Bae KS, Oh HW, Swings J (2006a) *Formosa agariphila* sp. nov., a budding bacterium of the family Flavobacteriaceae isolated from marine environments, and emended description of the genus *Formosa*. Int J Syst Evol Microbiol 56:161-167

Nedashkovskaya OI, Vancanneyt M, Cleenwerck I, Snauwaert C, Kim SB, Lysenko AM, Shevchenko LS, Lee KH, Park MS, Frolova GM, Mikhailov VV, Bae KS, Swings J (2006b) *Arenibacter palladensis* sp. nov., a novel marine bacterium isolated from the green alga *Ulva fenestrata*, and emended description of the genus *Arenibacter*. Int J Syst Evol Microbiol 56:155-160

Nedashkovskaya OI, Vancanneyt M, De Vos P, Kim SB, Lee MS, Mikhailov VV (2007) *Maribacter polysiphoniae* sp. nov. isolated from a red alga. Int J Syst Evol Microbiol 57:2840-2843

Nedashkovskaya OI, Suzuki M, Kim SB, Mikhailov VV (2008a) *Kriegella aquimaris* gen. nov., sp. nov., isolated from marine environments. Int J Syst Evol Microbiol 58:2624-2628

Nedashkovskaya OI, Kwon KK, Yang SH, Lee HS, Chung KH, Kim SJ (2008b) *Lacinutrix algicola* sp. nov. and *Lacinutrix mariniflava* sp. nov., two novel marine alga-associated bacteria and emended description of the genus *Lacinutrix*. Int J Syst Evol Microbiol 58:2694-2698

Nedashkovskaya OI, Suzuki M, Lee J-S, Lee KC, Shevchenko LS, Mikhailov VV (2009) *Pseudozobellia thermophila* gen. nov., sp. nov., a bacterium of the family Flavobacteriaceae, isolated from the green alga *Ulva fenestrata*. Int J Syst Evol Microbiol 59:806-810

Nedashkovskaya OI, Kim SB, Mikhailov VV (2010) *Maribacter stanieri* sp. nov., a marine bacterium of the family Flavobacteriaceae. Int J Syst Evol Microbiol 60:214-218

Nedashkovskaya OI, Kukhlevskiy AD, Zhukova NV (pub ahead) *Winogradskyella ulvae* sp. nov., a novel epiphyte of a Pacific seaweed and emended descriptions of the genus *Winogradskyella* and *Winogradskyella thalassocola*, *Winogradskyella echinorum*, *Winogradskyella exilis* and *Winogradskyella eximia*. Int J Syst Evol Microbiol (pub ahead) doi:10.1099/ijs.0.032219-0

Nelson WA, Knight GA (1996) Life history in culture of the obligate epiphyte *Porphyra subtumens* (Bangiales, Rhodophyta) endemic to New Zealand. Phycol Res 44:19-25

Neuhauser S, Bulman S, Kirchmair M (2010) Plasmodiophoroids: The challenge to understand soil-borne, obligate biotrophs with a multiphasic life cycle. Pp. 51-78. In: Gherbary Y, Voigt K (2010) Molecular identification of fungi. Springer-Verlag Berlin Heidelberg

Neuhauser S, Kirchmair M, Gleason FH (2011a) Ecological roles of the parasitic phytomyxids (plasmodiophorids) in marine ecosystems – A review. Mar Freshwater Res 62:365-371

Neuhauser S, Kirchmair M, Gleason FH (2011b) The ecological potentials of Phytomyxea (‘plasmodiophoroids’) in aquatic food webs. Hydrobiologia 659:23-35

Nishijima M, Takadera T, Imamura N, Kasai H, An KD, Adachi K, Nagao T, Sano H, Yamasato K (2009) *Microbulbifer variabilis* sp. nov. and *Microbulbifer epialgicus* sp. nov., isolated from Pacific marine algae, possess a rod-coccus cell cycle in association with the growth phase. Int J Syst Evol Microbiol 59:1696-1707

Nylund GM, Pavia H (2005) Chemical versus mechanical inhibition of fouling in the red alga *Dilsea carnosa*. Mar Ecol Prog Ser 299:111-121

Nylund GM, Cervin G, Hermansson M, Pavia H (2005) Chemical inhibition of bacterial colonization by the red alga *Bonnemaisonia hamifera*. Mar Ecol Prog Ser 302:27-36

Nylund GM, Persson F, Lindegarth M, Cervin G, Hermansson M, Pavia H (2010) The red alga *Bonnemaisonia asparagoides* regulates epiphytic bacterial abundance and community composition by chemical defence. FEMS Microbiol Ecol 71:84-93

Ortiz J, Romero N, Robert P, Araya J, Lopez-Hernandez L, Bozzo C, Navarrete E, Osorio A, Rios A (2006) Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. Food Chem 99:98-104

Palacios L, Arahal DR, Reguera B, Marín I (2006) *Hoeflea Alessandri* sp. nov., isolated from the toxic dinoflagellate *Alexandrium minutum* AL1V. Int J Syst Evol Microbiol 56:1991-1995

Pankow H (1971) Algenflora der Ostsee. 1. Benthos. VEB Gustav Fischer, Jena. 419 pp.

Park CS, Kakinuma M, Amano H (2006) Forecasting infections of the red rot disease on *Porphyra yezoensis* Ueda (Rhodophyta) cultivation farms. J Appl Phycol 18:295-299

Park CS, Park KY, Baek JM, Hwang EK (2008) The occurrence of pinhole disease in relation to developmental stage in cultivated *Undaria pinnatifida* (Harvey) Suringar (Phaeophyta) in Korea. J Appl Phycol 20:485-490

Park SC, Baik KS, Kim MS, Kim SS, Kim SR, Oh M-J, Kim D, Bang B-H, Seong CN (2009) *Aequorivita capsosiphonis* sp. nov., isolated from the green alga *Capsosiphon fulvescens*, and emended description of the genus *Aequorivita*. Int J Syst Evol Microbiol 59:724-728

Paul NA, de Nys R, Steinberg PD (2006) Chemical defence against bacteria in the red alga *Asparagopsis armata*: Linking structure with function. Mar Ecol Prog Ser 306:87-101

Paul VJ, Puglisi MP (2004) Chemical mediation of interactions among marine organisms. Nat Prod Rep 21:189-209

Pearson GA, Serrao EA (2006) Revisiting synchronous gamete release by fucoid algae in the intertidal zone: Fertilization success and beyond? Integr Comp Biol 46:587-597

Penesyan A, Marshall-Jones Z, Holmström C, Kjelleberg S, Egan S (2009) Antimicrobial activity observed among cultured epiphytic bacteria reflects their potential as source of new drugs. FEMS Microbiol Ecol 69:113-124

Penesyan A, Kjelleberg S, Egan S (2010) Development of novel drugs from marine surface associated microorganisms. Mar Drugs 8:438-459

Pereira RC, da Gama BAP (2008) Macroalgal chemical defenses and their roles in structuring tropical marine communities. Pp.25-55. In: Amsler CD (ed.) (2008) Algal chemical ecology, Springer-Verlag Berlin Heidelberg, 313 pp.

Perriere G, Gouy M (1996) WWW-query: an on-line retrieval system for biological sequence banks. Biochimie 78:364-369

Persson F, Svensson R, Nylund GM, Fredriksson NJ, Pavia H, Hermansson M (2011) Ecological role of a secondary metabolite for a colonizing bacterial community. Biofouling 27:579-588

Peters AF (1991) Taxonomic implications of gamete fusions in the parasitic brown alga *Herpodiscus durvillaeae*. Can J Bot 68:1398-1401

Peterson DM, Torrey JG (1968) Amino acid incorporation in developing *Fucus* embryos. Plant Physiol 43:941-947

Pinhassi J, Berman T (2003) Differential growth response of colony-forming Alpha- and Gamma-Proteobacteria in dilution culture and nutrient addition experiments from Lake Kinneret (Israel), the Eastern Mediterranean Sea, and the Gulf of Eilat. Appl Environ Microbiol 69:199-211

Polne-Fuller M, Gibor A (1987) Microorganisms as digestors of seaweed cell wall. Hydrobiologia 151/152:405-409

Potin P, Patier P, Floc'h JY, Yvin JC, Rochas C, Kloareg B (1992) Chemical characterization of cell-wall polysaccharides from tank-cultivated and wild platns of *Delesseria sanguinea* (Hudson) Lamouroux (Ceramilaes, Delesseriaceae): Culture patterns and potent anticoagulant activity. J Appl Phycol 4:119-128

Potter MC (1889) On the structure of the thallus of *Delesseria sanguinea* Lamour. Jour Mar Biol Assoc UK 1:171-172

Prokic I, Brümmer F, Brigge T, Görtz HD, Gerdts G, Schutt C, Elbrächterd M, Müller WEG (1998) Bacteria of the genus *Roseobacter* associated with the toxic dinoflagellate *Prorocentrum lima*. Protist 149:347-357

Puglisi MP, Engel S, Jensen PR, Fenical W (2007) Antimicrobial activities of extracts from Indo-Pacific marine plants against marine pathogens and saprophytes. Mar Biol 150:531-540

Qu Z, Li Z, Zhang X, Zhang XH (pub ahead) *Salinicoccus qingdaonensis* sp. nov., a novel bacterium isolated from coastal seawater of Qingdao during a bloom of green algae. Int J Syst Evol Microbiol (pub ahead) doi:10.1099/ijs.0.030551-0

Raghukumar S (2002) Ecology of the marine protests, the Labyrinthulomycetes (Thraustochytrids and Labyrinthulids). Eur J Protistol 38:127-145

Ramaiah N (2006) A review on fungal diseases of algae, marine fishes, shrimps and corals. Indian J Mar Sci 35:380-387

Ramírez ME, Santelices B (1991) Catálogo de algas marinas bentónicas de la costa temperada del Pacífico de Sudamérica. Facultad de Ciencias Biológicas, Universidad Católica de Chile. Monografías Biológicas 5

Rao D, Webb JS, Kjelleberg S (2005) Competitive interactions in mixed-species biofilms containing the marine bacterium *Pseudoalteromonas tunicata*. Appl Environ Microbiol 71:1729-1736

Rao D, Webb JS, Kjelleberg S (2006) Microbial colonization and competition on the marine alga *Ulva australis*. Appl Environ Microbiol 72:5547-5555

Rao D, Webb JS, Holmström C, Case R, Low A, Steinberg P, Kjelleberg S (2007) Low densities of epiphytic bacteria from the marine alga *Ulva australis* inhibits settlement of fouling organisms. Appl Environ Microbiol 73:7844-7852

Rasconi S, Jobard M, Sime-Ngando T (2011) Parasitic fungi of phytoplankton: Ecological roles and implications for microbial food webs. Fungal Biol Rev 22:17-25

Riemann L, Steward GF, Azam F (2000) Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. Appl Environ Microbiol 66:578-587

Rindi F, Guiry MD (2004) Composition and spatio temporal variability of the epiphytic macroalgal assemblage of *Fucus vesiculosus* Linnaeus at Clare Island, Mayo, western Ireland. J Exp Mar Biol Ecol 311:233-252

Roberts M (1979) Observations on cell structure and cytology in the genus *Durvillaea* Bory. NZ J Bot 17:241-249

Rogerson A (1991) On the abundance of marine naked amoeba on the surfaces of five species of macroalgae. FEMS Microb Ecol 85:301-312

Romanenko LA, Zhukova NV, Lysenko AM, Mikhailov VV, Stackebrandt E (2003) Assignment of "*Alteromonas marinoglutinosa*" NCIMB 1770 to *Pseudoalteromonas mariniglutinosa* sp. nov., nom. rev., comb. nov. Int J Syst Evol Microbiol 53:1105-1109

Romano I, Lama L, Nicolaus B, Poli A, Gambacorta A, Giordano A (2006) *Oceanobacillus oncorhynchi* subsp. *incaldanensis* subsp. nov., an alkalitolerant halophile isolated from an algal mat collected from a sulfurous spring in Campania (Italy), and emended description of *Oceanobacillus oncorhynchi*. Int J Syst Evol Microbiol 56:805-810

Roos H (1957) Untersuchungen über das Vorkommen antimikrobieller Substancen in Meeresalgen. Kieler Meeresforschung 13:41-58

Rueness J (1977) Norsk algeflora. Universitetsforlaget. Oslo, Bergen, Tromsø. 266 pp.

