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Epiphytic and endophytic microbiome of the seagrass *Zostera marina*: Do they contribute to pathogen reduction in seawater?

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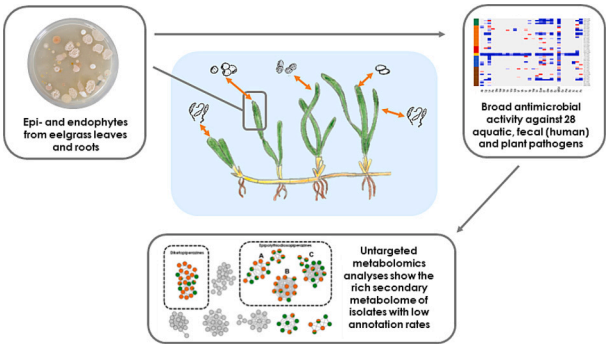
HIGHLIGHTS

- Inhibitory potential of eelgrass microbiome against aquatic and fecal pathogens
- Isolation of epiphytes and endophytes associated with eelgrass leaves and roots
- Particularly leaf epibiotic bacteria exhibit significant antimicrobial activity.
- Rich secondary metabolite composition by untargeted metabolomics
- Potential involvement of eelgrass microbiome in seagrass ecosystem services

GRAPHICAL ABSTRACT



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ABSTRACT

Seagrass meadows provide crucial ecosystem services for coastal environments and were shown to reduce the abundance of waterborne pathogens linked to infections in humans and marine organisms in their vicinity. Among potential drivers, seagrass phenolics released into seawater have been linked to pathogen suppression, but the potential involvement of the seagrass microbiome has not been investigated. We hypothesized that the microbiome of the eelgrass *Zostera marina*, especially the leaf epiphytes that are at direct interface between the seagrass host and the surrounding seawater, inhibit waterborne pathogens thereby contributing to their removal. Using a culture-dependent approach, we isolated 88 bacteria and fungi associated with the surfaces and inner tissues of the eelgrass leaves (healthy and decaying) and the roots. We assessed the antibiotic activity of microbial extracts against a large panel of common aquatic, human (fecal) and plant pathogens, and mined the metabolome of the most active extracts. The healthy leaf epibiotic bacteria, particularly *Streptomyces* sp. strain 131, displayed broad-spectrum antibiotic activity superior to some control drugs. Gram-negative bacteria abundant on healthy leaf surfaces, and few endosphere-associated bacteria and fungi also displayed remarkable activities. UPLC-MS/MS-based untargeted metabolomics analyses showed rich specialized metabolite repertoires with low annotation rates, indicating the presence of many undescribed antimicrobials in the extracts. This study

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contributes to our understanding on microbial and chemical ecology of seagrasses, implying potential involvement of the seagrass microbiome in suppression of pathogens in seawater. Such effect is beneficial for the health of ocean and human, especially in the context of climate change that is expected to exacerbate all infectious diseases. It may also assist future seagrass conservation and management strategies.

1. Introduction

Seagrasses are marine plants (angiosperms) that form extensive underwater meadows in temperate, subtropical and tropical coastal ecosystems and represent a widespread foundation species. They provide vital habitats and nursery ground for many species, including economically important fish species (Jeyabaskaran et al., 2018; Conte et al., 2021). Being key benthic ecosystem engineers, seagrasses stabilize soft sediments and store large quantities of CO_2 as blue carbon, contributing to the mitigation of anthropogenic emissions (Duarte et al., 2010; de Los Santos et al., 2019). A recently discovered ecosystem service of seagrass meadows is the suppression of pathogenic bacteria in the water column. Lamb et al. (2017) showed that mixed seagrass meadows effectively reduced the abundance of human fecal bacteria (enterococci) and a variety of marine pathogens (e.g., *Vibrio* spp.) that are harmful for fish, marine invertebrates and marine mammals. The authors investigated over 8000 reef-building corals in Indonesian coastal waters and reported two-fold lower levels of disease on reefs with adjacent seagrass meadows. Several subsequent studies carried out on seagrass meadows in other parts of the world also confirmed lower levels of pathogens in their vicinity, including the fecal pathogens *E. coli* and various enterococci (Palazón et al., 2018), *Salmonella* spp. (Deng et al., 2021) and *Vibrio* spp. (Reusch et al., 2021). The underlying reason(s) for this 'sanitary' effect is currently unclear but it has been partly linked to the high content of seagrass phenolics (e.g., flavonoids and other phenylpropanoids) that are well-known for their antimicrobial effects and involvement in seagrass chemical defense (Migliore et al., 2007; Guan et al., 2017; Papazian et al., 2019; Mannino and Micheli, 2020; Conte et al., 2021; Deng et al., 2021).

Seagrasses harbor complex assemblies of microorganisms on their surfaces and in their inner tissues, and live intimately with both beneficial and harmful microorganisms in their environment (Hurtado-McCormick et al., 2019; Conte et al., 2021). Although some interactions between seagrasses and sediment microbiota have been demonstrated (Bourque et al., 2015; Sun et al., 2015; Brodersen et al., 2018; Martin et al., 2018a, 2018b; Conte et al., 2021), the influence of seagrass microbiome on the surrounding water column has remained unexplored. Here we investigated whether seagrass microbiomes, particularly the leaf epibionts that constitute a direct interface between the seagrass and the surrounding seawater, inhibit aquatic and other pathogens 'introduced' into the sea by natural and anthropogenic effects, thereby potentially contribute to the 'sanitary' effects of seagrasses. A culture-based approach allowed isolation and identification of 88 epiphytic and endophytic bacteria and fungi associated with the healthy leaves, decaying leaves and the roots of the eelgrass *Zostera marina* in Baltic Sea, plus 19 microbes deriving from surrounding seawater and sediment. All 107 isolates were cultivated in two different growth media. Ethyl acetate (EtOAc) extracts of 214 microbial cultures were assessed for their antimicrobial effect against 28 aquatic, fecal, human and plant pathogens. The most active 88 extracts were investigated by an UPLC-MS/MS-based untargeted metabolomics approach to identify their chemical constituents that may contribute to the observed antimicrobial effects. We show both fungal and bacterial isolates, particularly those originating from the healthy leaf surfaces of *Z. marina*, to have antipathogenic effects and rich secondary metabolomes, suggesting their potential involvement in the eelgrass sanitation services.

2. Materials and methods

2.1. Collection of Z. marina

Plant material was collected at Falckenstein Beach, Kiel Fjord, Baltic Sea (N 54°23′37.5, E 10°11′23.3) in August 2017. Environmental parameters, i.e., seawater temperature (17 °C), pH (pH 7, determined by pH-Fix indicator strips, Carl Roth GmbH, Karlsruhe, Germany) and salinity (13.3 psu, determined by conductivity measurement using a fast protein liquid chromatography system, GE ÄKTA Purifier FPLC System, GE Healthcare, Braunschweig, Germany) were measured. Eelgrass plants were collected from several spots of the same seagrass meadow by snorkeling (approx. 1.5 m depth). Seawater and surface sediment in immediate vicinity of the sampled seagrass shoots were collected as reference. For sampling eelgrass and seawater, sterile 50 ml reaction tubes were used. Sediment reference samples were collected by scraping off the first centimeter of surface sediment with sterile 50 ml reaction tubes. Gloves were worn throughout the sampling procedure and freshly disinfected immediately before each sampling. All samples were transported in a cooler box to laboratory and processed on the same day.

2.2. Isolation of Z. marina associated microbes

Among nine different agar media used for isolation of microorganisms, we applied six standard media for cultivation of marine-derived microorganisms: Marine Broth Agar (MA; 3.74 % Marine Broth 2216, Becton Dickinson, Sparks, MD, USA; 1.5 % agar bacteriology grade, AppliChem, Darmstadt, Germany), Potato-Dextrose-Agar (PDA; Oppong-Danquah et al., 2018 with 2 % glucose instead of 0.4 %), modified Wickerham medium (WSP30; adjusted to pH 5.8, 3 % Instant Ocean, Blacksburg, VA, USA; Silber et al., 2013), modified Wickerham medium prepared with Baltic seawater instead of Instant Ocean (WSP + BSW), Tryptic Soy Broth agar (TSB3 + 10; Utermann et al., 2020) and TSB agar prepared with Baltic seawater instead of sodium chloride (TSB + BSW). Modified Melin-Norkrans-Agar was used for cultivation of fungi (https://www.uamh.ca/-/media/uamh/OrderCultures/Documents/Media.pdf). In order to mimic the natural conditions in the seagrass meadow, two "*Z. marina* media" for cultivation of bacteria (ZMB) and fungi (ZMF) were designed: freeze-dried eelgrass leaves were ground into powder (Pulverisette 14, Fritsch, Idar-Oberstein, Germany). 1 % of the resulting seagrass powder and 1.5 % agar were added to Baltic seawater (ZMB). A penicillin-streptomycin mixture (Gibco™, Thermo Fisher Scientific, Dreieich, Germany) was added to the fungal ZMF medium to prevent bacterial growth.

For inoculation, plant samples were divided into roots (R) and leaves (L). Based on previous reports showing significant changes in the *Z. marina* leaf microbiome during aging (Newell, 1981; Sanders-Smith et al., 2020), the leaves were further split into mature healthy green (HL) and decaying brown leaves (DL) based on their appearance (no young leaves were used). Prior to dissection, plants were thoroughly rinsed with artificial sterile seawater (1.8 % Instant Ocean). For inoculation of epibionts from the plant surfaces (encoded by "S" for each plant organ, i.e., DLS, HLS, RS), two different methods were applied, namely surface swabbing and imprinting. For swabbing, surfaces were wiped with a sterile cotton swab, placed in sterile saline (1.8 % NaCl) and vortexed for 5 min. For imprinting, tissues were cut into 1 cm pieces, the surface was wiped with a circular movement on the agar surface and finally left on the agar plate. For isolating *endo*-microbiota (encoded by "I" (for inner) for each plant tissue, i.e., DLI, HLI, RI), surface disinfection was

performed using 70 % EtOH. Subsequently, the surface sterilized tissue pieces of approximately 1 cm length were ground with a sterile pestle in a reaction tube containing sterile saline. The homogenized material was vortexed for 5 min and 100 µl were plated on the different media (undiluted, 1:10 dilutions).

Sediment samples were resuspended in sterile saline and mixed for 5 min before plating 100 µl suspension (undiluted and 1:10 dilutions) on agar plates. Undiluted seawater samples were plated using two different volumes (100 µl, 500 µl). Samples were incubated in the dark at 22 °C. Morphologically different colonies were picked after 7 and 21 days of incubation. Purified strains were cryopreserved at −80 °C using the Microbank™ system (Pro-Lab Diagnostics, Richmond Hill, ON, Canada).

2.3. Molecular identification of isolates

DNA extraction of pure bacterial strains was done by an established freeze-and-thaw procedure, while DNA from fungal cultures was obtained by mechanical disruption (Utermann et al., 2018). If initial DNA extraction failed, the extraction process was repeated with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany; Utermann et al., 2020). Amplification of bacterial and fungal DNA was performed with universal primers targeting the 16S rRNA gene or the ITS1–5.8S-ITS2 region by using established standard protocols (Utermann et al., 2018; Utermann et al., 2020). Successfully amplified DNA fragments were sequenced (Sanger et al., 1977) at IKMB (Institute of Clinical Molecular Biology, Kiel University, Kiel, Germany) or LGC Genomics GmbH (Berlin, Germany) using primers 1387R (5'-CGGGCGGWGTGTACAAGGC-3′) or Eub27F (5'-GAGTTTGATCMTGGCTCAG-3′) for the bacterial 16S rRNA gene and ITS1 (5'-TCCGTAGGTGAACCTGCGG-3′) for the fungal ITS fragment. Sequences were trimmed (ChromasPro V1.33, Technelysium Pty Ltd., South Brisbane, Australia) and FASTA files were submitted to BLAST (Basic Local Alignment Search Tool; Altschul et al., 1990) at NCBI (National Center for Biotechnology Information). DNA sequences are available under the accession numbers OR400253-OR400328 (bacteria) and OR400216-OR400246 (fungi) at NCBI GenBank.