Saha M, Rempt M, Grosser K, Pohnert G, Weinberger F (2011) Surface-associated fucoxanthin mediates settlement of bacterial epiphytes on the rockweed *Fucus vesiculosus*. Biofouling 27:423-433

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406-425

Sakai T, Kimura H, Kato I (2002) A marine strain of Flavobacteriaceae utilizes brown seaweed fucoidan. Mar Biotechnol 4:399-405

Sánchez-Saavedra MP, Licea-Navarro A & Bernáldez-Sarabia J (2010) Evaluation of the antibacterial activity of different species of phytoplankton. Rev Biol Mar Oceanogr 45:531-536

Sandsdalen E, Haug T, Stensvag K, Styrvold OB (2003) The antibacterial effect of a polyhydroxylated fucophlorethol from the marine brown alga, *Fucus vesiculosus*. World J Microbiol Biotechnol 19:777-782

Santelices B, Castilla JC, Cancino J, Schmiede P (1980) Comparative ecology of *Lessonia nigrescens* and *Durvillaea antarctica* (Phaeophyta) in Central Chile. Mar Biol 59:119-132

Sawabe T, Makino H, Tatsumi M, Nakano K, Tajima K, Iqbal MM, Yumoto I, Ezura Y, Christen R (1998) *Pseudoalteromonas bacteriolytica* sp. nov., a marine bacterium that is the causative agent of red spot disease of *Laminaria japonica*. Int J Syst Bacteriol 48:769-774

Sawabe T, Tanaka R, Iqbal MM, Tajima K, Ezura Y, Ivanova EP, Christen R (2000) Assignment of *Alteromonas elyakovii* KMM162^T and five strains isolated from spot wounded fronds of *Laminaria japonica* to *Pseudoalteromonas elyakovii* comb. nov. and the extended description of the species. Int J Syst Bacteriol 50:265-271

Schäfer H, Abbas B, Witte H, Muyzer G (2002) Genetic diversity of “satellite” bacteria present in cultures of marine diatoms. FEMS Microbiology Ecology 42:25-35

Schiel DR, Nelson WA (1990) The harvesting of macroalgae in New Zealand. Hydrobiologia 204/205:25-33

Schneemann I, Kajahn I, Ohlendorf B, Zinecker H, Erhard A, Nagel K, Wiese J, Imhoff JF (2010a) Mayamycin, a cytotoxic polyketide from a *Streptomyces* strain isolated from the marine sponge *Halichondria panicea*. J Nat Prod 73:1309-1312

Schneemann I, Nagel K, Kajahn I, Labes A, Wiese J, Imhoff JF (2010b) Comprehensive investigation of marine *Actinobacteria* associated with the sponge *Halichondria panicea*. Appl Environ Microbiol 76:3702-3714

Schnepf E, Kühn SF, Bulman S (2000) *Phagomyxa bellerocheae* sp. nov. and *Phagomyxa odontellae* sp. nov., Plasmodiophoromycetes feeding on marine diatoms. Helgoland Mar Res 54:237-242

Schomburg I, Chang A, Ebeling C, Gremse M, Heldt C, Huhn C, Schomburg D (2004) BRENDA, the enzyme database: Updates and major new developments. Nucleic Acids Res 32:431-433

Sekimoto S, Yokoo K, Kawamura Y, Honda D (2008) Taxonomy, molecular phylogeny, and ultrastructural morphology of *Olpidiopsis porphyrae* sp. nov. (Oomycetes, straminipiles), a unicellular obligate endoparasite of *Bangia* and *Porphyra* spp. (Bangiales, Rhodophyta). Mycol Res 112:361-374

Semenova EV, Shlykova DS, Semenov AM, Ivanov MN, Shelyakov OV, Netrusov AI (2009) Bacteria epiphytes of brown macro alga in oil utilization in North Sea ecosystems. Moscow Univ Biol Sci Bull 64:107-110

SERNAGEOMIN (2003) Mapa geológico de Chile: Versión digital. Servicio Nacional de Geología y Minería. Publicación geológica digital n°4. CD-ROM Version 1.0, 2003, Santiago de Chile

Shiba TO, Taga N (1980) Heterotrophic bacteria attached to seaweeds. J Exp Mar Biol Ecol 47:251-258

Shiba T, Simidu U (1982) *Erythrobacter longus* gen. nov., sp. nov., an aerobic bacterium which contains bacteriochlorophyll a. Int J Syst Bacteriol 32:211-217

Simidu U, Kita-Tsukamoto K, Yasumoto T, Yotsu M (1990) Taxonomy of four marine bacterial strains that produce tetrodotoxin. Int J Syst Bacteriol 40:331-336

Simonato F, Gomez-Pereira PR, Fuchs BM, Amann R (2010) Bacterioplankton diversity and community composition in the Southern Lagoon of Venice. Syst Appl Microbiol 33:128-138

Skovhus TL, Holmström C, Kjelleberg S, Dahllöf I (2007) Molecular investigation of the distribution, abundance and diversity of the genus *Pseudoalteromonas* in marine samples. FEMS Microbiol Ecol 61:348-361

Sneed JM, Pohnert G (2011a) The green alga *Dictyosphaeria ocellata* and its organic extracts alter natural bacterial biofilms communities. Biofouling 27:347-356

Sneed JM, Pohnert G (2011b) The green macroalga *Dictyosphaeria ocellata* influences the structure of the bacterioplankton community through differential effects on individual bacterial phylotypes. FEMS Microbiol Ecol 75:242-254

Sohn JH, Lee JH, Yi H, Chun J, Bae KS, Ahn TY, Kim SJ (2004) *Kordia algicida* gen. nov., sp. nov., an algicidal bacterium isolated from red tide. Int J Syst Evol Microbiol 54:675-680

South GR (1974) *Herpodiscus* gen. nov. and *Herpodiscus durvilleae* (Lindauer) comb. nov., a parasite of *Durvillea antarctica* (Chamisso) Hariot, endemic to New Zealand. J Roy Soc New Zeal 4:455-461

Sparrow FK (1960) Aquatic phycomycetes. The University of Michigan Press, 1187 pp.

Sridhar KR, Vidyavathi N (1991) Antimicrobial activity of seaweeds. Acta Hydroch Hydrob 5:455-496

Staufenberger T, Thiel V, Wiese J, Imhoff JF (2008) Phylogenetic analysis of bacteria associated with *Laminaria saccharina*. FEMS Microb Ecol 64:65-77

Steinberg PD, de Nys R (2002) Chemical mediation of colonization of seaweeds surfaces. J Phycol 38:621-629

Steinberg PD, Schneider R, Kjelleberg S (1997) Chemical defenses of seaweeds against microbial colonization. Biodegradation 8:211-220

Stevens CL, Hurd CL, Smith M, Harder DL (2001) *Durvillaea antarctica*: the strongest kelp in the world? NWA Water Atmosphere 9:8-9

Strömpl C, Hold GN, Lunsdorf H, Graham J, Gallacher S, Abraham W-R, Moore ERB, Timmis KN (2003) *Oceanicaulis alexandrii* gen. nov., sp. nov., a novel stalked bacterium isolated from a culture of the dinoflagellate *Alexandrium tamarense* (Lebour) Balech. Int J Syst Evol Microbiol 53:1901-1906

Suzuki M, Nakagawa Y, Harayama S, Yamamoto S (2001) Phylogenetic analysis and taxonomic study of marine *Cytophaga*-like bacteria: proposal for *Tenacibaculum* gen. nov. with *Tenacibaculum maritimum* comb. nov. and *Tenacibaculum ovolyticum* comb. nov., and description of *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amylolyticum* sp. nov. Int J Syst Evol Microbiol 51:1639-1652

Suzuki T, Muroga Y, Takahama M, Nishimura Y (2000) *Roseibium denhamense* gen. nov., sp. nov. and *Roseibium hemelinense* sp. nov., aerobic bacteriochlorophyll-containing bacteria isolated from the east and west coasts of Australia. Int J Syst Evol Microbiol 50:2151-2156

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731-2739

Taylor ID, Schiel DR (2005) Self-replacement and community modification by the southern bull kelp *Durvillaea antarctica*. Mar Ecol Prog Ser 288:87-102

Tewari JP, Baines P (1983) Fungi associated with the roots of clover in Alberta. I. *Olpidium brassicae* and *Ligniera* sp. Can Plant Dis Surv 63:35-37

Thabard M, Gros O, Hellio C, Maréchal JP (2011) *Sargassum polyceratium* (Phaeophyceae, Fucaceae) surface molecule activity towards fouling organisms and embryogenic development of benthic species. Bot Mar 54:147-157

Thiel V (2006) Sponge-associated bacteria: Specificity, diversity and antimicrobial potential. Ph.D. Thesis, IFM-GEOMAR, University of Kiel, Germany. 106 pp.

Thomas D (2002) Seaweeds. The Natural History Museum, London. 96 pp.

Thomas D, Beltran J, Flores V, Contreras L, Bollmann E, Correa JA (2009) *Laminariocolax* sp. (Phaeophyceae) associated with gall developments in *Lessonia nigrescens* (Phaeophyceae). J Phycol 45:1252-1258

Thomas F, Hehemann J-H, Rebuffet E, Czjzek M, Michel G (2011) Environmental and gut Bacteroidetes: The food connection. Front Microbiol 2:93

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24:4876-4882

Tujula NA, Crocetti GR, Burke C, Thomas T, Holmström C, Kjelleberg S (2010) Variability and abundance of the epiphytic bacterial community associated with a green marine Ulvacean alga. ISME J 4:301-311

Ue H, Matsuo Y, Kasai H, Yokota A (2011) *Demequina globuliformis* sp. nov., *Demequina oxidasica* sp. nov. and *Demequina aurantiaca* sp. nov., actinobacteria isolated from marine environments, and proposal of Demequinaceae fam. nov. Int J Syst Evol Microbiol 61:1322-1329

Vairappan CS, Anangdan SP, Tan KL, Matsunaga S (2010) Role of secondary metabolites as defense chemicals against ice-ice disease bacteria in biofouler at carrageenophyte farms. J Appl Phycol 22:305-311

Vásquez JA, Buschmann AH (1997) Herbivore-kelp interactions in Chilean subtidal communities: a review. Rev Chil Hist Nat 70:41-52

Vásquez JA (2008) Production, use and fate of Chilean brown seaweeds: Re-sources for a sustainable fishery. J Appl Phycol 20:457-467

Villarreal-Gómez LJ, Soria-Mercado IE, Guerra-Rivas G, Ayala-Sánchez NE (2010) Antibacterial and anticancer activity of seaweeds and bacteria associated with their surface. Rev Biol Mar Oceanogr 45:267-275

Wada S, Aoki MN, Tsuchida Y, Sato T, Shinagawa H, Hama T (2007) Quantitative and qualitative analyses of dissolved organic matter released from *Ecklonia cava* Kjellman, in Oura bay, Shimoda, Izu Peninsula, Japan. J Exp Mar Biol Ecol 349:344-358

Wagner-Dobler I, Biebl H (2006) Environmental biology of the marine *Roseobacter* lineage. Ann Rev Microbiol 60:255-280

Wagner-Dobler I, Ballhausen B, Berger M, Brinkhoff T, Buchholz I, Bunk B, Cypionka H, Daniel R, Drepper T, Gerdts G, Hahnke S, Han C, Jahn D, Kalhoefer D, Kiss H, Klenk H-P, Kyrpides N, Liebl W, Liesegang H, Meincke L, Pati A, Petersen J, Piekarski T, Pommerenke C, Pradella S, Pukall R, Rabus R, Stackebrandt E, Thole S, Thompson L, Tielen P, Tomasch J, von Jan M, Wanphrut N, Wichels A, Zech H, Simon M (2010) The complete genome sequence of the algal symbiont *Dinoroseobacter shibae*: A hitchhiker's guide to life in the sea. ISME J 4:61-77

Wahl M (1989) Marine epibiosis I. Fouling and antifouling: some basic aspects. Mar Ecol Prog Ser 58:175-189

Wahl M (2008) Ecological lever and interface ecology: Epibiosis modulates the interactions between host and environment. Biofouling 24:427-438

Wahl M, Shahnaz L, Dobretsov S, Saha M, Symanowski F, David K, Lachnit T, Vasel M, Weinberger F (2010) Ecology of antifouling resistance in the bladder wrack *Fucus vesiculosus*: patterns of microfouling and antimicrobial protection. Mar Ecol Prog Ser 411:33-48

Wang G, Shuai L, Li Y, Lin W, Zhao X, Duan D (2008) Phylogenetic analysis of epiphytic marine bacteria on hole-rotten diseased sporophytes of *Laminaria japonica*. J Appl Phycol 20:403-409

Wang H, Zhang X, Yan S, Qi Z, Yu Y, Zhang XH (pub ahead) *Huaishuia halophila* gen. nov. sp. nov., a novel bacterium isolated from Yellow Sea coastal water in Qingdao, China. Int J Syst Evol Microbiol (pub ahead) doi:10.1099/ijs.0.025536-0

Wang Z, Xiao T, Pang S, Liu M, Yue H (2009) Isolation and identification of bacteria associated with the surfaces of several algal species. Chin J Oceanol Limnol 27:487-492

Weinberger F, Hoppe HG, Friedlander M (1997) Bacterial induction and inhibition of a fast necrotic response in *Gracilaria conferta* (Rhodophyta). J Appl Phycol 9:277-285

Weinberger F (2007) Pathogen-induced defense and innate immunity in macroalgae. Biol Bull 213:290-302

Westermeier R, Müller DG, Gomez I, Rivera P, Wenzel H (1994) Population biology of *Durvillaea antarctica* and *Lessonia nigrescens* (Phaeophyta) on the rocky shores of southern Chile. Mar Ecol Prog Ser 110:187-194

Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: The unseen majority. Proc Natl Acad Sci USA 95:6578-6583

Wiese J, Thiel V, Gärtner A, Schmaljohann R, Imhoff JF (2009a) *Kiloniella laminariae* gen. nov., sp. nov., a new alphaproteobacterium from the marine macroalga *Laminaria saccharina*. Int J Syst Evol Microbiol 59:350-356

Wiese J, Thiel V, Nagel K, Staufenberger T, Imhoff JF (2009b) Diversity of antibiotic active bacteria associated with the brown alga *Laminaria saccharina* from the Baltic Sea. Mar Biotechnol 11:287-300

Wilson S, Raftos DA, Corrigan SL, Nair SV (2010) Diversity and antimicrobial activities of surface-attached marine bacteria from Sydney Harbour, Australia. Microbiol Res 165:300-311

Womersley HBS (1984) The marine benthic flora of Southern Australia. Part I. Government Printer, South Australia

Wong TY, Preston LA, Schiller NL (2000) Alginate lyase: Review of major sources and enzyme characteristics, structure-function analysis, biological roles, and applications. Ann Rev Microbiol 54:289-340

Woollacott RM (1981) Association of bacteria with bryozoan larvae. Mar Biol 65:155-158

Yoon J, Matsuo Y, Adachi K, Nozawa M, Matsuda S, Kasai H, Yokota A (2008a) Description of *Persicirhabdus sediminis* gen. nov., sp. nov., *Roseibacillus ishigakijimensis* gen. nov., sp. nov., *Roseibacillus ponti* sp. nov., *Roseibacillus persicicus* sp. nov., *Luteolibacter pohnpeiensis* gen. nov., sp. nov. and *Luteolibacter algae* sp. nov., six marine members of the phylum 'Verrucomicrobia', and emended descriptions of the class Verrucomicrobiae, the order Verrucomicrobiales and the family Verrucomicrobiaceae. Int J Syst Evol Microbiol 58:998-1007

Yoon J, Matsuo Y, Katsuta A, Jang JH, Matsuda S, Adachi K, Kasai H, Yokota A (2008b) *Haloferula rosea* gen. nov., sp. nov., *Haloferula harenae* sp. nov., *Haloferula phyci* sp. nov., *Haloferula helveola* sp. nov. and *Haloferula sargassicola* sp. nov., five marine representatives of the family Verrucomicrobiaceae within the phylum 'Verrucomicrobia'. Int J Syst Evol Microbiol 58:2491-2500

Zhang GI, Hwang CY, Kang S-H, Cho BC (2009) *Maribacter antarcticus* sp. nov., a psychrophilic bacterium isolated from a culture of the Antarctic green alga *Pyramimonas gelidicola*. Int J Syst Evol Microbiol 59:1455-1459

Zheng L, Han X, Chen H, Lin W, Yan X (2005) Marine bacteria associated with marine macroorganisms: the potential antimicrobial resources. Ann Microbiol 55:119-124

ZoBell CE (1941) Studies on Marine Bacteria. I. The cultural requirements of heterotrophic aerobes. J Mar Res 4:42-75

12 LIST OF ABBREVIATIONS

Acc.-Nr.	Accession-Nummer
A.b.	*Algicola bacteriolytica*
blast	Basic Local Alignment Search Tool
B.a.	*Bacillus algicola*
bp	Base pair
B.s.	*Bacillus subtilis*
ca.	circa
CaCO3	Calcium carbonate
C.g.	*Candida glabrata*
cm2	Square centimetre
DGGE	Denaturing gradient gel electrophoresis
DNA	Desoxyribonucleic acid = Desoxyribonukleinsäure
dNTP	Desoxyribonukleosidtriphosphat
DOC	Dissolved organic carbon
DC	*Delesseria* Cauloid
DP	*Delesseria* Phylloid
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
E	East
E.a.	*Erwinia amylovora*
E.c.	*Escherichia coli*
EMBL	European Molecular Biology Laboratory
et al.	(‘*et alia*’) and colaborators
EtOAc	Ethyl acetate
F.a.	*Formosa algae*
Fig.	Figure
FISH	Fluorescence *in situ* hybridization
FC	*Fucus* Cauloid
FP	*Fucus* Phylloid
g	Gram
°C	Grad Celsius
GYM	Glucose Yeast Malt
h	hour = Stunde
H2O	Water
HCL	Hydrogen chloride
HPLC	high performance liquid chromatography
i.e.	(’*Id est*’) = this is
KiWiZ	Kieler Wirkstoff-Zentrum
km	Kilometer
MeOH	Methanol
mg	Milligram
µg	Mikrogram
MgSO4	Magnesium sulfate
min	Minute(n)
l	Liter
ml	Milliliter
µl	Mikroliter
mm	Millimeter
MTP	Microtiter plate
N	North
NaCl	Sodium chloride
nblast	nucleotide Basic Local Alignment Search Tool
NCBI	National Center for Biotechnology Information
nm	Nanometer

OD600	Optical Density by 600 nm
og	oogonium
op	conceptacles
P.l.	*Paenibacillus lautus*
PCR	Polymerase chain reaction = Polymerasekettenreaktion
P.e.	*Pseudoalteromonas elyakovii*
P.fl.	*Pseudomonas fluorescens*
P.m.	*Pseudomonas marincola*
rc	receptacles
RFLP	restriction fragment length polymorphism
rpm	rounds per minute = Umdrehung pro Minute
rDNA	ribosomal desoxyribonucleic acid = ribosomale Desoxyribonukleinsäure
rRNA	ribosomal ribonucleic acid = ribosomale Ribonukleinsäure
s	Seconds
S	South
S1	Summer 2008
S2	Summer 2009
SEM	Scanning electron microscopy = Rasterelektron Mikroskopie
S.l.	*Staphylococcus lentus*
sp.	Species
t°	Temperature
tRNA	transfer Ribonukleinsäure
TSB	tryptic soy broth
UV	Ultraviolett
V	Volt
W	West
W1	Winter 2009
W2	Winter 2010
%	percentage

13 ACKNOWLEDGMENTS

First I would like to thank the DAAD (German Academical Exchange Service) and the CONICYT (Comité Nacional de Ciencia y Tecnología, Chile) who financed my PhD. Especially I would like to thank Profesor Imhoff for giving me this excellent opportunity to write my PhD thesis at his lab. It has been a dream. I am immensely grateful to the entire PhD group at Marine Mikrobiology (IFM-GEOMAR) and especially to Herwig Heindl and Tim Staufenberger for their constant help in methodology and field work (and friendship!). To Dr. Rolf Schmaljohann and Arlette Erhard for the kind help in SEM and bioassay methodology, respectivelly. To Kerstin, Martin, Andrea, Nils, Johanna, Katrin, Anne… and to the entire Kiwiz, the best lab I have ever been, full with friends and knowledge!! Everyday eating together at 12:00 and making possible to have some laughs even in totally-stress-work-days! Especially I am deepely thankful to have had two outstanding supervisors Dr. Jutta Wiese and Dr. Antje Labes!! Their help and advises have make possible to finish my PhD on time!!

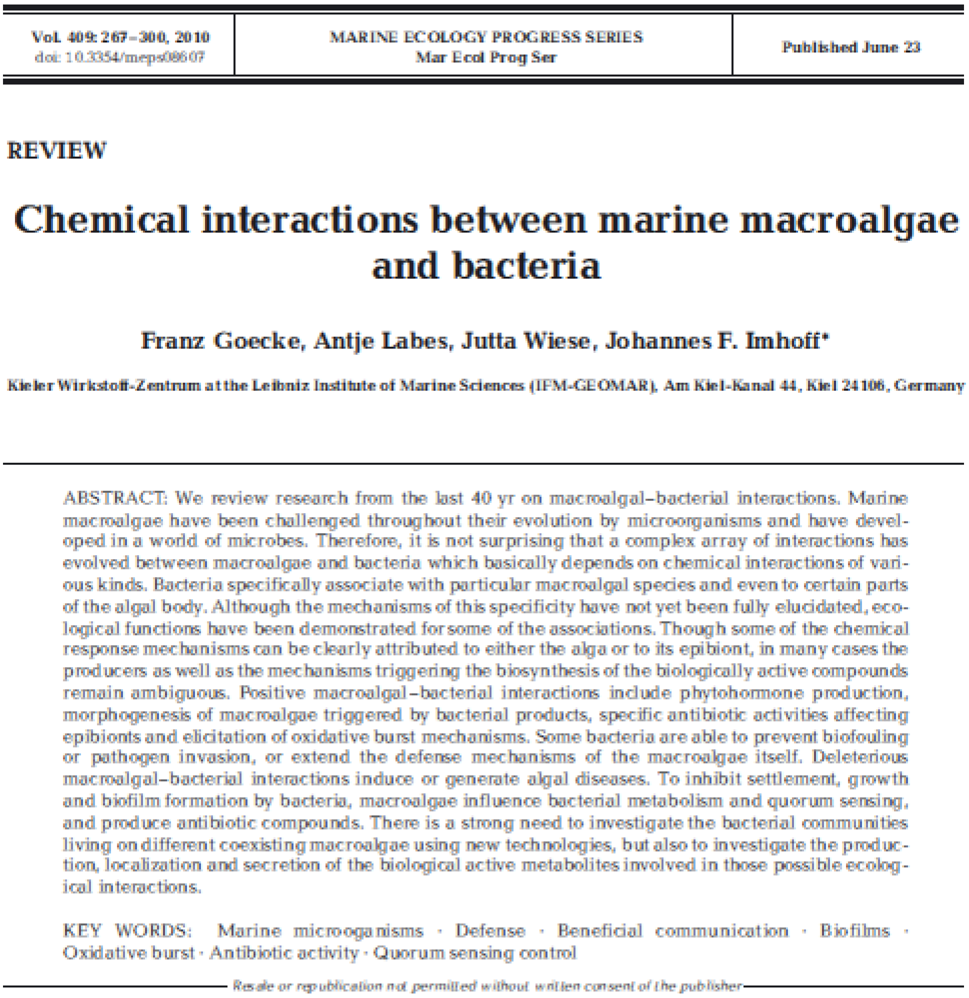
The support and love of Tonje and my family have been my strength through these 3.5 years, without it, it would have been impossible for me to be here in the first place. Thank you!

14 DECLARATION

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbständig und ohne unerlaubte Hilfe angefertigt habe und dass sie nach Form und Inhalt meine eigene Arbeit ist. Sie wurde keiner anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt und entstand unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft. Dies ist mein einziges und bisher erstes Promotionsverfahren. Die Promotion soll im Fach Mikrobiologie erfolgen. Des Weiteren erkläre ich, dass ich Zuhörer bei der Disputation zulasse.

(Franz Goecke)