2.4. Cultivation and extraction of isolated microorganisms

Selection for cultivation and extraction was based on phylogeny to reflect a broad phylogenetic diversity, and on safety level (exclusion of BSL-2 organisms according to German safety guidelines TRBA 460 and TRBA 466 (Technical rules for biological agents for fungi and for bacteria). In total, 76 bacterial strains and 31 fungal strains were selected for cultivation (Supplementary Tables S2, S3). To provide the microorganisms differential nutritional sources, hence to achieve a richer chemical inventory, two agar media each were selected for cultivation of fungal and bacterial strains. Fungal strains were cultivated on potato dextrose agar (PDA; potato infusion powder: 0.4 %, D-glucose monohydrate: 2 %) and casamino acids glucose medium (M34, casein hydrolysate: 0.25 %, D-glucose monohydrate: 4 %, magnesium sulfate: 0.01 %, potassium dihydrogen phosphate: 0.18 %). Bacterial strains were grown on MA and Glucose Yeast Malt Agar (GYM4; D-glucose monohydrate: 2 %, malt extract: 0.4 %, yeast extract: 0.4 %, calcium carbonate: 0.2 %). All cultivation media were supplied with 3.5 % Instant Ocean (Aquarium systems, Sarrebourg, France) prior to adjusting the pH. Isolate pre-cultures were grown on plates with 1.5 % Agar Bacteriology Grade (AppliChem); the respective main-culture plates for extraction contained 1.2 % Agar Noble (Becton Dickinson).

Per pre-culture, three plates were inoculated using one bead per plate from the cryopreservation. For main cultures, the ten best grown plates were chosen for extraction. Fungal cultures were grown for 14 d, while bacteria were grown for 7 d at 22 °C in darkness. Main cultures were extracted with EtOAc (Pestinorm grade ≥ 99,8 %; VWR, Leuven, Belgium) and water as described by Utermann et al. (2021). The EtOAc extracts were stored at −20 °C.

2.5. Antimicrobial activity assays

Supplementary Table S1 displays the list of all pathogens, which were purchased from Leibniz Institute DSMZ (Braunschweig, Germany), Institut Pasteur (Paris, France) or Westerdijk Fungal Biodiversity Institute (Utrecht, Netherlands). Assays were performed in 96-well microplates at the initial test concentration of 100 µg/ml. Extracts inhibiting >50 % of the pathogen growth were subjected to IC_{50} determinations.

2.5.1. Assays against pathogenic bacteria

The extracts were tested against 28 bacterial and fungal pathogens divided into four panels reflecting i) 16 aquatic pathogens, ii) 5 human fecal pathogens, iii) 1 human pathogen (MRSA), and iv) 6 phytopathogens. The aquatic panel includes the bacteria *Algicola bacteriolytica* CIP 105725, *Lactococcus garvieae* DSM 20684, *Leifsonia aquatica* DSM 20146, *Pseudoalteromonas elyakovii* CIP 105338, *Shewanella algae* DSM 9167, *Vibrio aestuarianus* DSM 19606, *Vibrio alginolyticus* DSM 2171, *Vibrio anguillarum* DSM 21597, *Vibrio cholerae* DSM 100200, *Vibrio coralliilyticus* DSM 19607, *Aliivibrio fischeri* DSM 507, *Vibrio harveyi* DSM19623, *Vibrio ichthyoenteri* DSM 14397, *Vibrio parahaemolyticus* DSM 11058, *Vibrio splendidus* DSM and *Vibrio vulnificus* DSM 10143. All strains were cultivated in MB medium (0.5 % peptone, Becton Dickinson; 0.1 % yeast extract, Merck, Darmstadt, Germany; 3 % artificial sea salt Instant Ocean) except L. *garvieae*, *L. aquatica* and *V. vulnificus,* which were cultivated in M92 medium (3 % tryptic soy broth, Becton Dickinson; 0.3 % yeast extract). The human fecal panel covers *Enterococcus casseliflavus* DSM 7370, *E. faecalis* DSM 20478, *E. faecium* DSM 20477, *E. hirae* DSM 27815 and *Escherichia coli* DSM 1576. All enterococci were cultivated in M92 medium. TSB12 medium (1.2 % tryptic soy broth, 0.5 % NaCl) was used for *E. coli* and MRSA DSM 18827. Phytopathogenic bacteria *Pseudomonas syringae* DSM 50252, *Xanthomonas campestris* DSM 2405, *Erwinia amylovora* DSM 50901 were cultivated in TSB12 medium, whereas *Ralstonia solanacearum* DSM 9544 *R* was grown in M186 medium containing 1 % glucose, 0.5 % peptone from soybeans, 0.3 % yeast extract and 0.3 % malt extract (Becton Dickinson). To evaluate the antimicrobial activity of the crude extracts, a stock solution (20 mg/ml) in DMSO was prepared and transferred in duplicates into a 96-well microplate. 200 µl of the respective test organism were added to each well after diluting an overnight culture to an optical density (600 nm) of 0.01–0.03. Microplates containing bacteria of the phytopathogenic and aquatic panels were incubated for 5–6 h at 28 °C and shaken at 200 rpm, with the exception of *V. splendidus* (22 °C). *Vibrio ichthyoenteri, A. bacteriolytica* and *R. solanacearum* were cultivated for 18 h. L. *garvieae* and enterococci were incubated for 5–7 h at 37 °C without shaking, while MRSA and *E. coli* were incubated with shaking at 200 rpm. Subsequently, 10 µl resazurin solution (0.3 mg/ml in phosphate buffer) was added to each well and the microplates were incubated again for 5–60 min before measuring fluorescence (560/590 nm) using a microplate reader (Tecan Infinite M200, Tecan, Männedorf, Switzerland). For enterococci and L. *garvieae*, the pH indicator bromocresol purple was used to determine color/pH change due to acidification caused by the test strain growth. The color change was detected by absorbance (600 nm/reference 690 nm). The percentage of inhibition was calculated based on a negative control (no extract) and compared to a positive control. The broad-spectrum antibiotic chloramphenicol was used as positive control for most pathogens. Exceptions were made for enterococci and L. *garviae* (ampicillin) and *R. solanacearum* (tetracycline) based on literature and long-term experience in our routine bioassay pipeline (e.g., Oppong-Danquah et al., 2018, Utermann et al., 2021, Marino et al., 2021, Oppong-Danquah et al., 2023). To determine the IC_{50} values, a 1:2 dilution series of the extracts was prepared and tested as described above. A concentration depending graph was created with Excel and the IC_{50} value was calculated.

2.5.2. Assays against pathogenic fungi and oomycetes

The phytopathogenic fungus *Magnaphorte grisea* DSM 62938 and the

oomycete *Phytophthora infestans* CBS 120920 were cultivated on agar medium for 2 weeks until sporulation. *P. infestans* was inoculated on carrot medium (4 % grated carrot, 1.5 % agar) and *M. grisea* on GPY medium (0.1 % glucose, 0.05 % peptone, 0.01 % yeast extract, 1.5 % agar). A suspension of 1–5 × 10^4 spores/ml was prepared for each pathogen in liquid medium. Pea medium (150 g peas cooked, filtrated, filled up to 1 L with H_2O and 5 g glucose added, pH 6.5) was used for *P. infestans* and M186 medium for *M. grisea*. Crude extracts were prepared in microplates as described above and a volume of 200 µl of the spore suspension was added to each well. Microplates were incubated at 22 °C for 3 (*M. grisea*) or 4 days (*P. infestans*), and the absorbance was measured at 600 nm. The percentage inhibition and IC_{50} determinations were done as described above.

2.6. UPLC-ESI-QToF-MS/MS analyses

Ultra-high-performance liquid chromatography analysis of selected crude extracts (concentration 1 mg/ml in MeOH) was performed on an Acquity UPLC I-Class System (Waters, Milford, MA, United States) coupled to a Xevo G2-XS QToF Mass Spectrometer (Waters) operated in fast data-dependent acquisition (DDA) mode controlled by MassLynx version 4.2. ULC-MS grade solvents were purchased from Biosolve Chimie, Dieuze, France. The volume injected was set to 0.3 µl for bacterial crude extracts and 0.6 µl for fungal crude extracts. All samples were analysed in triplicate. Chromatographic separation was achieved on a CORTECS UPLC C18 column (1.7 mm, 100 × 2.1 mm, Waters) at a temperature of 40 °C. The mobile phase was composed of a mixture of (A) water with 0.1 % formic acid (*v*/v) and (B) acetonitrile with 0.1 % formic acid and pumped at a rate of 0.4 mL/min. The elution gradient was set as follows: 0.0–8.5 min, gradient from 30 % to 99 % B; 8.5–14.5 min, isocratic 99 % B; back to initial condition in 0.1 min, for 5 min. Mass spectrometry data in the range of *m/z* 50–1200 Da were acquired with electrospray ionization source (ESI) in positive ion detection mode with the following parameters: spray voltage of 3 kV, cone gas flow of 50 L/h, desolvation gas flow of 1000 L/h, source temperature of 150 °C, desolvation temperature of 500 °C with sampling cone and source offset at 40 and 60, respectively. The MS/MS experiments were carried out in tandem with ramp collision energy (CE): Low CE from 6 to 60 eV and a high CE of 9 to 80 eV. The solvent (MeOH) was run under the same conditions.

2.6.1. UPLC–MS/MS data processing and molecular networking

UPLC–MS/MS raw data were converted to mzXML file format (MSconvert, Chambers et al., 2012) and then processed in batch mode with the software MZmine 3.2.8 (Schmid et al., 2023). The output files (.csv and .mgf) were exported to GNPS (Global Natural Products Social Molecular Networking) and networks were created with the feature-based molecular networking (FBMN) workflow (Wang et al., 2016; Nothias et al., 2020). Networks were generated with the following parameters: a precursor ion mass tolerance 0.02 Da, MS/MS fragment ion tolerance 0.02 Da, cosine score > 0.6, minimum number of matched peaks ≥6, maximum number of neighbour nodes = 10, maximum number of nodes in a single network = 100. The spectra in the networks were searched against GNPS spectral libraries. A score above 0.6 and at least 6 matching peaks were required to keep matches between network spectra and library spectra. Generated networks were visualized using Cytoscape version 3.9.1 (Shannon et al., 2003) with the edges modulated by cosine score. The analysis of the networks was simplified showing only nodes representing ions in a *m/z* range of 180–1200 Da. Nodes originating from the solvent (MeOH) were removed from the MN. In the networks, node colors were mapped based on the source of the spectra files or on the culture conditions. For compound annotation, GNPS library search was combined with automated, manual, and *in-silico* dereplication tools (SIRIUS with CANOPUS and CSI:FingerID tools, plus MolDiscovery (v.1.0.0) and DEREPLICATOR+ tool, available on GNPS (Dührkop et al., 2015; Djoumbou Feunang et al., 2016; Dührkop et al., 2019; Cao et al., 2021; Dührkop et al., 2021; Kim et al., 2021)). Additionally, we searched putative molecular formulae, predicted with MassLynx version 4.1, against natural product databases such as COCONUT (https://coconut.naturalproducts.net/ accessed 11 August 2023, Sorokina et al., 2021), LOTUS, (https://lotus.naturalproducts.net/ accessed 11 August 2023, Rutz et al., 2022) MarinLit (http://pubs.rsc.org/marinlit/ accessed 11 August 2023), Dictionary of Natural Products (https://dnp.chemnetbase.com accessed 11 August 2023), The Natural Products Atlas (https://www.npatlas.org/joomla/index.php/search/basic-search accessed 11 August 2023, van Santen et al., 2019) and Scifinder (https://scifinder-n.cas.org accessed 11 August 2023).