15 SUPPORTING INFORMATION



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REVIEW

Chemical interactions between marine macroalgae and bacteria

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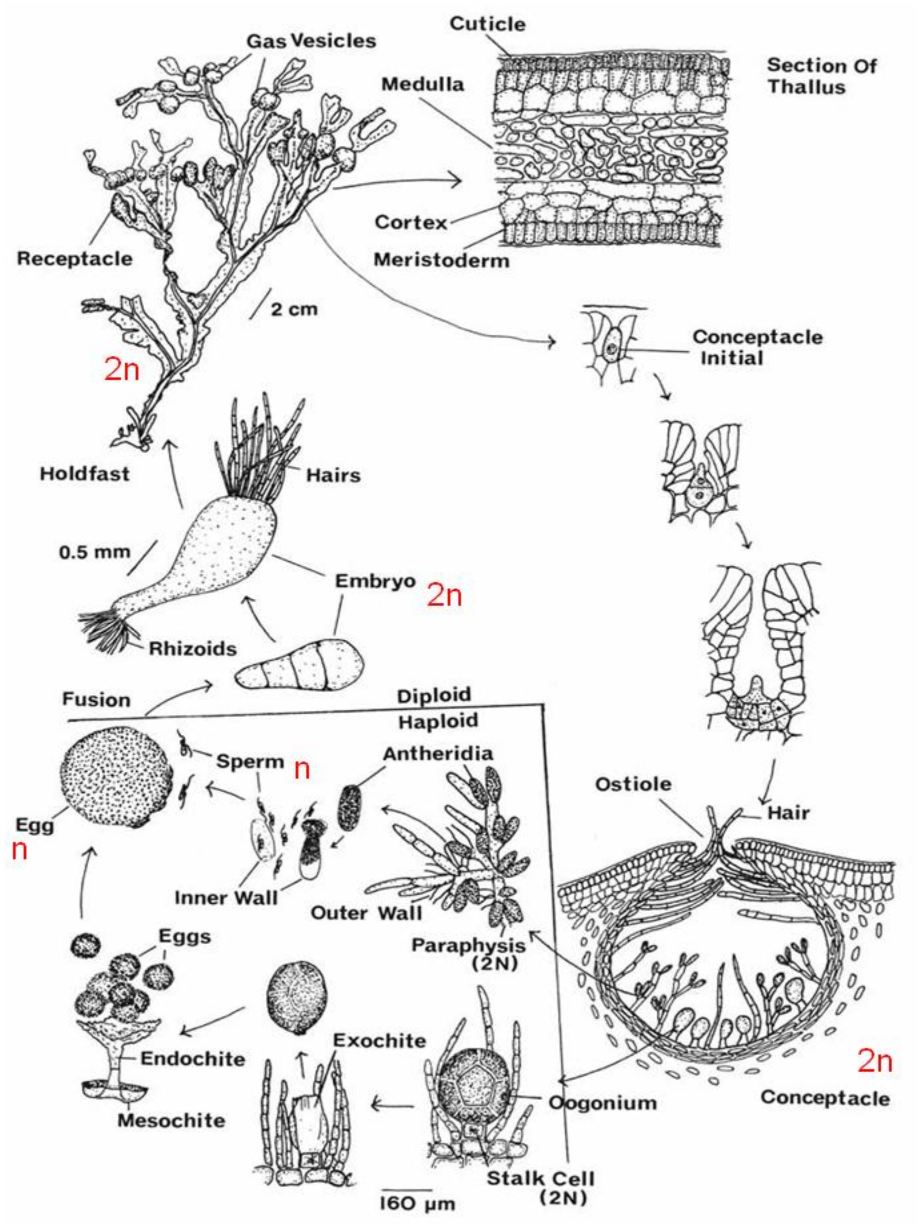
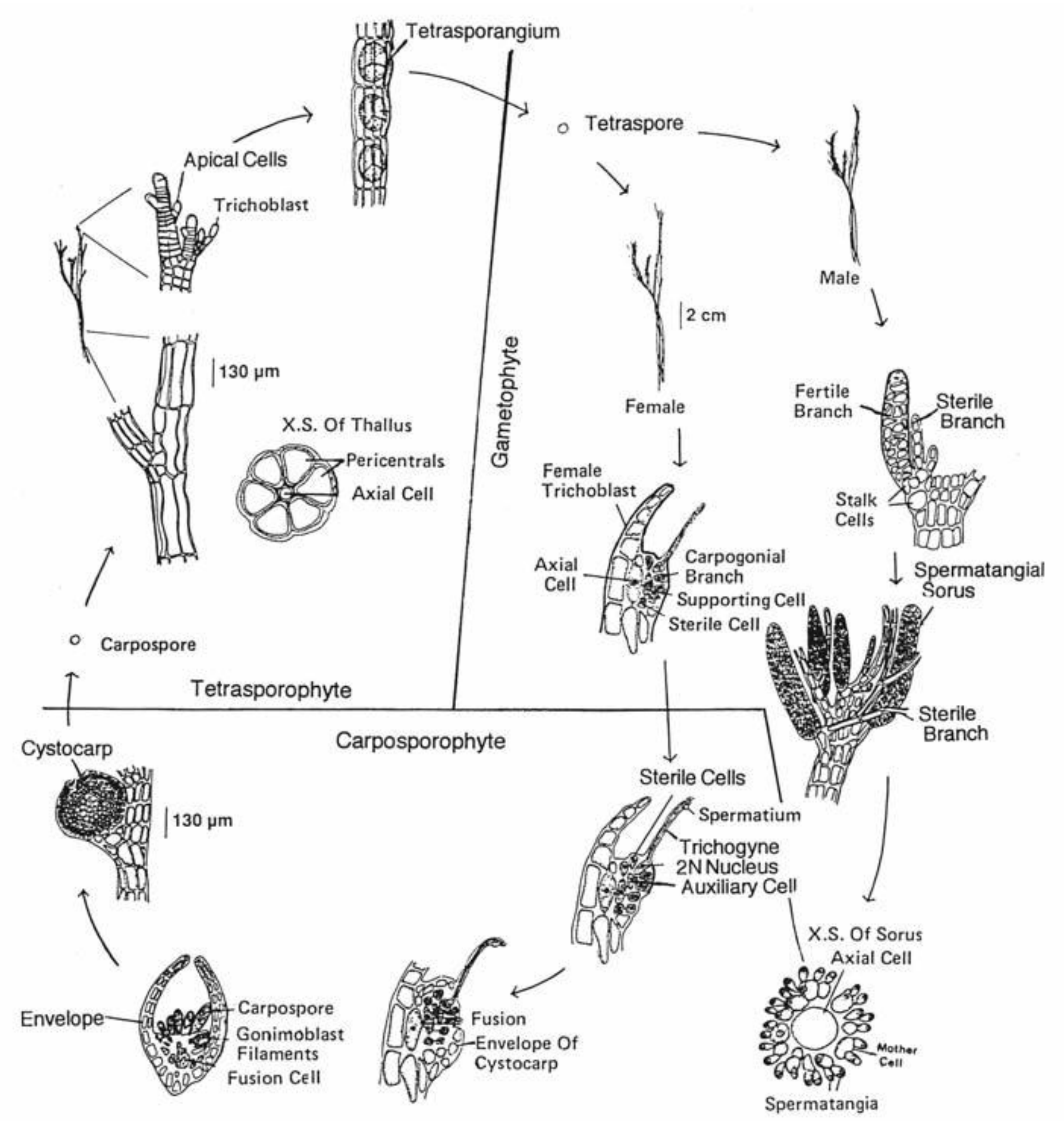
ABSTRACT: We review research from the last 40 yr on macroalgal–bacterial interactions. Marine macroalgae have been challenged throughout their evolution by microorganisms and have developed in a world of microbes. Therefore, it is not surprising that a complex array of interactions has evolved between macroalgae and bacteria which basically depends on chemical interactions of various kinds. Bacteria specifically associate with particular macroalgal species and even to certain parts of the algal body. Although the mechanisms of this specificity have not yet been fully elucidated, ecological functions have been demonstrated for some of the associations. Though some of the chemical response mechanisms can be clearly attributed to either the alga or to its epibiont, in many cases the producers as well as the mechanisms triggering the biosynthesis of the biologically active compounds remain ambiguous. Positive macroalgal–bacterial interactions include phytohormone production, morphogenesis of macroalgae triggered by bacterial products, specific antibiotic activities affecting epibionts and elicitation of oxidative burst mechanisms. Some bacteria are able to prevent biofouling or pathogen invasion, or extend the defense mechanisms of the macroalgae itself. Deleterious macroalgal–bacterial interactions induce or generate algal diseases. To inhibit settlement, growth and biofilm formation by bacteria, macroalgae influence bacterial metabolism and quorum sensing, and produce antibiotic compounds. There is a strong need to investigate the bacterial communities living on different coexisting macroalgae using new technologies, but also to investigate the production, localization and secretion of the biological active metabolites involved in those possible ecological interactions.

KEY WORDS: Marine microoganisms · Defense · Beneficial communication · Biofilms · Oxidative burst · Antibiotic activity · Quorum sensing control

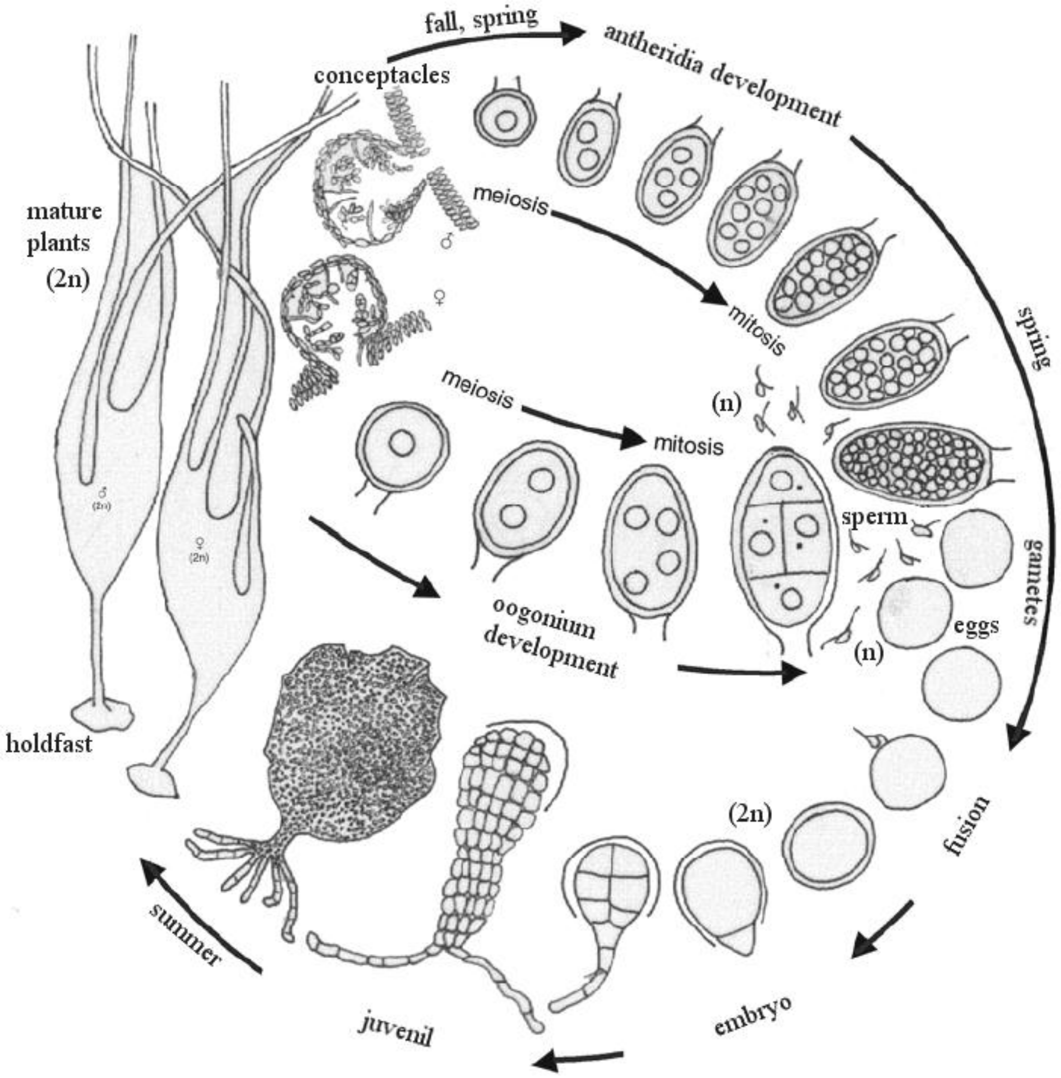
S1. **Paper I. Goecke *et al*. 2010.** (available online or by the authors)



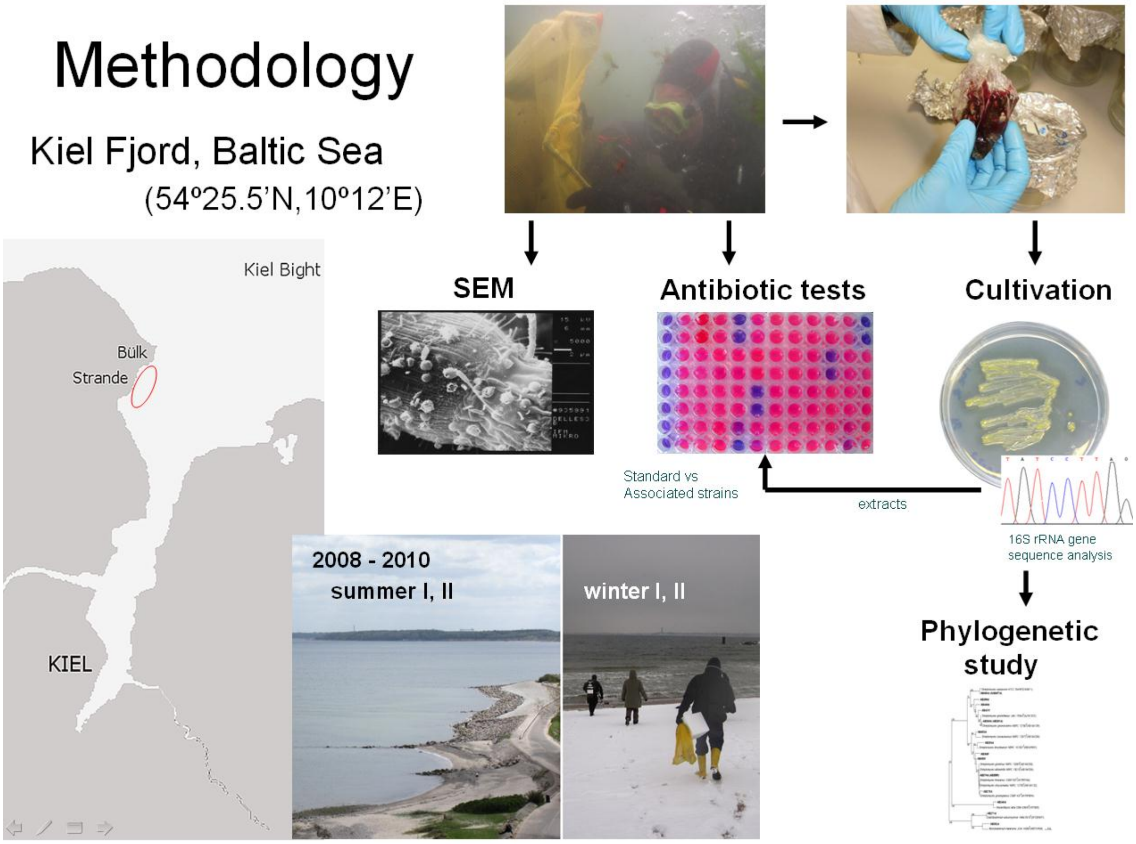
S2. Figure of the life cycle of Fucales adapted from Lee 2008.