3. Results

3.1. Isolation and identification of microorganisms

By using 9 isolation media, >300 microbial strains were retrieved from the surfaces and the inner tissues of healthy and decaying eelgrass leaves, eelgrass roots, plus the sediment and seawater references. The strain number was reduced to 107 by exclusion of strains with identical sequence or biological safety level 2. The majority of strains were bacteria (76 strains, 71 %) and only 31 (29 %) were fungi (Fig. 1, Supplementary Tables S2 and S3). The MA medium afforded the largest portion of bacterial strains (28 %), while PDA and the *Z. marina* media ZMB and ZMF were the most prolific media for fungal growth yielding altogether 68 % of fungal isolates.

Bacterial strains derived from the healthy leaf surfaces (HLS, 31 strains) dominated the isolates (41 %; Fig. 1). Additional 19 bacterial strains were obtained from decaying leaf surfaces (DLS, 7 strains) and root surfaces (RS, 12 strains), while 20 strains were retrieved from inner tissues. The striking difference in the number of isolates between the HLS (31 strains) and HLI (5 strains) indicated the abundance of the epiphytic bacterial community on eelgrass phyllosphere. Most fungal strains were obtained from DLS (11), whereas inner tissues of decaying leaves (DLI) afforded a single fungal strain. No fungi were isolated from the inner tissues of healthy leaves or roots. Altogether 6 bacterial and 13 fungal strains were retrieved from the reference samples. Overall, the *Z. marina* epibiome showed the highest microbial diversity (63 % of all strains). The eelgrass endosphere was much lesser colonized by bacteria and fungi (20 % of all strains).

Bacterial isolates belonged to four phyla, Proteobacteria, Actinomycetota, Bacteroidota and Firmicutes (Supplementary Fig. S1A). Proteobacteria dominated most seagrass sources (HLS: 58 %, DLS: 71 %, DLI: 40 %, RS: 50 %, RI: 80 %). Proteobacteria were assigned either to the class of Gammaproteobacteria (66 % of all proteobacterial strains) or Alphaproteobacteria (34 %) with healthy blades being the richest source for both classes. All bacterial endophytes of the healthy eelgrass blades were assigned to Firmicutes, which showed either low abundance (HLS, RS, DLI) or were absent (DLS, RI) in other samples. Bacteroidota were isolated from all surfaces (HLS: 19 %, DLS: 14 %, RS: 33 %), however endophytic Bacteroidota were only detected in decaying leaf tissues (20 %). The largest number of Actinomycetes was obtained from HLS (5 strains). While absent from HLI, Actinomycetota was the sole or main component of the microbiota of the reference samples (W: 100 %, S: 80 %).

Ascomycota clearly dominated the culturable eelgrass mycobiome (67–100 %, Supplementary Fig. S1B), and was the sole component of both healthy (3 strains) and decaying leaf surfaces (11 strains) and the endosphere of the decaying leaves (1 strain). The fungal root epiphytes were assigned to Ascomycota (2 strains) and Basidiomycota (1 strain). Seawater (W)-derived fungi showed a similar distribution. The comparably rich mycobiome of the sediment (S) reference was dominated by Ascomycota (89 %). One strain belonged to the phylum Mucoromycota.

Bacterial strains isolated from *Z. marina* and reference samples were affiliated to 49 genera (Fig. 2A, Supplementary Table S2). Overall, there was little overlap between different eelgrass tissues, since the majority

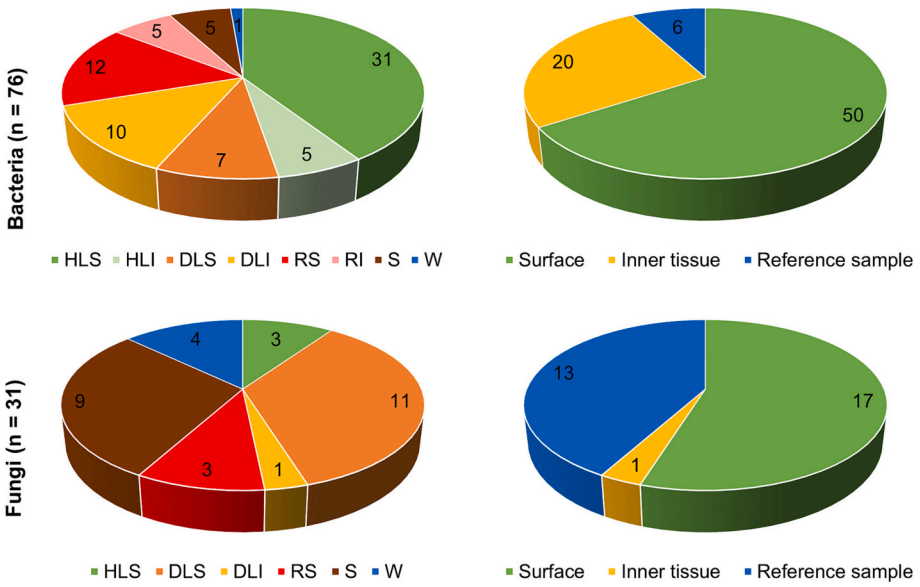

Fig. 1. Isolation source of bacterial (top) and fungal (bottom) strains from eelgrass and reference samples. HLS: Healthy leaf surface, HLI: Healthy leaf inner tissue, DLS: Decaying leaf surface, DLI: Decaying leaf inner tissue, RS: Root surface, RI: Root inner tissue, W: Seawater reference, S: Sediment reference.

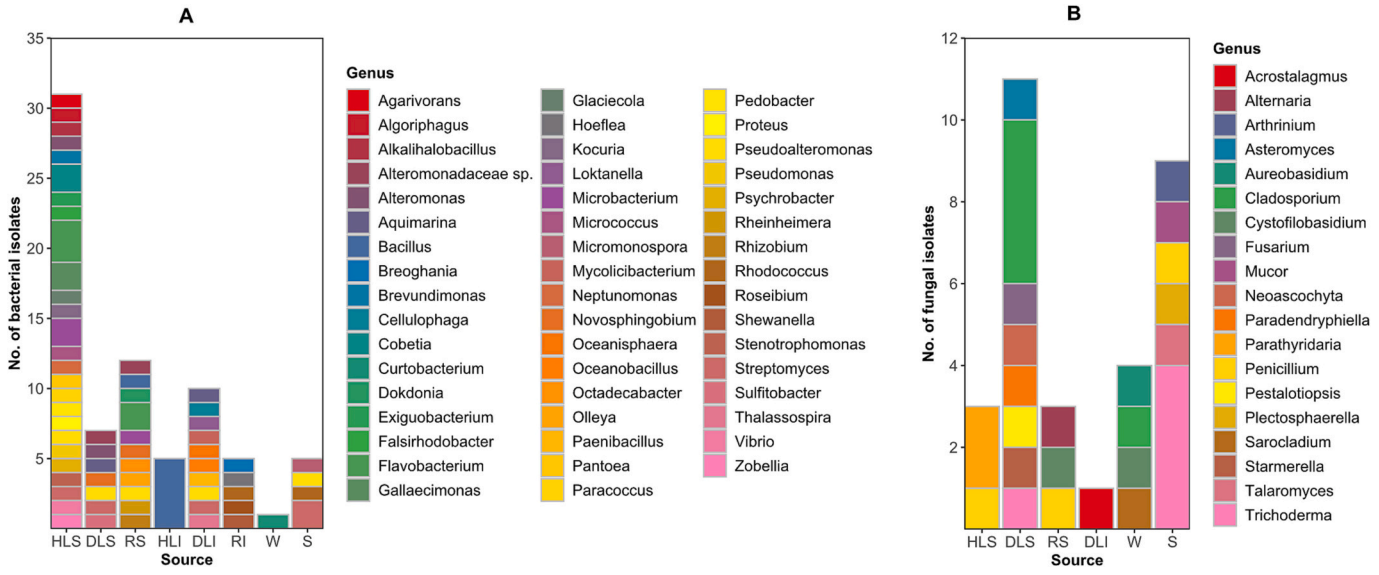

Fig. 2. Diversity of all bacterial (**A**) and fungal (**B**) strains at genus level. HLS: Healthy leaf surface, HLI: Healthy leaf inner tissue, DLS: Decaying leaf surface, DLI: Decaying leaf inner tissue, RS: Root surface, RI: Root inner tissue, W: Seawater reference, S: Sediment reference.

of bacterial genera were exclusive to one sample source (82 %) and no bacterial genus was common to all eelgrass samples. With 26 different genera, HLS were by far the most diverse source, with 21 genera being exclusive to it (e.g., *Exiguobacterium*, *Glaciecola*, *Micrococcus*, *Stenotrophomonas*, *Vibrio*). Only five genera were shared with other surface samples: *Flavobacterium* and *Microbacterium* (roots), *Alteromonas* and *Streptomyces* (decaying leaves) and ubiquitous *Pseudoalteromonas* sp. from all surfaces. Root surfaces yielded 11 genera, five of which being unique to RS (e.g., *Dokdonia*, *Octadecabacter*). Bacteria associated with DLS generally resembled to other plant tissues, *Sulfitobacter* was the only genus exclusive to DLS. Notably, the root and leaf endosphere (both healthy and decaying) were much less diverse than the eelgrass surfaces. All five strains obtained from HLI were identified as *Bacillus* spp., overall the most abundant bacterial genus in this study. Root endophytic bacteria were assigned to 5 genera exclusive to RI. Notably, DLI and DLS shared several bacterial genera common to the marine habitat (*Aquimarina*, *Pseudoalteromonas*, *Streptomyces*). In addition, the DLI hosted seven genera (e.g., *Mycolicibacterium*, *Thalassospira*) that are absent from all other eelgrass tissues.

Reference samples had very low bacterial diversity: *Curtobacterium herbarum* 218 was the only bacterium obtained from seawater (W, Fig. 2A, Supplementary table S2). Sediment-associated (S) bacteria were assigned to four different genera, of which all but one (*Micromonospora*) were detected also in eelgrass samples

The cultivable eelgrass mycobiome was considerably less diverse, yielding only 19 different genera (Fig. 2B, Supplementary Table S3). Decaying leaf surfaces were the richest source of fungal strains (8 genera) hosting six genera exclusively. Surfaces of healthy leaves and roots showed comparably lower fungal abundance. The phylloplane of healthy blades hosted the fungal genera *Parathyridaria* and *Penicillium*. The root phylloplane mycobiome was composed of *Penicillium glabrum* (strain 403), *Cystofilobasidium bisporidii* (strain 417) and *Alternaria* sp.

(strain 903a). Notably, culturable fungi were absent from the endosphere of healthy leaves and roots. Only one fungal endophyte (*Acrostalagmus luteoalbus* strain 720) was isolated from decaying leaves. Accordingly, DLS afforded the most diverse fungal consortium.

S-associated fungi (Fig. 2B, Supplementary Table S3) were, with six different genera, the second most diverse sample source dominated by *Trichoderma* sp. This genus and *Penicillium* were also detected in the phylloplane, while all other genera were exclusive to the sediment reference. W-reference yielded only four fungal isolates, two of which (*Aureobasidium*, *Sarocladium*) being unique for it. Despite some overlap, the majority of seagrass-associated taxa were absent in reference samples indicating a seagrass-specific mycobiome.