S3. *Delesseria sanguinea* present the same life cycle as *Polysiphonia*: The tetrasporophyte is a diploid, corticated filament within which meiosis occurs and produces haploid tetraspores. The tetraspore germinates into a haploid filament that is isomorphic with the tetrasporophyte. The gametophytes are dioecious with spermatangial (male) and carpogonial (female) plants (figure from Lee 2008).



S4. Figure of the life cycle of *Durvillaea antarctica* (Fucales) after Collantes *et al*. (2002).



S5. Summary of the research activities.

S6. List of the congress and scientific meetings where part of the present work has been presented:

2012 **Goecke F.**, V. Thiel, J. Wiese, A. Labes & J.F. Imhoff. Phylogenetic relationships among bacteria described from algae: Algae are a distinct source of new taxa. 3th Latin-American Congress of Algal Biotechnology, *Universidad de Concepción*, Concepción, Chile, 16-18 Jan. Poster. (accepted)

2012 **Goecke F.**, T. Staufenberger, J. Wiese, A. Labes & J.F. Imhoff. Diferencias de asociación bacteriana en macroalgas son producto de interacciones químicas y ambientales 3th Latin-American Congress of Algal Biotechnology, *Universidad de Concepción*, Concepción, Chile, 16-18 Jan. Oral presentation. (accepted)

2011 **Goecke F.**, A. Labes, J. Wiese & J.F. Imhoff. Bacteria associations with co-occurring macroalgae: Host, epiphyte and environmental factors. 5th European Phycological Congress. Rhodes Island, Greece, 04-09 Sept. Oral presentation. (Observation: G. Nikolaidis Travel Award 2011)

2011 **Goecke F.**, A. Labes, J. Wiese & J.F. Imhoff. Insights supporting the proposal of possible 'symbiotic role' of marine bacteria on macroalgae. MIMAS Simposium: Microbial Interactions in Marine Systems. *Alfried-Krupp-Wissenschaftskolleg*, Greifswald, Germany, 06-08 Jul. Poster.

2011 Goecke F., A. Labes, J. Wiese & J.F. Imhoff. Bacteria associated with coexisting macroalgae: Seasonal, interspecies variation and antibiotic effects. Annual Conference of the Association for General and Applied Microbiology (VAAM), *Karlsruhe Institute of Technology*, Karlsruhe, Germany, 03 -06 Apr. Poster.

2010 **Goecke F.**, J. Wiese, A. Labes & J.F. Imhoff. Interactions between macroalgae and bacteria: bacterial communities associated to two Baltic Sea macroalgae. 5th International Student Conference: Biodiversity and Functioning of Aquatic Ecosystems in the Baltic Sea Region. *Klaipeda Universtity*, Lithuania, 06-08 Oct. Oral presentation. (Observation: First price oral presentation)

2009 **Goecke F.**, T. Staufenberger, J. Wiese, A. Gärtner, V. Thiel, K. Nagel, M. Blümel, R. Schmaljohan & J.F. Imhoff. Macroalgae as source of new species of microorganisms. 11th Young Systematists Forum (YSF), *Natural History Museum*, London, England, 02 Dec. Poster.

2009 **Goecke F.**, J. Wiese, A. Labes & J.F. Imhoff. Variability of the algae-associated bacterial communities among coexisting *Fucus vesiculosus* (Phaeophyceae) and *Delesseria sanguinea* (Rhodophyta) in the Baltic Sea. Annual Conference of the Association for General and Applied Microbiology (VAAM), *Ruhr-Universität Bochum*, Bochum, Germany, 08 -11 Mar. Poster.

S7. Table of the different bacterial taxa which affiliated to type strains previously described as associated with algae that have been detected by molecular (16S rRNA clone libraries, DGGE), cultivation (culti) or optical methods from other algal sources. (Supporting information Chapter I).

Next related type strain	%	Sample	Method	Source	Reference
Bacillus algicola	99	*Fucus vesiculosus*	cult	*Fucus evanescens*	Goecke et al. 2011
Dinoroseobacter shibae	99	microalgal sources	16S lib	*Prorocentrum lima*	Wagner-Dobler et al. 2011
Erythrobacter longus	99	*Saccharina latissima*	16S lib	*Enteromorpha linza*	Staufenberger et al. 2008
	98	*Laminaria hyperborea*	16S lib	*Enteromorpha linza*	Bengtsson et al. 2011
Leucothrix mucor	nd	various macroalgae	optical	*Monostroma* sp.	Johnson et al. 1971 Bland and Brock 1973
Marinovum algicola	(94)	*Alexandrium lusitanicum*	DGGE	*Prorocentrum lima*	Hold et al. 2001
	(95)	*Prorocentrum lima*	DGGE	*Prorocentrum lima*	Prokic et al. 1998
	97	*Pfisteria*-like dinoflagellate	16S lib	*Prorocentrum lima*	Alavi et al. 2001
	97	*Gymnodinium catenatum*	cult	*Prorocentrum lima*	Green et al. 2004
	100	diatom bloom	DGGE	*Prorocentrum lima*	Riemann et al. 2000
P. atlantica	99	*Saccharina latissima*	cult	*Lessonia* sp.	Wiese et al. 2009b
'*P. porphyrae*'	99	*Saccharina japonica*	cult	*Porphyra yezoensis*	Dimitrieva et al. 2006
Ulvibacter litoralis	nd	*Fucus serratus*	cult	*Ulva fenestrata*	Saha et al. 2011

References

Alavi M, Miller T, Erlandson K, Schneider R, Belas R (2001) Bacterial community associated with *Pfiesteria*-like dinoflagellate cultures. Environ Microbiol 3:380-396.

Bengtsson MM, Sjøtun K, Storesund JE, Øvreås L (2011) Utilization of kelp-derived carbon sources by kelp surface-associated bacteria. Aquat Microb Ecol 62:191-199.

Bland JA, Brock TD (1973) The marine bacterium *Leucothrix mucor* as an algal epiphyte. Mar Biol 23:283-292.

Dimitrieva GY, Crawford RL, Yüksel GU (2006) The nature of plant growth-promoting effects of a pseudoalteromonad associated with the marine algae *Laminaria japonica* and linked to catalase excretion. J Appl Microbiol 100:1159-1169.

Goecke F, Labes A, Wiese J, Imhoff JF. Chapter II in the present work.

Green DH, Llewellyn LE, Negri AP, Blackburn SI, Bolch CJ (2004) Phylogenetic and functional diversity of the cultivable bacterial community associated with the paralytic shellfish poisoning dinoflagellate *Gymnodinium catenatum*. FEMS Microbiol Ecol 47:345-357.

Hold GL, Smith EA, Rappe MS, Maas EW, Moore ERB, Stroempl C, Stephen JR, Prosser JI, Birkbeck TH, Gallacher S (2001) Characterisation of bacterial communities associated with toxic and non-toxic dinoflagellates: *Alexandrium* spp. and *Scrippsiella trochoidea*. FEMS Microbiol Ecol 37:161-173.

Johnson P, Sieburth J, Sastry A, Arnold C, Doty M (1971) *Leucothrix mucor* infestation of benthic crustacea, fish eggs and tropical algae. Limnol Oceanogr 16:962-969.

Prokic I, Brümmer F, Brigge T, Görtz HD, Gerdts G, Schutt C, Elbrächterd M, Müller WEG (1998) Bacteria of the genus *Roseobacter* associated with the toxic dinoflagellate *Prorocentrum lima*. Protist 149:347-357.

Riemann L, Steward GF, Azam F (2000) Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. Appl Environ Microbiol 66:578-587.

Saha M, Rempt M, Grosser K, Pohnert G, Weinberger F (2011) Surface-associated fucoxanthin mediates settlement of bacterial epiphytes on the rockweed *Fucus vesiculosus*. Biofouling 27:423-433.

Staufenberger T, Thiel V, Wiese J, Imhoff JF (2008) Phylogenetic analysis of bacteria associated with *Laminaria saccharina*. FEMS Microbiol Ecol 64: 65-77.

Wagner-Dobler I, Ballhausen B, Berger M, Brinkhoff T, Buchholz I, Bunk B, Cypionka H, Daniel R, Drepper T, Gerdts G, Hahnke S, Han C, Jahn D, Kalhoefer D, Kiss H, Klenk H-P, Kyrpides N, Liebl W, Liesegang H, Meincke L, Pati A, Petersen J, Piekarski T, Pommerenke C, Pradella S, Pukall R, Rabus R, Stackebrandt E, Thole S, Thompson L, Tielen P, Tomasch J, von Jan M, Wanphrut N, Wichels A, Zech H, Simon M (2010) The complete genome sequence of the algal symbiont *Dinoroseobacter shibae*: A hitchhiker's guide to life in the sea. ISME J 4:61-77.

Wiese J, Thiel V, Nagel K, Staufenberger T, Imhoff JF (2009b) Diversity of antibiotic active bacteria associated with the brown alga *Laminaria saccharina* from the Baltic Sea. Mar Biotechnol 11:287-300.

S8. Classification of bacterial strains from the two macroalgae based on 16S rRNA gene sequences analysis. The affiliation to phylotypes (Ph) determined by similarity matrix (Phylip). *Fucus vesiculosus* (Fu), *Delesseria sanguinea* (De), GPY/TM/SCA/HWO correspond to the nutrient media; and the season is represented by (S1), summer I, (W1) winter 1, (S2) summer 2 and (W2) winter 2. The antibiotic activity is shown, where pp >80%, p>50%, and (p)>20% inhibition respectivelly, 0 = no activity, and nd: not determined. The test strains were *Bacillus subtilis* (Bs), *Staphylococcus lentus* (Sl), *Escherichia coli* (Ec), *Candida glabrata* (Ca), *Algicola bacteriolytica* (Ab), *Pseudoalteromonas elyakovii* (Pe), members of the genera *Bacillus algicola* (Ba), and *Formosa algae* (Fa), respectivelly. (Supporting information of the Chapter II)