3.2. Assessment of antimicrobial activity of the isolates

In total, 214 crude microbial EtOAc extracts (152 bacterial, 62 fungal) were assessed for their in vitro antimicrobial activity against 28 bacterial, fungal or oomycete pathogens belonging to 4 panels (Supplementary Tables S4-S11). The first test panel (aquatic) included common pathogens of the aquatic/marine environments affecting marine animals (fish, shellfish, shrimps, corals, sea urchins) and seaweeds, also causing illness in humans through ingestion (seafood consumption, e.g., *V. parahaemolyticus*, drinking of contaminated water, e.g., *V. cholerae*) or skin contact (e.g., *V. vulnificus*) (Baker-Austin et al., 2018). The fecal pathogen panel included commensal intestinal bacteria

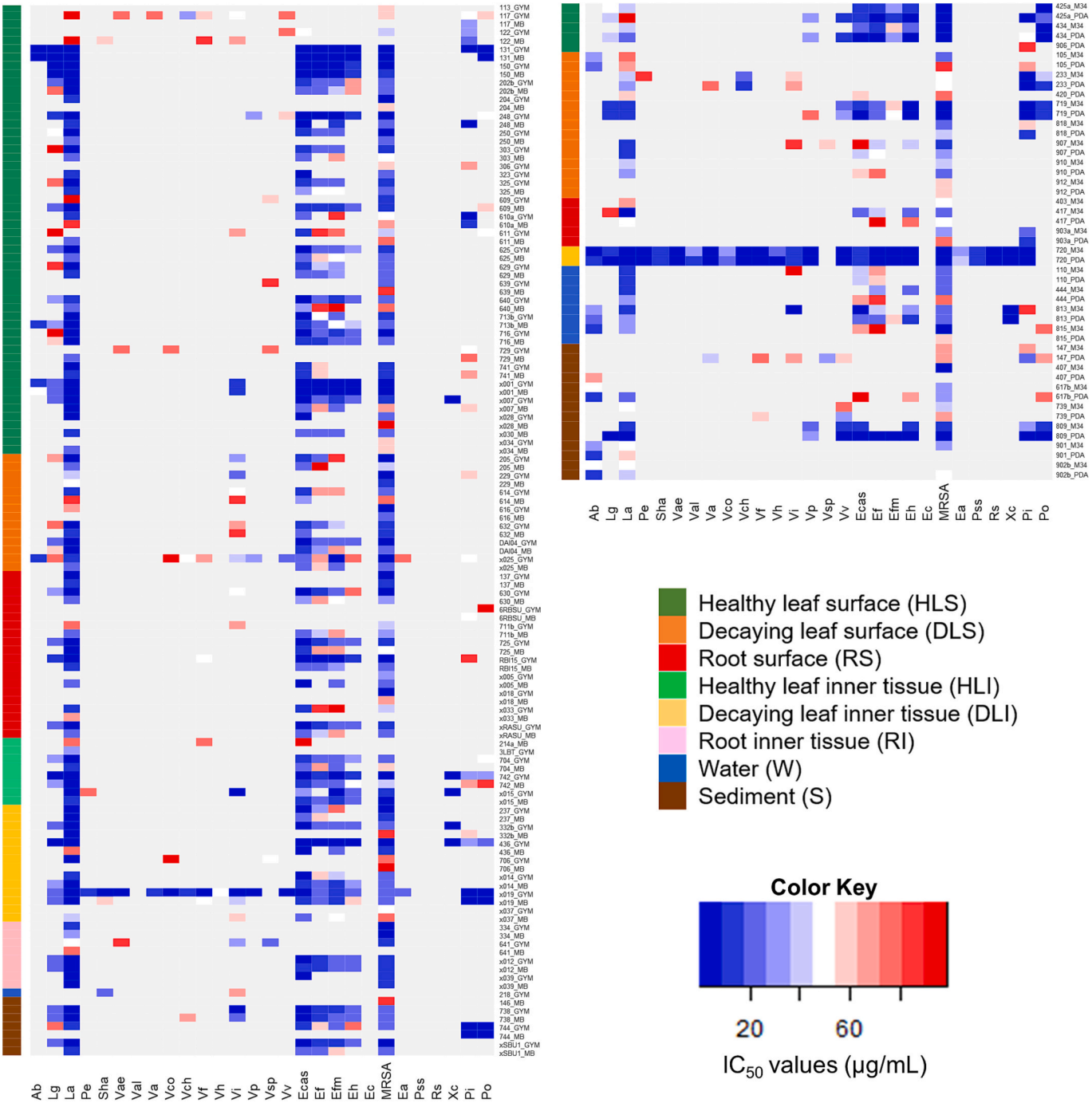

Fig. 3. Antimicrobial activity of bacterial (left) and fungal (right) extracts with IC_{50} values ≤100 µg/ml against test pathogens. Abbreviations for test pathogens are shown in the Supplementary Table S1.

that are mainly discarded to seawater by humans (*Enterococcus* spp., *E. coli*; Noble et al., 2003). Contact with seawater has also been associated with an enhanced risk of *Staphylococcus* infections, including MRSA, which is shed by human skin, feces or by wastewater contamination, and transmitted back to human by sea bathing (Levin-Edens et al., 2011; Plano et al., 2011). MRSA is often detected in surface- and wastewaters (Denissen et al., 2022), hence was included as the third 'human' pathogen panel. The pathogens of fecal and human panels cause gastrointestinal and other critical infections in human (Ascioti et al., 2022). The last panel entailed economically important terrestrial plant pathogens, some belonging to genera reportedly affecting seagrasses, e.g., *Phytophora* (Govers et al., 2016; Sullivan et al., 2018), which are washed into the sea by large watersheds and riverine input.

Due to the large number of extracts and test pathogens, we visualize the antimicrobial activity of the isolates in heatmaps (Fig. 3) displaying the extracts with IC_{50} value (i.e., inhibiting the growth of the test pathogen (≥50 %) at the initial screening at 100 μg/ml concentration). For simplicity, we use, whenever appropriate, abbreviations showing the origin (e.g., HLS) and the medium (e.g., GYM) together to describe the extract of an isolate, e.g., HLS-GYM bacterium.

3.2.1. Bioactivity of epiphytic bacterial extracts

The **GYM** extract of **HLS**-associated (HLS-GYM) *Streptomyces* sp. strain 131 was most active against two test organisms in the **aquatic panel**, *L. aquatica* (La) and the seaweed pathogen *Algicola bacteriolytica* (Ab, both IC_{50}s 1.8 μg/ml, Fig. 3, Supplementary Table S4). Gammaproteobacteria dominated the observed activities of the HLS-GYM bacterial extracts. For example, *Psychrobacter nivimaris* 741 and *Pseudomonas* sp. x028 inhibited only La, while *Stenotrophomonas* sp. 150 was equally active against La and Lg (IC_{50}s 6.6–8.6 μg/ml). *Cobetia* sp. x001 inhibited Ab and *V. ichthyoenteri* (Vi) with moderate IC_{50} values and *Vibrio* sp. 248 had good activity against La, Lg, *V. parahaemolyticus* (Vp) and *V. vulnificus* (Vv). The **MA** extract of HLS bacterium *Streptomyces* sp. 131 inhibited the same aquatic pathogens with higher potency (Fig. 3, Supplementary Table S6). Its potency towards the gram-negative seaweed pathogen Ab was approx. 4 times greater than the standard drug chloramphenicol (IC_{50}s 0.8 and 2.9 μg/ml, resp.). Seven HLS originated Gammaproteobacteria exerted low potential against La. *Proteus* sp. 713b and *Cobetia* sp. x001, inhibited La, Lg and Ab. The best anti-*Vibrio* effect was shown by the MA extract of *Cobetia* sp. x001 against Vi.

The majority of **HLS-GYM** extracts had remarkable activity against multiple **fecal pathogens**, particularly against *Enterococcus casseliflavus* (Ecas), *E. faecalis* (Ef) and *E. faecium* (Efm, Fig. 3, Supplementary Table S5). HLS-GYM extract of *Streptomyces* sp. 131 inhibited the growth of all fecal *Enterococcus* spp. (IC_{50}s 1.6–6.4 μg/ml). Half of the bacterial **HLS-MA** extracts inhibited at least two **fecal pathogens** (Fig. 3, Supplementary Table S7). HLS-MA extract of *Streptomyces* sp. 131 potently inhibited the growth of all enterococci with equal (against *E. hirae*, Eh) or 8 and 2.5-times higher efficacy (against Ecas and Ef, resp.) than the positive control ampicillin. Both GYM and MA extracts of many Gammaproteobacteria, *Stenotrophomonas* sp. 150, *Vibrio* sp. 248 and *Cobetia* sp. x001, had anti-*Enterococcus* potential (Fig. 3, Supplementary Tables S5 and S7). The MA extract of the latter exerted equal potency as ampicillin (IC_{50} 2.4 μg/ml) against Ecas. *Escherichia coli* (Ec) was not susceptible to any bacterial or fungal extract.

The **human pathogen MRSA** was susceptible to **HLS-GYM** extracts, many of which showed cross activity against enterococci (Fig. 3, Supplementary Table S5). Except for three isolates, all HLS-GYM extracts inhibited MRSA. The anti-MRSA activity of the Gammaproteobacterium *Pantoea* sp. 716 was higher than of the reference compound chloramphenicol (IC_{50}s 0.9 and 1.5 μg/ml, resp.), followed by *Streptomyces* sp. 131 and *Vibrio* sp. 248. Twenty-four **HLS-MA** bacterial extracts were active against MRSA (Fig. 3, Supplementary Table S7). Again *Streptomyces* sp. 131 showed extraordinary anti-MRSA activity with almost 3-fold greater potency than chloramphenicol (IC_{50}s 0.6 and 1.5 μg/ml, resp.). *Cobetia* sp. x001 was the second most active strain, while the remaining bacterial extracts had lower inhibitory potentials. **Phytopathogens** were less susceptible, *Phytophthora infestans* (Pi) and *Magnaphorte grisea* (Mg) were inhibited by few GYM extracts (Fig. 3, Supplementary Table S5). The GYM-HLS *Streptomyces* sp. 131 was highly effective versus Mg (IC_{50} 1.2 μg/ml). Only the GYM-HLS extract of *Alkalihalobacillus hwajinpoensis* x007 inhibited *Xanthomonas campestris* (Xc, IC_{50} 6.2 μg/ml). The **MA** extract of *Streptomyces* sp. 131 was active against Mg with an IC_{50} value close to the reference drug nystatin (IC_{50}s 0.5 and 0.3 μg/ml, resp., Fig. 3, Supplementary Table S7).

All **DLS-GYM** extracts inhibited the growth of at least one **aquatic pathogen** (Fig. 3, Supplementary Table S4). The most sensitive aquatic pathogen, La, which was efficiently inhibited by multiple Gammaproteobacteria, e.g., Alteromonadaceae sp. 205, *Alteromonas stellipolaris* 632, *Pseudoalteromonas* sp. DAI04 (IC_{50}s 6.2 to 9.1 μg/ml). Five bacteria inhibited Lg and Vi with moderate potency. The GYM extract of *Streptomyces griseorubens* x025 had the broadest activity against six *Vibrio* spp., besides Ab and La. Most **DLS-MA** extracts exerted some activity against **aquatic pathogens** La, Lg and *V. harveyi* (Vh), with *Pseudoalteromonas* sp. DAI04 being the most active strain (Fig. 3, Supplementary Table S6).

Most of the **DLS-GYM** extracts inhibited **fecal pathogens** with *Streptomyces griseorubens* x025 and *Pseudoalteromonas* sp. DAI04 being the most active (Fig. 3, Supplementary Table S5). All DLS-GYM extracts had anti-MRSA activity, and the lowest IC_{50} values were shown by *S. griseorubens* x025 and *Sulfitobacter pontiacus* 616. The other GYM or MA extracts exhibited low or no activity towards the remaining pathogen panels (Supplementary Table S7).

Half of the **GYM** extracts of root surface (**RS**) bacteria inhibited the **aquatic panel** (Fig. 3, Supplementary Table S4). Besides few Gammaproteobacteria (e.g., *Pseudoalteromonas* sp. RBI15), the Alphaproteobacterium *Rhizobium* sp. 137 and *Bacillus* sp. 725 exhibited good activity against La, while several strains inhibited Lg, *Aliivibrio fischeri* (Vf) and *V. ichthyoenteri* (Vi). A similar trend was observed against **fecal pathogens**, *Pseudoalteromonas* sp. RBI15 being the most active against Ecas (Fig. 3, Supplementary Table S5). *Bacillus* sp. 725 was the only gram-positive bacterium inhibiting all enterococci. The majority of **GYM-RS** bacteria inhibited **MRSA**, and four Proteobacteria were the most active ones. **RS-MA** extracts had moderate activity versus La and **enterococci** (Fig. 3, Supplementary Tables S6 and S7).

3.2.2. Bioactivity of endophytic bacterial extracts

All five healthy leaf endophytic (**HLI**) bacteria belonged to *Bacillus* spp. Few **HLI-GYM** extracts showed low μg/ml level inhibition against **aquatic pathogens** La and Lg. *Bacillus* sp. strain x015 had good activity against Vi (Fig. 3, Supplementary Table S4). Similar bioactivity profile was obtained **HLI-MA** extracts (Fig. 3, Supplementary Table S6). Three GYM-HLI isolates inhibited multiple **enterococci** and **MRSA**, with *Bacillus* sp. 742 being most potent (Fig. 3, Supplementary Table S5). The GYM extracts of *Bacillus* spp. 742 and x015 had notable **anti-phytopathogenic** effect towards *X. campestris*. Most **HLI-MA** extracts were moderately active against **fecal pathogens**. HLI-MA *Bacillus* sp. x015 had comparable anti-**MRSA** potency to that of chloramphenicol (IC_{50}s 2.2 and 1.5 μg/ml, resp., Fig. 3, Supplementary Table S7).

Approximately the half of the **DLI-GYM** bacteria inhibited **aquatic pathogens** La and Lg, some showing low activity against *V. coralliilyticus* (Vc) and *V. splendidus* (Vs; Fig. 3, Supplementary Table S4). *Streptomyces* sp. x019 stood out for its broadest spectrum activity against many aquatic pathogens, plus strong activity towards six *Vibrio* spp. (IC_{50} values 1.5 to 2.2 μg/ml). It was the only bacterial DLI-GYM extract that inhibited *Shewanella algae* (Sha, IC_{50} 6.6 μg/ml). A similar bioactivity profile was observed with the **DLI-MA** extracts, many inhibiting La. DLI-MA *Streptomyces* sp. x019 exerted the highest potential against La and to some extent against Lg, and Gram-negative pathogens Sha, Vf and Vi (Fig. 3, Supplementary Table S6). Half of the both DLI-GYM and MA extracts inhibited **enteric pathogens** and **MRSA**, with *Paenibacillus*

terrae 436 being the most active (Fig. 3, Supplementary Tables S5, S7). Several gram-positive isolates showed prominent activity against **phytopathogens**. Both media extracts of DLI bacterium *Streptomyces* sp. x019 showed comparable or better activity against Mg (IC_{50}s 0.6 (GYM) and 0.2 μg/ml (MA)) than the positive control nystatin (IC_{50} 0.3 μg/ml).

A similar activity profile was seen with both media extracts of root-endophytic (**RI**) bacteria, most inhibiting **aquatic panel**, with Alphaprotobacteria *Hoeflea alexandrii* x039, *Breoghania corrubedonensis* 334, and Gammaprotobacterium *Shewanella* sp. x012 being the most active (Fig. 3, Supplementary Tables S4-S7). The RI-GYM extracts had broader activity profile, e.g., *Roseibium marinum* 641 moderately inhibited *V. splendidus* (Vs), Vi and *V. aestuarianus* (Vae). *Shewanella* sp. x012 inhibited all four enterococci regardless of the medium, while the GYM-RI *Hoeflea alexandrii* x039 targeted Ecas (IC_{50} 4.4 μg/ml; Fig. 3, Supplementary Tables S5 and S7). The majority of RI bacteria were active against **MRSA** but not against **phytopathogens**.

Sediment-derived (**S**) bacteria extracts had broad activity when cultured in GYM medium (Fig. 3, Supplementary Tables S4-S7). *Streptomyces* sp. 738 exerted best activity against La, Vi and Ecas, and *S. scopiformis* 744 against phytopathogens Pi and Po in both media. The GYM extract of *Pseudoalteromonas ulvae* xSBU1 inhibited La, Ecas, MRSA but none of the phytopathogens.

3.2.3. Bioactivity of epiphytic fungal extracts

HLS-associated *Parathyridaria* spp. mildly inhibited **aquatic pathogens** La or Lg in both media (Fig. 3, Supplementary Tables S8, S10). Their PDA extracts showed also a moderate anti-*Vibrio* activity against Vp and Vv. Both media extracts of *Parathyridaria* spp. inhibited fecal panel but the efficacy was higher in the PDA extracts (Fig. 3, Supplementary Tables S9 and S11). The same trend applied towards **MRSA** (IC_{50} values 2.1 to 6.5 μg/ml). M34 media extracts had a notable activity against **phytopathogen** Mg (IC_{50}s 2.9 and 4.5 μg/ml), and the PDA extracts against Pi and Mg.

With 10 strains, **DLS**-associated fungi represented the largest cohort with moderate activity (Fig. 3, Supplementary Tables S8-S11). Within the **aquatic panel**, e.g., La, Lg, Ab and few *Vibrio* spp. were inhibited modestly by *Cladosporium halotolerans* 233, *Cladosporium* sp. 907 and *Fusarium* sp. 719. The activities against **fecal** pathogens were more potent and broader when PDA medium was used. *Fusarium* sp. 719 showed the best potential towards Ecas and Efm. Most DLS-derived fungi exhibited anti-**MRSA** activity in both media, with *Fusarium* sp. 719 exhibiting the best activity. The **phytopathogens** Pi and Mg were inhibited by *C. halotolerans* 233 and *Fusarium* sp. 719 with PDA extracts being more potent (IC_{50}s 0.7 and 2.6 μg/ml, resp.)

Rhizoplane (**RS**) derived fungi were represented by 3 strains, but only the M34 extract of *Cystofilobasidium bisporidii* 417 displayed some activity against aquatic pathogen La, whereas the activities against other panel pathogens were low (Fig. 3, Supplementary Tables S8-S11).

3.2.4. Bioactivity of endophytic fungal extracts

Regardless of the medium, the only fungal isolate of decaying leaf-endosphere (**DLI**), *Acrostalagmus luteoalbus* 720, inhibited all aquatic pathogens, except for *V. splendidus*, with comparable IC_{50} values against La, Pe, Vch, Vf, Vp and Vv (Fig. 3, Supplementary Tables S8 and S10). *Acrostalagmus* sp. inhibited all 4 fecal pathogens (Supplementary Tables S9 and S11). The activity of both media extracts against Ef (IC_{50}s 0.8 and 1.0 μg/ml) was comparable to that of the ampicillin (IC_{50} 0.5 μg/ml). With IC_{50} values of 0.2 μg/ml (M34) and 0.5 μg/ml (PDA) against MRSA, it was 7- and 3-times more potent than chloramphenicol (IC_{50} 1.5 μg/ml). It also inhibited phytopathogen Pi (IC_{50}s 0.5 and 0.7 μg/ml).

Of the seawater-associated (**W**) fungi, *Aureobasidium pullulans* 813 gave the best activity profile (Fig. 3, Supplementary Tables S8-S11). When cultured in M34 medium, it killed reasonably the **aquatic pathogens** Vi, La and Ab. Most of the W-derived fungi inhibited multiple enterococci. Towards Ecas, the M34 extract of *A. pullulans* was as active as ampicillin (IC_{50}s 2.8 and 2.4 μg/ml, resp.). Independent of the growth medium, all four fungi were moderately active against **MRSA**. *A. pullulans* had equipotent activity against **phytopathogen** Xc in both media.

Only few sediment (***S***)-derived fungi showed activity (Fig. 3, Supplementary Tables S8-S11). *Penicillium olsonii* 809 stood out for its broad activity against **aquatic pathogens** La, Lg, Vv and Vp, especially when cultured in PDA medium. Its PDA extract inhibited all 4 **fecal pathogens** with comparable activity with some of the reference compounds, and **MRSA** with a better activity than chloramphenicol (IC_{50}s 1.0 and 1.5 μg/ml, resp.). Also *Trichoderma viride* 407 had anti-MRSA activity. Against the **phytopathogen** Mg, the PDA extract of *P. olsonii* 809 was more potent than the standard nystatin (IC_{50}s 0.2 and 0.3 μg/ml, resp.).

3.3. Untargeted metabolomics analyses

Next, we wanted to shed light into chemical composition of the bioactive *Zostera*-associated microorganisms and identify secondary metabolites that may underlie the observed antimicrobial activities. We set the bioactivity threshold (IC_{50} value) to 10 μg/ml -against any pathogen and from either culture medium-, and mined the metabolome of 88 extracts (71 bacterial and 17 fungal, Supplementary Tables S12 and S13) by an untargeted metabolomics strategy using UPLC-QToF-MS/MS-based Feature-Based Molecular Networking (FBMN, Nothias et al., 2020) workflow combined with other tools explained in material and methods section. Microbial extracts were divided into groups, epiphytes and endophytes, and further into bacterial and fungal cultures. Seawater and sediment references were analysed together with epiphytes. The metabolome of both media extracts obtained are presented together, the relative abundance of metabolites expressed in different media are shown in Supplementary Figs. S2-S4. Supplementary Tables S14-S17 and Supplementary Fig. S5 show further relevant information on putatively annotated compounds. Primary metabolite clusters (e.g., lipids, amino acids) are colored grey to emphasize molecular families (MFs) of secondary metabolites that are most likely to contribute to the detected antimicrobial activities. Annotated MFs are highlighted in frames.

3.3.1. Metabolome of epiphytic bacteria

Only one Gram-positive HLS associated bacterium (*Streptomyces* sp. strain 131) returned hits in the metabolome analyses and the majority of the annotated MFs were exclusive to this strain. The first MF belonged to streptophenazines where we could annotate streptophenazines B, C, E, G, H, I (Fig. 4, cluster A) and two oxo-streptophenazines (A and G, Fig. 4, cluster B). Streptophenazines (but not oxo-streptophenazines) possess activity versus Gram-positive pathogens (Liang et al., 2017; Bauman et al., 2019) and were most abundant in the MA medium extract (Supplementary Fig. S2).

The second MF was assigned to protonated (clusters A and E) and sodiated (clusters B-G) adducts of homononactic and nonactic acid type polyketides (Fig. 4). Clusters A and D included linear ones (secodinactin, homononactyl homononactate, bonactin, nonactyl nonactoate, ethyl homononactyl nonactate), while cluster B, C, *E*-G included the cyclic derivatives (monactin, nonactin, dinactin, trinactin, tetranactin, macrotetrolide C, Fig. 4 and Supplementary Table S14). Homononactic and nonactic acid are the building units of the macrocyclic tetralactones (also called as macrotetrolide antibiotics or nactins). Nactins are ionophoric compounds that potently inhibit Gram-positive bacteria, including *S. aureus*, *E. faecalis* (Kusche et al., 2009; Shishlyannikova et al., 2017) and pathogens of plants, fish and vertebrates (Islam et al., 2016). Tetranactin (cluster C) and dinactin (cluster F) are potent inhibitors of fungal phytopathogens (Liu et al., 2019; Zhang et al., 2020). Bonactin, a linear homononactic acid derivative, exhibits strong antibiotic activity towards Gram-positive and -negative bacteria, fungi (Schumacher et al., 2003; Huang et al., 2015) and the phytopathogen *M. oryzae* (Rabby et al., 2022). These metabolites can be linked the observed bioactivity of *Streptomyces* sp. 131 against enterococci, MRSA