Ph	**Isolate**	**Next related type strain**	**Sample**	**Test strains**							
				Bs	**Sl**	**Ec**	**Ca**	**Ab**	**Pe**	**Ba**	**Fa**
P1	AB308f	*Aeromonas bivalvium*	FUGPY7 S2	0	0	0	0	0	0	0	0
P2	AB267d	*Algoriphagus yeomjeoni*	DEGPY7 W1	0	0	0	0	0	0	pp	0
P3	AB304d	*Bacillus aerophilus*	DEGPY6 S2	0	0	0	0	0	0	0	0
P4	AB341d	*Bacillus algicola*	DETM5 S2	0	0	0	0	0	(p)	0	0
	AB357d	*Bacillus algicola*	DEHWO1 S2	0	0	0	0	0	0	0	0
	AB423f	*Bacillus algicola*	FUTM23 W2	0	0	0	0	0	(p)	0	0
P5	AB305f	*Bacillus altitudinis*	FUSCA2 S2	nd	nd	nd	nd	nd	nd	nd	nd
P6	AB335f	*Bacillus aerophilus*	FUTM5 S2	0	0	0	0	0	0	0	(p)
	AB196d	*Bacillus altitudinis*	DETM2 S1	0	0	0	0	0	0	pp	0
	AB243d	*Bacillus altitudinis*	DEHWO13W1	0	nd	0	0	nd	nd	nd	nd
	AB244d	*Bacillus altitudinis*	DEGPY1 W1	0	0	0	0	0	0	pp	0
	AB247f	*Bacillus altitudinis*	FUSCA2 W1	0	0	0	0	0	0	pp	(p)
	AB249d	*Bacillus altitudinis*	DEGPY2 W1	0	0	0	0	0	0	pp	0
	AB412d	*Bacillus pumilus*	DETSB15 W2	0	0	0	0	0	(p)	0	0
	AB438f	*Bacillus pumilus*	FUTM48 W2	0	0	0	0	0	0	(p)	0
	AB200d	*Bacillus safensis*	DETM6 S1	0	0	0	0	0	0	0	0
	AB208f	*Bacillus safensis*	FUSCA5 S1	0	0	0	0	0	0	p	(p)
	AB224d	*Bacillus safensis*	DEGPY2 S1	0	0	0	0	0	0	pp	0
	AB235f	*Bacillus safensis*	FUSCA2 S1	0	0	0	0	0	0	0	(p)
	AB212f	*Bacillus safensis*	FUTM12 S1	0	nd	0	0	nd	nd	nd	nd
P7	AB202d	*Bacillus amyloliquefaciens*	DETM8 S1	0	0	0	0	0	0	0	0
	AB263f	*Bacillus amyloliquefaciens*	FUSCA5W1	0	nd	0	0	nd	nd	nd	nd
	AB306f	*Bacillus amyloliquefaciens*	FUGPY3 S2	0	0	0	0	0	0	0	0
	AB321d	*Bacillus amyloliquefaciens*	DEGPY1 S2	0	0	0	0	0	(p)	0	0
	AB332d	*Bacillus amyloliquefaciens*	DETM4 S2	0	0	0	0	0	0	0	0
	AB339d	*Bacillus amyloliquefaciens*	DEHWO2S2	0	0	0	0	0	0	0	0
	AB363f	*Bacillus amyloliquefaciens*	FUTMb S2	0	0	0	0	0	0	0	0
	AB256d	*Bacillus subtilis*	DETM11 W1	0	nd	0	0	nd	nd	nd	nd
	AB230d	*Bacillus subtilis*	DEGPY9 S1	0	0	0	0	0	(p)	(p)	0
	AB270f	*Bacillus vallismortis*	FUGPY1 W1	0	0	0	0	0	0	nd	0
	AB227d	*Bacillus subtilis*	DEGPY5 S1	0	0	0	0	0	0	p	0
	AB209f	*Bacillus subtilis*	FUSCA6 S1	0	0	0	0	0	0	pp	0
	AB410d	*Bacillus subtilis*	DETSB12 W2	0	0	0	0	0	(p)	0	0
	AB194d	*Bacillus vallismortis*	DESCA1 S1	p	0	0	0	0	0	0	0
	AB242d	*Bacillus vallismortis*	DETM1 W1	0	0	0	0	0	0	nd	0
	AB273d	*Bacillus vallismortis*	DETSB5 W1	0	nd	0	0	nd	nd	nd	nd
P8	AB289d	*Bacillus anthracis*	DETM7 S2	0	0	0	0	0	0	0	0
	AB315f	*Bacillus anthracis*	FUTSB1 S2	nd	nd	nd	nd	nd	nd	nd	nd
	AB197d	*Bacillus cereus*	DETM3 S1	0	0	0	0	0	0	0	0
	AB250d	*Bacillus mycoides*	DEHWO3 W1	0	0	0	0	0	0	nd	0
P9	AB358d	*Bacillus anthracis*	DETM10 S2	nd	nd	nd	nd	nd	nd	nd	nd
P10	AB409d	*Bacillus aquimaris*	DETSB11 W2	0	0	0	0	0	0	0	0
P11	AB286d	'*Bacillus baekryungensis*'	DEHWO4 S2	0	0	0	0	0	0	0	0
P12	AB471d	*Bacillus hwajinpoensis*	DESCA62 W2	0	0	0	0	0	0	pp	0
	AB419f	*Bacillus hwajinpoensis*	FUTM29 W2	0	0	0	0	0	(p)	0	0
	AB309f	*Bacillus hwajinpoensis*	FUGPY5 S2	0	0	0	0	0	0	0	0
	AB257f	*Bacillus hwajinpoensis*	FUSCA6 W1	p	nd	0	0	nd	nd	nd	nd
	AB342d	*Bacillus hwajinpoensis*	DEGPY7 S2	0	0	0	0	0	(p)	0	0
	AB352d	*Bacillus hwajinpoensis*	DEGPY13 S2	0	0	0	0	0	0	0	0
	AB343d	*Bacillus hwajinpoensis*	DESCA1 S2	nd	nd	nd	nd	nd	nd	nd	nd
P13	AB217f	*Bacillus infantis*	FUTM7 S1	p	0	0	0	0	0	pp	0
	AB265f	*Bacillus infantis*	FUTM1 W1	0	0	0	0	0	0	pp	0

P14	AB213f	*Bacillus licheniformis*	FUTM3 S1	0	0	0	0	0	0	0	0
P15	AB201d	*Bacillus licheniformis*	DETM7 S1	0	0	0	0	0	0	pp	0
	AB203d	*Bacillus licheniformis*	DETM9 S1	nd	0	nd	nd	0	0	pp	0
	AB246d	*Bacillus licheniformis*	DEHWO1 W1	0	0	0	0	0	0	pp	0
	AB266f	*Bacillus licheniformis*	FUSCA1 W1	p	0	0	0	0	0	pp	0
	AB353f	*Bacillus licheniformis*	FUHWO6 S2	0	0	0	0	0	0	0	0
	AB424f	*Bacillus licheniformis*	FUGPY34 W2	0	0	0	0	0	0	0	0
	AB464f	*Bacillus licheniformis*	FUSCA36 W2	0	0	0	0	0	0	0	0
P16	AB366d	*Bacillus safensis*	DETSB6 bl S2	0	(p)	0	0	0	0	0	0
P17	AB297f	*Bacillus subtilis*	FUTSB4 S2	0	0	0	0	0	(p)	0	0
P18	AB195d	*Brevibacterium frigoritolerans*	DETM1 S1	0	0	0	0	0	0	pp	0
P19	AB218f	*Brevibacterium frigoritolerans*	FUTM8 S1	0	0	0	0	0	0	pp	0
P20	AB252f	*Cellulophaga baltica*	FUGPY4 W1	nd	(p)	nd	nd	0	0	pp	0
	AB262f	*Cellulophaga baltica*	FUTM2 W1	p	nd	0	0	nd	nd	nd	nd
	AB405d	*Cellulophaga baltica*	DEHWO7 W2	0	0	0	0	0	0	0	0
	AB420f	*Cellulophaga baltica*	FUGPY31 W2	0	0	0	0	0	0	0	0
	AB359d	*Cellulophaga baltica*	DESCA8 S2	nd	nd	nd	nd	nd	nd	nd	nd
P21	AB290f	*Cellulophaga baltica*	FUTM9 S2	0	0	0	0	0	0	0	0
	AB299d	*Cellulophaga baltica*	DEGPY3 S2	0	0	0	0	0	0	0	0
P22	AB261d	*Cellulophaga fucicola*	DEHWO4 W1	0	0	0	0	0	0	nd	0
P23	AB204d	*Cellulophaga tyrosinoxydans*	DETM10 S1	pp	nd	0	0	nd	nd	nd	nd
P24	AB320f	*Cobetia marina*	FUTM2 S2	0	0	0	0	0	0	pp	0
P25	AB312d	*Cobetia marina*	DETSB2b S2	0	0	0	0	0	0	0	0
	AB324d	*Cobetia marina*	DEGPY2 S2	0	0	0	0	0	0	0	0
	AB350f	*Cobetia marina*	FUTM8 S2	0	0	0	0	0	0	0	0
P26	AB294d	*Cyclobacterium amurskyense*	DESCA4bS2	0	0	0	0	0	(p)	0	0
	AB348f	*Cyclobacterium amurskyense*	FUTM7 S2	0	0	0	0	0	0	0	p
P27	AB322f	*Formosa algae*	FUGPY4 S2	0	0	0	0	0	(p)	0	0
	AB356f	*Formosa algae*	FUSCA3 S2	(p)	0	0	0	0	(p)	pp	0
P28	AB437f	*Glaciecola mesophila*	FUTM47 W2	0	0	0	0	0	0	0	0
P29	AB205d	*Hoeflea alexandrii*	DEGPY1 S1	0	0	0	0	0	0	pp	0
P30	AB432f	*Hydrogenophaga taeniospiralis*	FUTSB42W2	0	0	0	0	0	0	0	0
P31	AB434f	*Labrenzia marina*	FUGPY44 W2	0	0	0	0	0	0	0	0
P32	AB233d	*Olleya marilimosa*	DEHWO4 S1	0	0	0	0	0	0	pp	0
	AB429f	*Olleya marilimosa*	FUSCA39 W2	0	0	0	0	0	0	pp	0
P33	AB292d	*Olleya marilimosa*	DESCA12 S2	nd	nd	nd	nd	nd	nd	nd	nd
P34	AB214f	*Maribacter aquivivus*	FUTM4 S1	0	nd	0	0	nd	nd	nd	nd
P35	AB216f	*Marinomonas dokdonensis*	FUTM6 S1	(p)	0	0	0	0	0	pp	0
P36	AB287f	*Marinomonas dokdonensis*	FUSCA8 S2	nd	nd	nd	nd	nd	nd	nd	nd
	AB337d	*Marinomonas dokdonensis*	DETSB6na S2	0	0	0	0	0	0	0	0
P37	AB433f	*Marinomonas polaris*	FUTSB43 W2	0	0	0	0	0	(p)	0	0
	AB465f	*Marinomonas polaris*	FUSCA38 W2	0	0	0	0	nd	nd	nd	nd
P38	AB314f	*Marinomonas pontica*	FUTSBbS2	0	0	0	0	0	0	0	0
P39	AB302d	*Microbacterium hatanonis*	DETSB4 S2	0	0	0	0	0	0	0	p
P40	AB443f	*Microbulbifer epialgicus*	FUTM53 W2	0	0	0	0	nd	nd	nd	nd
	AB454d	*Microbulbifer epialgicus*	DETM71 W2	0	0	0	0	nd	nd	nd	nd
P41	AB259d	*Microbulbifer thermotolerans*	DEHWO5 W1	0	0	0	0	0	(p)	pp	0
	AB425f	*Microbulbifer thermotolerans*	FUGPY35 W2	0	0	0	0	pp	0	pp	0
	AB470d	*Microbulbifer thermotolerans*	DEHWO54W2	0	0	0	0	0	0	0	0
P42	AB240d	*Nocardiopsis alba*	DETSB12 W1	0	0	0	0	0	0	pp	0
P43	AB236d	*Paenibacillus lautus*	DETSB4 W1	0	pp	0	0	0	0	pp	0
	AB241d	*Paenibacillus lautus*	DESCA3 W1	nd	pp	nd	nd	0	0	pp	0
P44	AB355d	*Paenibacillus xylanexedens*	DETSB7 S2	0	0	0	0	0	0	0	0
P45	AB365d	*Pantoea agglomerans*	DESCA5 S2	(p)	0	0	0	0	0	pp	0
P46	AB219f	*Paracoccus marcusii*	FUGPY1 S1	0	0	0	0	0	0	pp	0
P47	AB277f	*Paracoccus marinus*	FUGPY6 W1	0	0	0	0	0	0	pp	0
	AB307d	*Paracoccus marinus*	DETM1 S2	0	0	0	0	0	0	0	0
	AB300d	*Rhodobacter azotoformans*	DEGPY5 S2	0	0	0	0	0	0	0	(p)
	AB407d	*Rhodobacter azotoformans*	DEHWO9 W2	0	0	0	0	0	0	0	0
P48	AB238d	*Photobacterium halotolerans*	DETSB6 W1	nd	nd	nd	nd	nd	nd	nd	nd
	AB248d	*Photobacterium halotolerans*	DEGPY3 W1	0	pp	0	0	(p)	(p)	p	0
	AB272d	*Photobacterium halotolerans*	DESCA6 W1	nd	nd	nd	nd	nd	nd	nd	nd
P49	AB408d	*Photobacterium halotolerans*	DETSB10 W2	0	0	0	0	0	(p)	0	0
P50	AB416d	*Pseudoalteromonas arctica*	DESCA26W2	0	0	0	0	0	0	pp	0
	AB293f	*Pseudoalteromonas atlantica*	FUSCA4 S2	nd	nd	nd	nd	nd	nd	nd	nd
	AB296d	*Pseudoalteromonas atlantica*	DESCA2 S2	pp	(p)	0	0	0	0	pp	(p)
	AB330f	*Pseudoalteromonas atlantica*	FUHWO5 S2	0	0	0	0	0	0	0	0