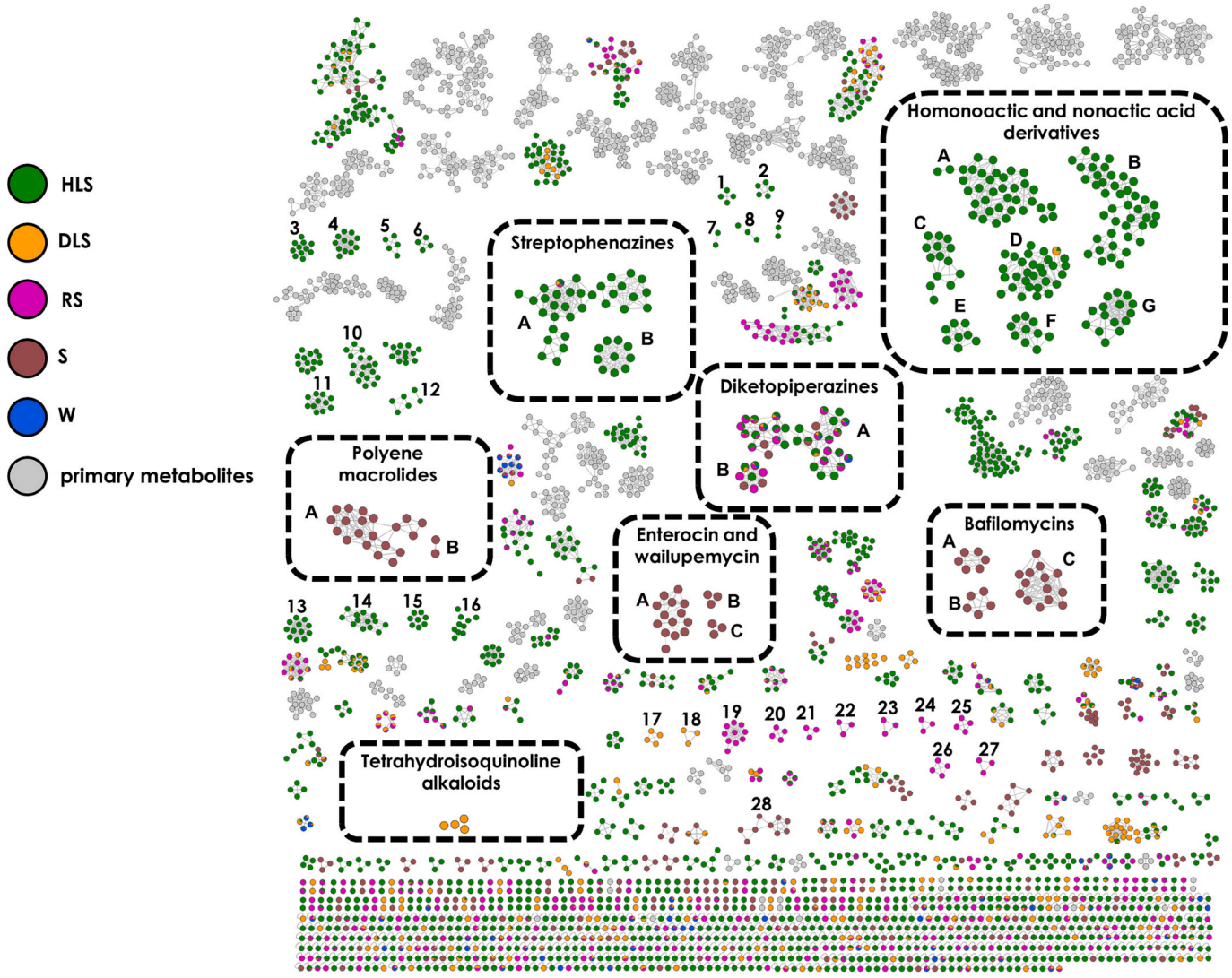

Fig. 4. Feature-based molecular network analysis of the two media extracts of the most active bacteria isolated from the surface of *Zostera marina* healthy leaves (HLS), decaying leaves (DLS), roots (RS), as well as the sediment (S) and seawater (W) references. Clusters of primary metabolites are shown in grey color. Annotated molecular families (MF) are framed.

and phytopathogens. The majority of the nodes were exclusive or more abundant in the MA extract, which possibly underlies the stronger activity observed (Supplementary Fig. S2, Supplementary Table S12). Many additional MFs exclusive to *Streptomyces* sp. 131 were found but none of them returned hits in databases (Fig. 4 and Supplementary Fig. S2).

The other HLS-derived Gram-positive bacterium, *Alkalihalobacillus hwajinpoensis* strain x007, accounted for three unique MFs, produced only in MA medium (Fig. 4 and Supplementary Fig. S2, clusters 7–9), but no compounds could be annotated. Eighteen HLS-associated Gram-negative bacterial extracts produced mainly fatty acids, phospho- and aminolipids, the abundant components of Gram-negative bacteria (Chianese et al., 2018). Many unannotated MFs (detected in both media extracts) were produced by *Vibrio metschnikovii* 248 (Fig. 4 and Supplementary Fig. S2, clusters 10–15) or *Cobetia* sp. x001 (Fig. 4 and Supplementary Fig. S2, cluster 16).

Two clusters shared by many HLS-, DLS-, RS- and reference isolates were annotated as diketopiperazines expressed in both culture media (Supplementary Fig. S2). From cluster A, we annotated cyclo(L-Val-L-Phe), cyclo(L-Pro-L-Leu), cyclo(L-Pro-L-Val), and cyclo(L-Trp-L-Pro) from cluster B (Fig. 4). Diketopiperazines are cyclic-dipeptides, displaying antibacterial, antifungal and anticancer activities (Borthwick, 2012; Bojarska et al., 2021). Cyclo(L-Pro-L-Val) and cyclo(L-Trp-L-Pro) show antibiotic activity against various bacteria including *S. aureus* (Borthwick, 2012; Alshaibani et al., 2017). Cyclo(L-Trp-L-Pro) inhibits human pathogenic fungi (Borthwick, 2012; Nishanth Kumar et al., 2014) while cyclo(L-Val-L-Phe) is a quorum sensing regulator (Guo et al., 2011). Cyclo(L-Pro-L-Leu) potently inhibits vancomycin-resistant enterococci (VRE) and fungal phytopathogens including *M. oryzae* (Rhee, 2002; Rhee, 2003).

DLS-associated bacteria presented several MFs, some of which were shared with the HLS-isolates. The majority of the MFs remained unannotated (Fig. 4). One cluster unique to GYM-DLS extract of *Streptomyces griseorubens* x025 was attributed to tetrahydroisoquinoline alkaloids (Fig. 4 and Supplementary Fig. S2). Two nodes were assigned as naphthyridinomycin *m/z* 418.1969, and bioxalomycin β2 *m/z* 400.1866 that are potent and broad-spectrum antibiotics against e.g., MRSA and *E. faecium* (Bernan et al., 1994; Zaccardi et al., 1994; Scott and Williams, 2002). These putatively identified compounds could relate the observed moderate bioactivities towards test pathogens (Supplementary Table S12). A few clusters detected in DLS-derived Gram-negative bacterial extracts (Fig. 4, clusters 17–18) could not be annotated.

A few MFs were exclusive to bacterial RS-isolates and many clusters were shared with the bacterial isolates of HLS, DLS and sediment

(Fig. 4). The majority of the MFs remained unannotated (Fig. 4). One cluster was specific to the Gram-positive bacterium, *Bacillus* sp. 725 (Fig. 4, cluster 19). The MFs belonging to Gram-negative RS-derived bacteria were grouped in small clusters (Fig. 4, 20–27) or appeared as singletons, but none could be annotated.

Moving to reference isolates, several clusters were expressed solely in sediment-derived (S) bacterial extracts (Fig. 4). Most clusters remained unannotated. Two clusters, expressed in both media extracts of *Streptomyces scopiformis* 744 (Fig. 4, Supplementary Fig. S2) belonged to polyene macrolides, dermostatin B and PN00053 (Fig. 4, clusters B and A, resp.) that display broad-spectrum antifungal activity (Vartak et al., 2014). Three clusters unique to *S. scopiformis* 744 were annotated as bafilomycins, macrolide antibiotics inhibiting Gram-positive bacteria, fungal human and phytopathogens (Werner et al., 1984; Zhang et al., 2011).

Three α-pyrone containing polyketide clusters (i.e., enterocin and wailupemycins) were exclusive to the sediment-associated *Streptomyces* sp. 738 (Fig. 4). From cluster A, we annotated 8-deoxyenterocin (*m/z* 429.1186) and enterocin (*m/z* 445.1130), which is a bacteriostatic agent (Miyairi et al., 1976). In the other two clusters, we annotated the bacteriostatic agents wailupemycins F (cluster B) and G (cluster C, Fig. 4, Sitachitta et al., 1996; Xiang et al., 2002).

3.3.2. *Metabolome of endophytic bacteria*

The extracts of HLI- and DLI-derived bacteria dominated the metabolome of the endophytic bacteria (Fig. 5). Two HLI-derived Gram-positive strains (*Bacillus* spp. x015 and 742) returned hits in database searches. Putatively identified compounds for *Bacillus* sp. x015 included cyclic (bacillomycin and surfactin clusters) and linear (gageostatin cluster) lipopeptides (Fig. 5). C15-bacillomycin D (Peypoux et al., 1984) was annotated only in the MA medium (Supplementary Fig. S3). Bacillomycins and gageostatins strongly inhibit MRSA (Nam et al., 2021) or pathogenic bacteria and phytopathogenic fungi (Tareq et al., 2014), and may underlie the observed antibacterial properties of the MA extract (Supplementary Table S12). The surfactin MF, solely produced by *Bacillus* sp. x015 in both culture media, was clustered in six networks comprising different adducts, i.e., $[M + NH_4]^+$ (cluster A), $[M + H]^+$ (clusters B, D, E), and $[M + Na]^+$ (clusters C). This cyclic peptidolipids were shown to inhibit Gram-positive, Gram-negative and phytopathogenic bacteria (Kim et al., 2009; Bartal et al., 2023), in line with the present study (Supplementary Table S12). The cyclic imine heptapeptides koranimine and koranimine B were exclusive to *Bacillus* sp. 742, being particularly abundant in its MA extract (Fig. 5 and Supplementary Fig. S3). Koranimine (*m/z* 804.5026) is a potent nematocidal (Evans et al., 2011; Montecillo and Bae, 2022). The GYM extract of HLI-isolate, *Bacillus* sp. 704, had one unannotated cluster (Fig. 5, cluster 27).

Two clusters, shared by all endophytic bacteria, but present more abundantly in DLI isolates, belonged to diketopiperazines (Fig. 5). We annotated cyclo(L-Val-L-Phe), cyclo(L-Pro-L-Leu), cyclo(L-Pro-L-Val) in cluster A and cyclo(L-Trp-L-Pro) in cluster B. The majority of the nodes