	AB333f	*Pseudoalteromonas atlantica*	FUTSB3 S2	pp	pp	0	0	0	0	pp	pp
	AB360f	*Pseudoalteromonas atlantica*	FUHWO2 S2	0	0	0	0	0	0	0	0
	AB462f	*Pseudoalteromonas elyakovii*	FUTM52 W2	0	0	0	0	0	0	0	0
	AB466f	*Pseudoalteromonas elyakovii*	FUSCA40 W2	0	0	0	0	0	0	pp	(p)
	AB474f	*Pseudoalteromonas elyakovii*	FUTM68 W2	0	0	0	0	nd	nd	nd	nd
P51	AB276f	*Pseudoalteromonas mariniglutinosa*	FUSCA4W1	pp	nd	0	0	nd	nd	nd	nd
P52	AB291d	*Pseudoalteromonas mariniglutinosa*	DETSB3 S2	nd	nd	nd	nd	nd	nd	nd	nd
P53	AB231d	*Pseudoalteromonas tunicata*	DEHWO2 S1	0	0	0	0	0	0	0	0
P54	AB198d	*Pseudoalteromonas ulvae*	DETM4 S1	pp	pp	0	0	0	0	0	pp
	AB199d	*Pseudoalteromonas ulvae*	DETM5 S1	0	nd	0	0	nd	nd	nd	nd
	AB228d	*Pseudoalteromonas ulvae*	DEGPY6 S1	nd	pp	nd	nd	0	0	pp	0
	AB229d	*Pseudoalteromonas ulvae*	DEGPY8 S1	nd	pp	nd	nd	0	0	pp	pp
P55	AB251f	*Pseudomonas marincola*	FUTSB2 W1	0	0	0	0	0	0	(p)	0
P56	AB319d	*Pseudorhodobacter ferrugineus*	DETM8 S2	0	0	0	0	0	0	0	0
	AB347d	*Pseudorhodobacter ferrugineus*	DETM9 S2	0	0	0	0	0	0	pp	0
P57	AB415d	*Pseudorhodobacter ferrugineus*	DESCA24 W2	0	0	0	0	0	0	0	0
P58	AB260d	*Psychrobacter maritimus*	DESCA4 W1	pp	0	0	0	0	0	pp	0
P59	AB402d	*Rheinheimera pacifica*	DEGPY2 W2	pp	0	0	0	0	0	0	0
P60	AB271d	*Salinibacterium amurskyense*	DESCA2 W1	0	0	0	0	0	pp	pp	0
P61	AB411d	*Shewanella baltica*	DETSB13 W2	0	0	0	0	0	0	0	0
P62	AB274d	*Streptomyces alboviridis*	DEGPY8 W1	0	0	0	0	0	0	nd	0
P63	AB275d	*Streptomyces chrysomallus*	DEHWO8 W1	nd	0	nd	nd	0	0	pp	0
P64	AB453d	*Streptomyces ciscaucasicus*	DETSB69 W2	pp	pp	0	p	0	0	0	0
P65	AB254d	*Streptomyces drozdowiczii*	DEGPY9 W1	nd	pp	nd	nd	0	0	nd	0
P66	AB450f	*Streptomyces fimicarius*	FUSCA66 W2	0	0	0	0	pp	0	pp	pp
P67	AB368f	*Streptomyces griseinus*	FUTM4 S2	0	0	0	0	0	0	pp	p?
P68	AB431f	*Streptomyces griseoflavus*	FUTSB41 W2	0	0	0	0	0	0	0	0
P69	AB255f	*Streptomyces griseoplanus*	FUHWO4 W1	0	0	0	0	0	0	pp	0
P70	AB269d	*Streptomyces griseorubens*	DESCA12 W1	0	nd	0	0	nd	nd	nd	nd
	AB281d	*Streptomyces griseorubens*	DEGPY10 W1	0	pp	0	0	0	0	pp	0
P71	AB448d	*Streptomyces griseorubens*	DETM64 W2	pp	pp	0	0	0	(p)	pp	pp
P72	AB288d	*Streptomyces griseorubens*	DETSB5 S2	0	pp	0	0	0	pp	pp	pp
P73	AB460d	*Streptomyces sampsonii*	DETSB14 W2	0	0	0	0	0	0	0	0
	AB457d	*Streptomyces sampsonii*	DEGPY4 W2	0	0	0	0	0	0	0	0
P74	AB445d	*Sulfitobacter guttiformis*	DEGPY55 W2	0	0	0	0	0	0	0	0
P75	AB401d	*Tenacibaculum adriaticum*	DEGPY1 W2	0	0	0	0	0	0	0	0
P76	AB346f	*Thalassobacter arenae*	FUSCA12 S2	nd	nd	nd	nd	nd	nd	nd	nd
P77	AB344f	*Vibrio natriegens*	FUSCA13 S2	nd	nd	nd	nd	nd	nd	nd	nd
	AB334d	*Vibrio rotiferianus*	DETSB2 S2	0	0	0	0	0	0	0	0
	AB336d	*Vibrio rotiferianus*	DESCA9 S2	0	0	0	0	0	0	0	pp
	AB394f	*Vibrio rotiferianus*	FUSCA9 S2	nd	nd	nd	nd	nd	nd	nd	nd
P78	AB310d	*Vibrio rotiferianus*	DEGPY15 S2	0	0	0	0	0	(p)	0	0
P79	AB367f	*Vibrio rumoiensis*	FUSCA7 S2	nd	nd	nd	nd	nd	nd	nd	nd
P80	AB222f	*Winogradskyella echinorum*	FUHWO3 S1	0	0	0	0	0	0	pp	0
P81	AB264d	*Zobellia amurskyensis*	DETM3 W1	0	0	0	0	0	0	pp	0
P82	AB237f	*Zobellia galactanivorans*	FUGPY3 W1	0	0	0	0	0	0	p	0
	AB446d	*Zobellia uliginosa*	DETM57 W2	0	(p)	0	0	0	0	0	0

S9. Number of different phylotypes exhibiting antibiotic active members with respect to their origin obtained in four sampling periods in two years. *Fucus vesiculosus* (Fv) and *Delesseria sanguinea* (Ds) were the macroalgae investigated. The specific activity against standard test strains: *Bacillus subtilis*, *Staphylococcus lentus*, *Escherichia coli*, and *Candida glabrata* ("Standard set"), surface-associated isolates: *Bacillus algicola* and *Formosa algae* ("Associated set"), and macroalgal pathogenic bacteria: *Algicola bacteriolytica* and *Pseudoalteromonas elyakovii* ("Pathogenic set") is given.

Seasonal	**Standard set**		**Associated set**		**Pathogenic set**		**Associated and/or pathogenic set**	
	Fv	**Ds**	**Fv**	**Ds**	**Fv**	**Ds**	**Fv**	**Ds**
Summer 1	2	3	8	7	0	1	7	7
Winter 1	4	5	6	13	0	3	8	12
Summer 2	2	5	6	6	2	6	6	12
Winter 2	0	3	4	4	5	4	7	6

S10. Antibacterial activity reported for the studied algal species in previously investigations around the world. The origin of the activity is expressed as P: plant, E: extract, C: compound. The solvent used for the extraction procedure is given. Numbers in brackets indicate reference.

Alga	Origin	Location	Target strains	Ref.
Fucus vesiculosus	P, E (aqueous)	Germany (Baltic Sea)	*Bacillus subtilis* *Staphylococcus aureus*	(9)
	P, E (ethanol)	Germany (North Sea)	*Bacillus cereus* *B. subtilis* *Sarcina lutea*	(3)
	P	Great Britain	-	(5)
	E (methanol)	New York, USA	*Micrococcus imfimus*	(8)
	C (polyhydroxylated fucophlorethol)	Tromsø, Norway	*Escherichia coli* *Pseudomonas aeruginosa* *S. aureus* *Staphylococcus epidermis* *Proteus mirabilis*	(10)
	E (methanol)	Mediterranean Sea, Morocco	*E. coli* *S. aureus*	(6)
	E (methanol/ dichloromethane)	Germany (Baltic Sea)	*B. subtilis* *Staphylococcus lentus*	(2)
	E (methanol/hexane)	Germany (Baltic Sea)	Bacterial fouling community determined DGGE	(7)
	E (methanol)	USA	-	(1)
	E (aqueous, dichloromethane, ethanol)	Brittany, France	-	(4)
	E (methanol/hexane)	Germany (Baltic Sea)	*Bacillus aquimaris* *Cytophaga* sp. *Flavobacterium* sp. *Pseudoalteromonas* sp. *Pseudoalteromonas tunicata* *Rheinheimera pacifica* *Tenacibaculum adriaticum*	(11)
Delesseria sanguinea	P, E (aqueous)	Germany (Baltic Sea)	*B. subtilis* *E. coli* *S. aureus*	(9)
	P, E (ethanol)	Germany (North Sea)	*B. subtilis* *B. cereus* *S. lutea*	(3)
	P	Great Britain	*S. aureus* *E. coli* *B. subtilis* *Streptococcus pyogenes* *Proteus morganii*	(5)

References.

(1) Betz J, Der Marderosian AH (1991) Antimicrobial activity of marine organisms. Mar Technol Soc J 25:57-64.

(2) Goecke F, Labes A, Wiese J, Imhoff JF (unpubl. data). Chapter IV.

(3) Glombitza KW (1969) Antibakterielle Inhaltsstoffe in Algen. Helgol Wissenschaftliche Meeresunters 19:376-384.

(4) Hellio C, Bremer G, Pons AM, Le Gal Y, Bourgougnon N (2000) Inhibition of development of microorganisms (bacteria and fungi) by extracts of marine alga from Brittany, France. Appl Microbiol Biotechnol 54:543-549.

(5) Hornsey IS, Hide D (1974) The production of antimicrobial compounds by British marine algae. I. Antibiotic producing marine algae. Br Phycol J 9:353-361.

(6) Ibtissam C, Riadi H, Martínez-López J, Domínguez Seglar JF, Gómez-Vidal JA, Bouziane H, Kadiri M (2009) Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco. Afr J Biotechnol 8:1258-1262.

(7) Lachnit T, Wahl M, Harder T (2010) Isolated thallus-associated compounds from the macroalga *Fucus vesiculosus* mediate bacterial surface colonization in the field similar to that on the natural alga. Biofouling 26:247-255.

(8) Lustigman B, Lee LH, Thees N, Masucci J (1992) Production of antibacterial substances by macroalgae of the New York/New Jersey Coast, USA. Bull Environ Contam Toxicol 49:743-749.

(9) Roos H (1957) Untersuchungen über das Vorkommen antimikrobieller Substancen in Meeresalgen. Kiel Meeresforsch 13:41-58.

(10) Sandsdalen E, Haug T, Stensvag K, Styrvold OB (2003) The antibacterial effect of a polyhydroxylated fucophlorethol from the marine brown alga, *Fucus vesiculosus*. World J Microbiol Biotechnol 19:777-782.

(11) Wahl M, Shahnaz L, Dobretsov S, Saha M, Symanowski F, David K, Lachnit T, Vasel M, Weinberger F (2010) Ecology of antifouling resistance in the bladder wrack *Fucus vesiculosus*: patterns of microfouling and antimicrobial protection. Mar Ecol Prog Ser 411:33-48.

S11. Supporting Information Scanning electron microscopy (SEM)

SEM of macroalgal surfaces

Three algal individuals from *F. vesiculosus* and *D. sanguinea*, respectively, from each sampling time were investigated by scanning electron microscopy to observe the distribution of microorganisms on the surface of the macroalgae according to Heindl et al. (2010). Briefly, samples were prepared by fixation with 1% formaldehyde in sterile seawater. After dehydration in a gradient ethanol series (30%, 50%, 70%, 90%, and 100%; v/v) the samples were critical point dried with carbon dioxide (Balzers CPD030) and sputter coated with gold–palladium (Balzers Union SCD004). The specimens were examined in triplicate in the phylloid area (thallus blade) and in the cauloid area (algal stalk) per sample with a scanning electron microscope (Zeiss DSM960). Pictures were taken with a Contax SLR camera. Bacterial morphotypes observable on the surface of the macroalgal tissue were counted directly from SEM picture at 5000 times magnification according to Rogerson (1991) and Armstrong et al. (2000). In all cases, bacterial counts were based on 10 random micrographs recorded at this magnification.

Results

Scanning electron microscopy was used to observe surface cell structure of the macroalgae and the microorganisms associated with it. Both algae presented relatively flat surfaces in the phylloid area, with cells displayed one next to the other (see W1 in Fig. 1), though the cellular structure of the surfaces of the cauloid differed between the two algae: *D. sanguinea* presented compact and smaller cells in the cauloid as compared to the phylloid. The surface of the cauloid of *F. vesiculosus* comprised large cells of irregular size giving a cracked appearance to the tissue (Fig. 2).

The microphotographs revealed a variety of microorganisms settled on both macroalgal surfaces (Figs. 1-3). In comparison, samples of *Fucus vesiculosus* contained a higher number of different morphological types of microorganisms as compared to *D. sanguinea*, including cocoids, rod-shaped bacteria and filamentous forms (Fig. 1: FP). The morphotypes associated with *D. sanguinea* appear to be more homogeneous and coccoid and rod-shaped bacteria were more common (Fig. 1: DP). An effect of the seasons on morphotypes could be inferred at least for *F. vesiculosus*: In the summer season the ‘morphotype diversity’ appeared to be higher than in winter (Fig. 1: FP).

Differences between the two sections (phylloid and cauloid) of the same macroalga were also noticed. With *F. vesiculosus*, the density of the epibiotic microorganisms was higher on the phylloid (with a calculated density of 32 to 67 x 10^6 cells cm^{-2}) but lower on the cauloid (18 to 51 x 10^6 cells cm^{-2}); the opposite was observed with *D. sanguinea* (1 to 23 against 26 to 47 x 10^6 cells cm^{-2}) (Fig. 2, Table 1). As observed in samples of *D. sanguinea*, some bacteria colonized the surface (Fig. 2), but others were able to penetrate the cell wall or grow embedded in the extracellular matrix of the macroalgae. *F. vesiculosus* presented microorganisms penetrating the cell wall as well (Fig. 3). In summer (2008), *Fucus vesiculosus* presented rod-shaped cells in multiple perforations (diameter 10-20 µm), which were free of attached bacteria in the closer surroundings (Fig. 3).