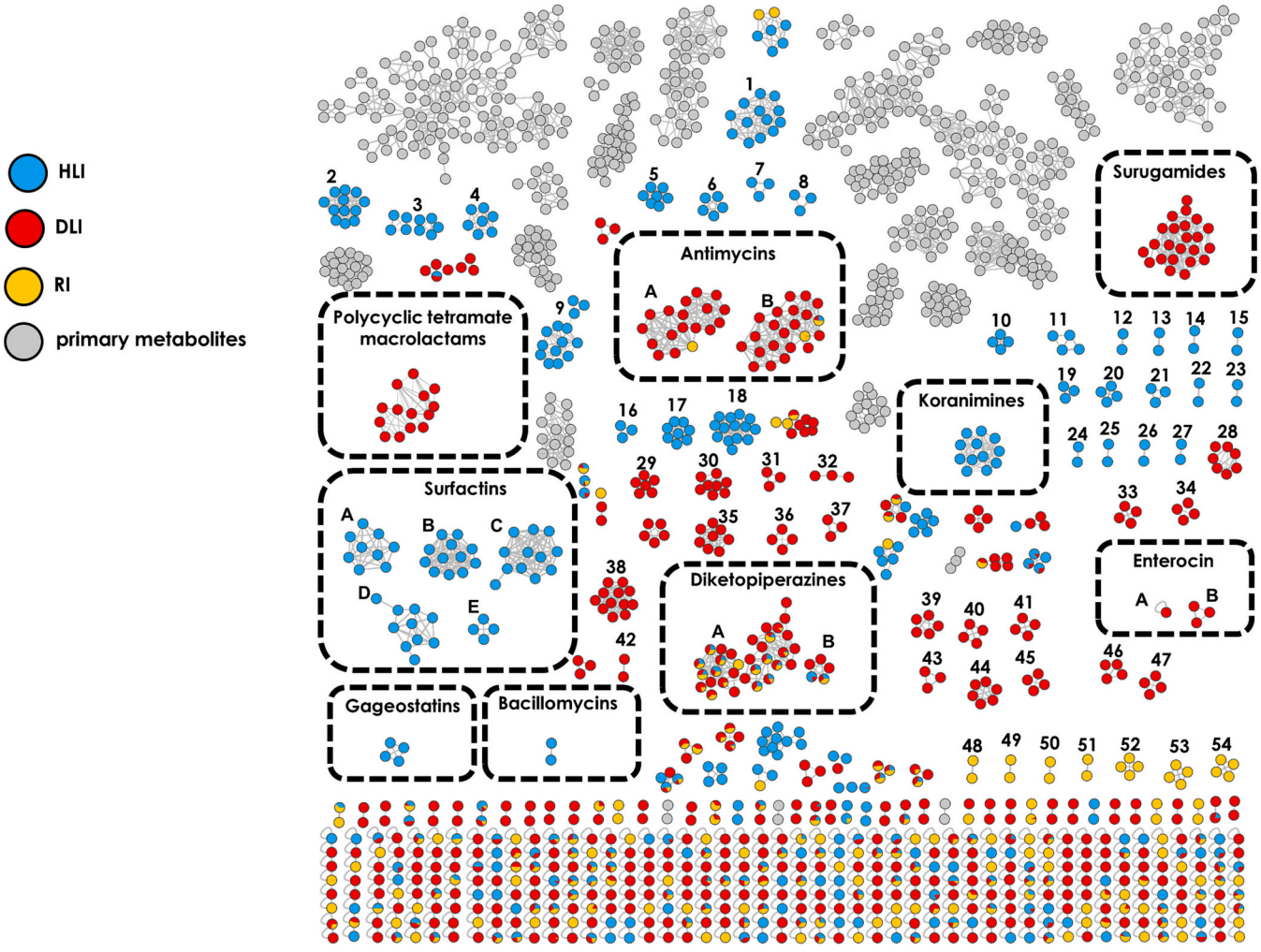

Fig. 5. Feature-based molecular network showing ions detected in the most active bacterial isolates from the inner tissues of *Zostera marina* healthy leaves (HLI), decaying leaves (DLI) and roots (RI) obtained from both culture media. Clusters grouping primary metabolites are colored in grey. Annotated molecular families (MF) are framed.

were exclusive to or more abundant in the MA medium extract (Supplementary Fig. S3).

Of the DLI-isolates analysed, *Streptomyces* sp. x019 showed the most interesting chemistry. A cluster belonging to surugamide type cyclic peptides (Almeida et al., 2019) was detected in both media extracts (Fig. 5, Supplementary Fig. S3). Two clusters were annotated as antimycin antibiotics and its deformylated derivatives (Fig. 5, clusters A and B, resp.), which are potent fungicides, especially against phytopathogens (Belakhov et al., 2018; Paul et al., 2022). Both clusters were dominant in the MA extract, possibly justifying the strong anti-phytopathogenic activity of this extract (Fig. 5, Supplementary Fig. S3, Supplementary Table S12). A further cluster belonged to polycyclic tetramate macrolactams, including ikarugamycin (*m/z* 479.2906), ikarugamycin epoxide that inhibit Gram-positive bacteria (Bertasso et al., 2003), and maltophilin and dihydromaltophilin that are broad spectrum fungicides (Fig. 5, Jakobi et al., 1996; Graupner et al., 1997). Finally, enterocin (GYM medium) and 8-deoxyenterocin (MA medium) were annotated (Fig. 5, cluster B and A, resp., Supplementary Fig. S3). The remaining DLI Gram-positive bacteria (*Paenibacillus terrae* 436 and *Oceanobacillus* sp. 332b) produced clusters 38–43, but no annotation was possible. Gram-negative bacterial DLI-isolates displayed many nodes clustered in lipid families and small unannotated clusters 44–47.

All selected bacterial root endophytes (RI) were Gram-negative, showing only few specific unannotated clusters (48–54). The majority of the MFs remained unannotated, except for three nodes in antimycin cluster and 18 nodes in diketopiperazine clusters (Fig. 5).

3.3.3. *Metabolome of epiphytic fungi*

Fig. 6 displays the global MN of all selected epiphytic fungi, while Supplementary Fig. S4 indicates the media effect on their metabolome. Of 12 clusters originating from the HLS fungi, three could be dereplicated as sodiated (cluster A) and protonated (clusters C, B) adducts of atranone type diterpenoids (Hinkley et al., 1999; Li et al., 2017) exclusive to *Parathyridaria* spp. 425a and 436. Some atranones exhibit antibacterial activity (Yang et al., 2019).

DLS-derived fungi produced many MFs, the few annotatable MFs deriving from *Fusarium* sp. 719. Naphthoquinone type mycotoxins clustered in two networks (Fig. 6, cluster A and B), some nodes were shared with the sediment-associated fungus *Penicillium olsonii* 809, as quinones are common constituents of filamentous fungi (Christiansen et al., 2021). Naphtoquinones, expressed in both culture media, are selective inhibitors of Gram-positive bacteria (Medentsev and

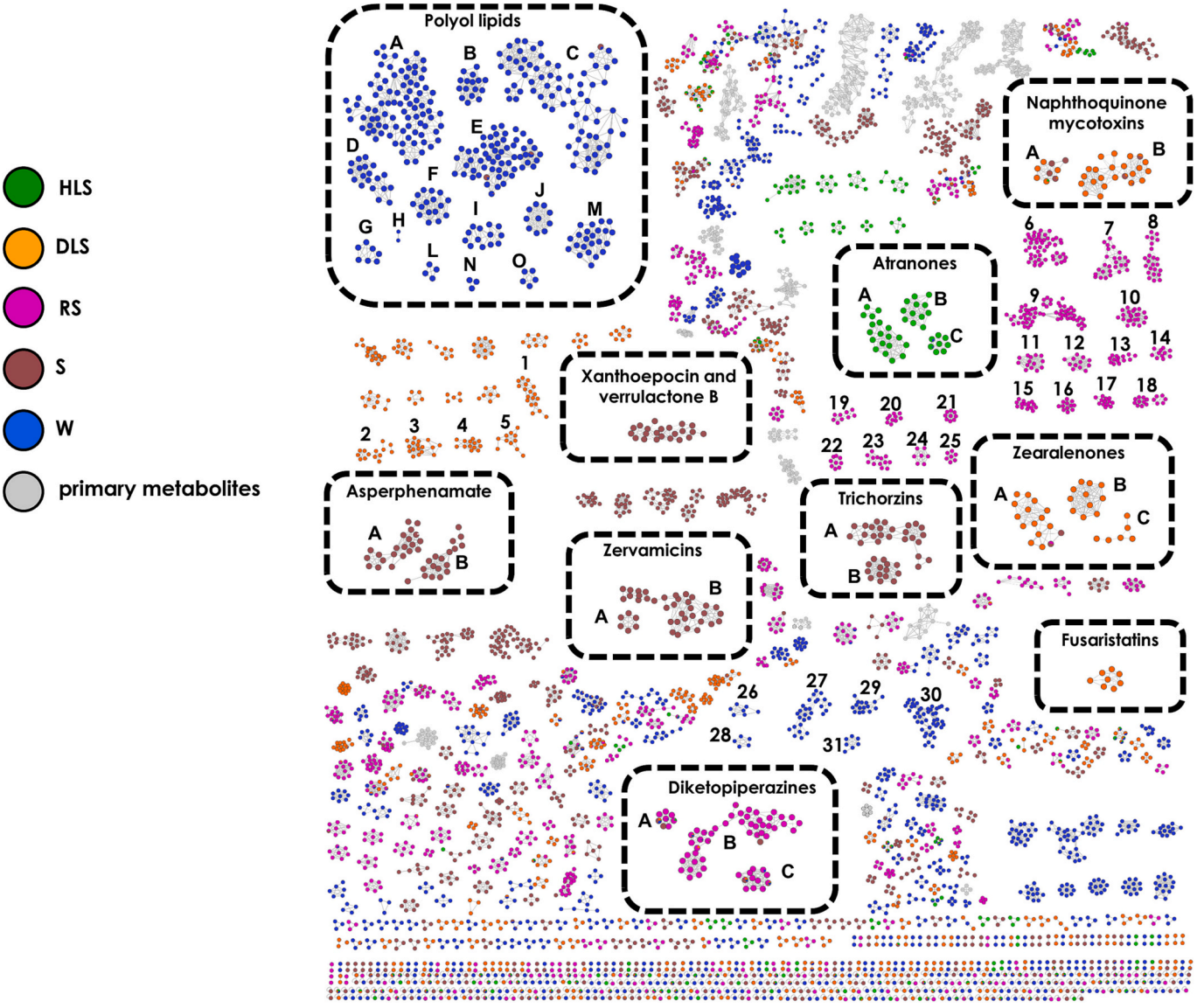

Fig. 6. FBMN showing ions detected in the most active fungi isolated from the surface of eelgrass healthy leaves (HLS), decaying leaves (DLS), roots (RS), and the sediment (S) and seawater (W) references obtained from both culture media. Annotated molecular families (MF) are framed.

Akimenko, 1998; Wang et al., 2019). Another network belonging to *Fusarium* sp. 719 was assigned to cyclic lipopeptides fusaristatin A and B, produced only in M34 medium. Fusaristatin A inhibits phytopathogens (Li et al., 2016), and may contribute to strong observed activity reported herein. Three MFs abundant in the M34 extract of *Fusarium* sp. 719 were annotated as zearalenones (Fig. 6, clusters A-C), polyketide mycotoxins, inhibitors of competing fungi and MRSA (Utermark and Karlovsky, 2007).

The only RS-isolate selected for metabolome analysis, *Cystofilobasidium bisporidii* 417, displayed unique MFs, but none were annotated (Fig. 6, clusters 6–25). Several diketopiperazine clusters that were shared by all surface associated fungi were more abundant in the M34 extract of *C. bisporidii* (Fig. 6, Supplementary Fig. S4). One of the annotated compounds, cyclo(L-Tyr-L-Pro) is a broad-spectrum antibiotic (Kumar et al., 2013; Wattana-Amorn et al., 2016).

Of many clusters expressed in the sediment-associated (S) reference fungal extracts, two were annotated as peptaibols (trichorzins and zervamicins) of *Trichoderma viride* 407; they are known for potent antibacterial and antiphytopathogenic activities (Duval et al., 1997; Goulard et al., 1995). The anticancer amino acid ester asperphenamate and its tyrosine derivative (Frisvad et al., 2013) were annotated from both media extracts of the S-derived fungus, *Penicillium olsonii* 809 (Fig. 6). The dimeric polyketide family (xanthoepocin and verrulactone B) was unique to *P. olsonii* 809 (Fig. 6). Xanthoepocin is known to inhibit pathogenic yeasts and multi-drug resistant bacteria (Igarashi et al., 2000; Vrabl et al., 2022). Also verrulactone B is a potent MRSA inhibitor (Kim et al., 2012). This MF was predominantly expressed in the PDA medium (Supplementary Fig. S4) and may contribute to the potent activity of *P. olsonii* 809 (Supplementary Table S13).