The bacterial cell density differed significantly between macroalgal species and season (Table 1). The surface of *Fucus vesiculosus* was covered completely (Table 1) in summer (Fig. 1: FPS1-S2). In comparison, *Delesseria sanguinea* was less colonized, and the macroalgal cells were always visible beyond the apparently thin biofilm on the surface (Fig. 1: DP, Table 1). In winter, samples of both macroalgae showed less epibiosis (varying from 32 to 58, and 1 to 13 x 10^6 cells cm^{-2} respectively for the brown and the red algae, Table 1). In addition to the associated bacteria, the presence of filamentous algae of the order Ectocarpales was constant on *Fucus vesiculosus* (data not shown), other epiphytic algae were found occasionally growing on both species. Fungi and protozoa as epiphytic organisms were rare. A few pennate diatoms, like *Coconeis* sp., *Grammatophora* sp. and *Licmophora* sp. (as identified by morphology) were found to be associated with the surface of both macroalgae (Fig. 1: FPS1), especially with the cauloid of *D. sanguinea* (data not shown).

As revealed by SEM observation, the surface colonization of both algae of our study showed a seasonal pattern with higher bacterial densityin summer (Table 1). Similar numbers of bacteria have been observed on the surface of other macroalgae including other *Fucus* species (see Koop et al. 1982, Corre & Prieur 1990, Rogerson 1991, Armstrong et al. 2000, Dobretsov et al. 2006b, Bengtsson et al. 2010). Seasonal dynamics of bacterioplankton and epiphytic bacterial communities in the Baltic Sea including *F. vesiculosus* have been previously observed (Andersson et al. 2010, Lachnit et al. 2011). Worldwide, the same pattern, a minimum number of epiphytic bacteria in winter and a maximum in summer, has been previously reported for *Fucus serratus* in Scotland (Armstrong et al. 2000), and for other brown macroalgae as for *Eisenia bicyclis* in Japan (Shiba & Taga 1980), *Saccharina longicruris* (as *Laminaria longicruris*) in Canada (Laycock 1974), *Laminaria pallida* and *Ecklonia maxima* in South Africa (Mazure & Field 1980), *Laminaria digitata* on the coast of Brittany, France (Corre & Prieur 1990), and *Laminaria hyperborea* in Norway (Bengtsson et al. 2010). Thus, both algae in the Baltic Sea reflect the influence of abiotic factors in their habitat. However, *Fucus* *vesiculosus* always presented a higher number of bacteria on its surface than *Delesseria sanguinea*, indicating specific factors of the macroalgae shaping the associated communities (Table 1).

Table 1: Number of bacteria (10^6 cells per cm^{-2}) on *Fucus vesiculosus* and *Delesseria sanguinea* determined by SEM on the different alga section (phylloid and cauloid) and the sampled season. Bacterial counts were based on 10 random micrographs, values represent the mean value, and standard deviation is given.

	Fucus vesiculosus		***Delesseria sanguinea***	
	Phylloid	**Cauloid**	**Phylloid**	**Cauloid**
Summer 1	66.40 ±19	33.53 ±11	15.97 ±6	47.08 ±10
Winter 1	58.64 ±26	25.69 ±21	13.03 ±5	26.56 ±9
Summer 2	67.36 ±26	51.17 ±20	23.67 ±8	35.89 ±7
Winter 2	32.17 ±7	18.25 ±8	1.69 ±3	26.97 ±10

Differences between both algae with respect to the surface colonization were shown for different parts of the macroalgae as well (Table 1). The phylloids of *Fucus vesiculosus* in general contained a higher number of bacteria than the cauloids, but the opposite was observed in *Delesseria sanguinea* (Fig. 1 and 2, Table 1). This is in accordance with previous investigations, which have noticed different bacterial communities associated with different parts of macroalgae (Corre & Prieur 1990, Dimitrieva & Dimitriev 1996, Staufenberger et al. 2008). These differences may be explained by different structural features of the specific parts of the algal thallus (as discussed below), age, and/or by a lack of vascular connections in the algal tissue with a resulting chemical differentiation (Goecke et al. 2010). For example, Lüning (1990) mentioned that in *D. sanguinea*, the storage materials (i.e. digeneaside, trehalose) are contained in the stipes and old midribs, where in this case, a more dense bacterial community was found (Fig. 2).

We observed in samples of reproductive plants of *Fucus vesiculosus* a female gametangium being released from a conceptacle already covered with bacteria on the surface (Fig. 6). This is the first observation of bacterial associations to the earlier stages of life of *F. vesiculosus*. Which effect microorganisms have at different stages in *Fucus* life histories is yet completely unknown.

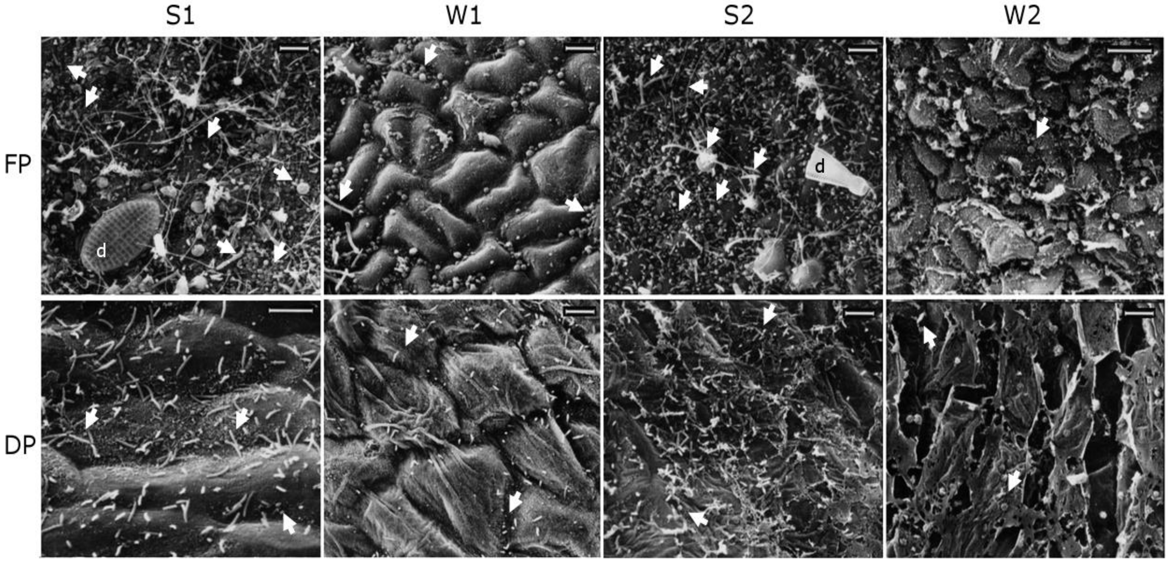


Figure 1. Scanning electron microscopic photomicrographs showing the microorganisms settled on the macroalgal surfaces. Occasionally diatoms (d) appear on the surfaces. Although bacteria are seen on the surfaces of all samples, the degree of microbial coverage was highly variable. Different morphological types of bacteria are observed on the surface of the macroalgae, including cocoids, rod-shaped bacteria and filamentous forms (see arrows). Presence of a film of organic matter can be observed in DPW2. (F) *Fucus vesiculosus*, (D) *Delesseria sanguinea*, phylloid (P), (S1) July 2008, (W1) January 2009, (S2) July 2009, (W2) January 2010. Scale bar = 5 µm.

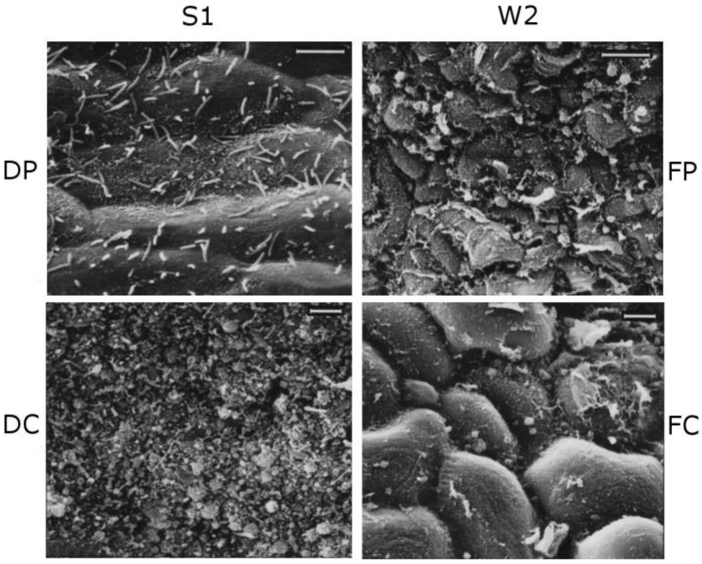


Figure 2. Scanning electron microscopic photomicrographs showing microorganisms settled on different parts of the macroalgal surfaces. The phylloid of *Delesseria sanguinea* (DPS1) and the cauloid of *Fucus vesiculosus* (FCW2) are less covered by microorganisms. (F) *Fucus vesiculosus*, (D) *Delesseria sanguinea*, phylloid (P), cauloid (C), July 2008 (S1), January 2010 (W2). Scale bar = 5 µm.

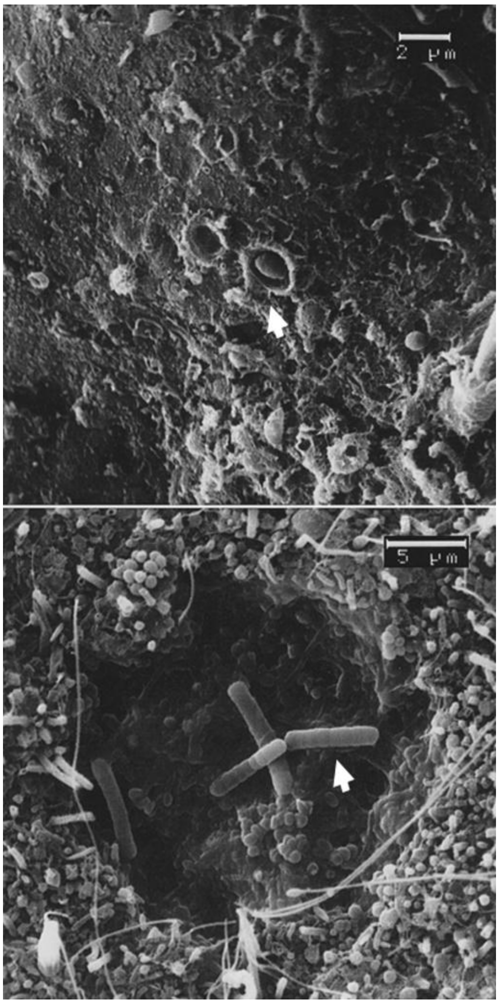


Figure 3. Scanning electron microscopic photomicrographs showing microorganisms included in the surface of the macroalgae. Above) Cuticle surface of *Delesseria sanguinea* with round bodies emerging. Below) Perforations on the cell wall of *Fucus vesiculosus* with filament-forming structures surrounded by the dense biofouled cuticle. Scale bar = 2 and 5 µm respectively.

S12. Individual scientific contribution to multiple author publication

The chapters of this thesis are partly published (Paper I), or submitted (Chapter I, II, III, and short note) papers of multiple authorships. The list serves as a clarification of personal contributions on each publication.

Chapter I

Phylogenetic analysis was done by Vera Thiel. Evaluation of the data and preparation of the manuscript was done by Franz Goecke. Critical revision of the manuscript was done by Antje Labes, Jutta Wiese and Johannes F. Imhoff.

Chapter II

Sampling was done by Tim Staufenberger, Franz Goecke and Herwig Heindl. Cultivation of the isolates, phylogenetic analysis, biological activity tests, and evaluation of the data was done by Franz Goecke. Scanning electron microscopy was done by Rolf Schmaljohann and Franz Goecke. Preparation of the manuscript was done by Franz Goecke, Antje Labes, Jutta Wiese. Critical revision of the manuscript was done by Johannes F. Imhoff.

Chapter III

Sampling, scanning electron microscopy and preparation of the manuscript was done by Franz Goecke. Critical revision of the manuscript was done by Jutta Wiese, Antje Labes, Rolf Schmaljohann and Johannes F. Imhoff.

Chapter IV

Sampling was done by Tim Staufenberger and Franz Goecke. The biological activity tests, evaluation of the data and preparation of the manuscript was done by Franz Goecke in close collaboration with Antje Labes and Jutta Wiese. Critical revision of the manuscript was done by Johannes F. Imhoff.

Chapter V

Sampling, scanning electron microscopy, evaluation of the data and preparation of the manuscript was done by Franz Goecke. Histological cuts by Alejandra Núñez and Franz Goecke. Critical revision of the manuscript was done by Antje Labes, Jutta Wiese and Johannes F. Imhoff.

Schemas and photography used in the present investigation were performance by Franz Goecke except for: Figures 3a, 3b, 5b, 8b, 10, 11b and 12b (where the sources are given).