Seawater (W) associated fungi produced many MFs (Fig. 6). The largest cluster, mainly identified in *Aureobasidium pullulans* 813, belonged to polyol lipids (glycolipids) e.g., liamocins, exophilins and halymecins, most abundantly present in M34 extracts (Fig. 6,). Liamocins are selective inhibitors of *Streptococcus* spp. (Price et al., 2013; Bischoff et al., 2015), while exophilins inhibit enterococci and *S. aureus* (Bischoff et al., 2015). Among the annotated halymecins, halymecin A inhibits Gram-positive and Gram-negative bacteria (Chen et al., 1996; Le Dang et al., 2014) and halymecin F is a strong inhibitor of phytopathogenic bacteria (Le Dang et al., 2014). These results are in line with the moderate to good bioactivity observed for *A. pullulans* extracts against aquatic (e.g., *Vibrio ichthyoenteri*), fecal (*Enterococcus casseliflavus*) and phytopathogenic (*Xanthomonas campestris*) bacteria (Supplementary Table S13). Six clusters (Fig. 6, clusters 26–31) were exclusive for another W-derived fungus, *Sarocladium* sp. 815, but none could be annotated.

3.3.4. Metabolome of endophytic fungi

The FBMN of the only bioactive DLI-associated fungus *Acrostalagmus luteoalbus* 720 (Fig. 7) contained a variety of the epipolythiodioxopiperazine type mycotoxins (11′-deoxyverticillin A, verticillin B, C, chetracin C and chetocin) known for remarkable antitumor activities (Li et al., 2012; Huber, 2022). Chaetocin A inhibits penicillin-resistant *S. aureus* and *E. faecalis* (Hauser et al., 1970; Dwibedi et al., 2023). The second MF was dereplicated as diketopiperazines, expressed more abundantly in the M34 medium (Fig. 7). We annotated cyclo(L-Pro-L-Val), cyclo(L-Pro-L-Leu), cyclo(L-Tyr-L-Pro) and cyclo(L-Phe-L-Pro), which potently inhibits VRE enterococci, *S. aureus* and fungal pathogens (Rhee et al., 2001; Ström et al., 2002). The broad and potent activity of *A. luteoalbus* extracts may rather represent a non-specific toxicity towards test pathogens, possibly originating from epipolythiodioxopiperazines and diketopiperazines.

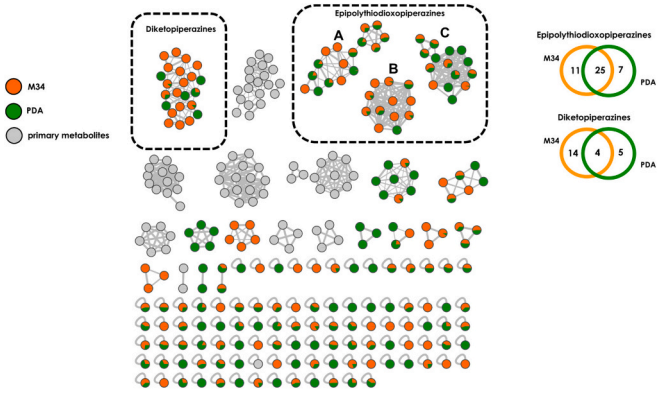

Fig. 7. FBMN showing ions detected in M34 and PDA media extracts of *Acrostalagmus luteoalbus* sourced from DLI. Clusters containing primary metabolites are colored in grey. Annotated molecular families (MF) are framed. The Venn diagram shows the number of exclusive and shared nodes of two culture media used for the annotated molecular families.

4. Discussion

4.1. Antipathogenic activity and chemical inventory of the eelgrass-associated microorganisms

The majority of eelgrass-associated microorganisms inhibited the growth of at least one pathogen. Gram-positive pathogens, such as the aquatic pathogen *Leifsonia aquatica*, fish pathogen *Lactococcus garvieae*, fecal *Enterococcus* spp. and human pathogen MRSA were highly susceptible towards eelgrass-associated bacteria and fungi. Gram-negative bacteria are generally difficult to inhibit, still, the seaweed pathogen *Algicola bacteriolytica,* fish pathogen *Shewanella algae* and multiple *Vibrio* spp. (e.g., *V. fischeri*, *V. ichthyoenteri*, *V. parahaemolyticus*) were hit. HLS-associated bacteria represented the largest and the most active group with broader antimicrobial activity. *Streptomyces* sp. 131 that exhibited potent antibiotic activity was an epiphyte of the healthy eelgrass leaves. Many gammaproteobacterial (Gram-negative) healthy leaf epiphytes, plus other surface associated bacteria (from DLS and RS) also proved active, albeit with lower potency. The inner tissues also hosted bacteria with antimicrobial properties. The most active fungi were those associated with DLS, e.g., *Cladosporium halotolerans* 233 and *Fusarium* sp. 719 being potently active against phytopathogens.

FBMN based untargeted metabolomics approach allowed comparative analysis of chemical machinery of the active strains isolated from different eelgrass parts, and the impact of the media on their biosynthetic capacity. Despite the use of massive computational, in silico and manual dereplication approaches, compound annotation rate was low, implying new chemistry in the extracts. Few taxa reputed with prolific chemistry, e.g., *Streptomyces*, *Penicillium* spp. returned hits in database searches, but only few nodes in large networks were dereplicated. Gram-negative bacteria were very rich in lipids, which may have masked the secondary metabolites.

The culture media used made significant differences in the chemical inventory (and the antibiotic activity) of the isolates. Nutrient-rich GYM medium with easily accessible carbon sources (glucose and malt extract) was favorable for overall bioactivity rate of bacteria, as the number of bioactive GYM extracts were 3-fold of the MA extracts, but the highest potency generally derived from the extracts of MA (nutrient-poor). In fungi, growth media had a less influence, but the PDA extracts often exerted better activity. This phenomenon (i.e., culture medium influencing the chemical composition and consequently the bioactivity of a microorganism) is well known in microbial drug discovery studies. It forms the basis of the popular OSMAC (One-Strain-Many-Compounds) strategy (Bode et al., 2002) that varies culture conditions, e.g., medium nutrient composition, salinity, temperature or pH to activate (silent)

biosynthetic gene clusters, thereby triggering the production of secondary metabolites (e.g., VanderMolen et al., 2013; Crüsemann et al., 2016; Utermann et al., 2021). The medium effect was notable in eelgrass-associated *Streptomyces* spp. (Fig. 3-5, Supplementary Tables S4-S7).

4.2. Pathogen reduction effect of seagrasses

Seagrass meadows influence their surrounding environments, and this includes the alteration of the microbial community structure, diversity and abundance in their vicinity (Webb et al., 2019; Mohapatra et al., 2022). Little is known on the effect of seagrass meadows on the seawater microbiome, but differences in bacterial taxa between eelgrass-present and eelgrass-absent seawater samples have been shown (Webb et al., 2019). A growing body of evidence show that seagrasses significantly reduce the load of various pathogenic bacteria in their surrounding water, thereby changing microbial community structure therein. By using a combination of 16S rRNA gene sequencing and enterococci assays, Lamb et al. (2017) showed that tropical eelgrass meadows (mixture of six seagrass species in the Spermonde Archipelago, Indonesia) significantly reduced bacterial pathogen load in their surrounding waters, and lowered the incidence of coral diseases in the neighboring coral reef. Based on the observed 50 % pathogen reduction in these coastal areas, seagrass meadows were attributed to provide a "sanitation service" as another benefit for the health of humans and marine organisms. A microbiological survey carried out in the coastal waters of 270 Spanish beaches found decreased concentrations of fecal bacteria (*E. coli* and enterococci) in the meadows of Mediterranean seagrass *Posidonia oceanica* (Palazón et al., 2018). A similar effect was detected in the South China Sea where *Salmonella* and *Vibrio* spp. were significantly reduced in the mixed seagrass meadows compared to non-vegetated sites (Deng et al., 2021). Another study reported substantially lower levels of pathogenic *Vibrio* spp. in the Baltic eelgrass meadows by applying a plate counting approach using pathogen selective agar media (Reusch et al., 2021).

Seagrasses such as *Z. marina* and their associated microbiota are known to produce antimicrobial metabolites (Kannan et al., 2010; Marhaeni et al., 2010; Ravikumar et al., 2012; Papazian et al., 2019; Petersen et al., 2019). The reduction of bacterial contamination in seawater by seagrasses has been attributed to various mechanisms, such as grazing (filter-feeding or direct consumption) of plankton and bacteria by seagrass epiphytic fauna (Petersen and Heck Jr, 2001; González-Ortiz et al., 2014; Reusch et al., 2021), trapping and enhanced sedimentation rate of organic particles associated with bacteria (Fonseca et al., 1982; Worcester, 1995; Deng et al., 2021) and exudation of antibacterial phytochemicals by seagrasses (Conte et al., 2021; Deng et al., 2021). Being sessile benthic marine plants, seagrasses are in constant contact with waterborne microbes, including harmful pathogens, hence they produce defensive secondary metabolites (Engel et al., 2002; Seymour et al., 2018). Phenolic compounds such as flavonoids and other phenylpropanoids that form the basis of defensive mechanisms in plants are abundant in seagrasses, providing protection against pathogen attacks, inhibiting microbial settlement or growth and controlling biofouling (Harrison, 1982; Engel et al., 2002; Laabir et al., 2013; Guan et al., 2017). A sulfated flavone glycoside (thalassiolin) has been shown to protect the healthy leaves of *Thalassia testudinum* from infestation by a thraustochytrid protist (Jensen et al., 1998). Zosteric acid (sulfated *p*-coumaric acid), a strong antiadhesive/antifouling seagrass metabolite, is known for preventing the attachment microorganisms, algae and bivalves to plant leaves (Vilas-Boas et al., 2017). By using metabolomics, mass spectrometry imaging coupled with bioassay-guided isolation and bioassays, we showed that surface deployed sulfated flavonoids and the depside rosmarinic acid control microfouling of bacteria and yeasts on the eelgrass leaf surface (Guan et al., 2017; Papazian et al., 2019). Also, seagrass tissue extracts exert antimicrobial effect against many environmental, marine and human pathogens (Puglisi et al., 2007; Kumar et al., 2008; Ross et al., 2008; Kannan et al., 2010; Trevathan-Tackett et al., 2015).

4.3. Seagrass microbiome and waterborne pathogens

Seagrass surfaces and internal tissues harbor an abundant and diverse microbiota essential for resource provision, plant growth and health (Ugarelli et al., 2017; Hurtado-McCormick et al., 2019; Tarquinio et al., 2019; Tarquinio et al., 2021). Seagrasses support heterotrophic epiphytic microbes with exudation of nutrients, and in turn receive several benefits e.g., nitrogen fixation and release of chemicals protecting the host from pathogens and biofouling (Cole and McGlathery, 2011; Seymour et al., 2018; Tarquinio et al., 2019). Due to their highly intimate spatial association with the seagrass host, endophytic microbes would also be expected to play a key role on seagrass health and physiology, but the current knowledge on their functions is scarce (Seymour et al., 2018). Endophytic bacteria promote the growth within reproductive tissues (Tarquinio et al., 2021), and based on common experience in terrestrial plants, endophytes are expected to produce defense chemicals for the host (Trivedi et al., 2020). Endophytic bacteria such as Actinobacteria, which are common in the root tissue of seagrasses (Jensen et al., 2007), are talented producers of potent antibiotics (Dalisay et al., 2013). Notably, the influence of the seagrass microbiome on the surrounding seawater microbiome, including the pathogens therein remain unstudied. Webb et al. (2019) have suggested the involvement of some microbial taxa (e.g., the genus *Tenacibaculum* identified in greater abundance in seawater samples from eelgrass-vegetated sites) in the sanitary effect of seagrasses. The surface of *Z. marina* blades has been found to contain high densities of (unidentified) bacteria that inhibit toxic dinoflagellates and algae causing harmful algal blooms (HABs; Imai et al., 2009; Onishi et al., 2014; Inaba et al., 2017), an effect also shown for *Z. marina* and *Z. noltii* extracts (Laabir et al., 2013). Few early studies on the extracts of seagrass epi- and endophytic bacteria show their antifouling and ecologically relevant activities e.g., towards fish pathogens (Marhaeni et al., 2010; Ravikumar et al., 2012). We recently reported the quorum quenching and antimicrobial activities of a small set of fungal strains isolated from the eelgrass phyllosphere and rhizosphere (Petersen et al., 2019). These studies altogether provide evidence that seagrass epiphytes and endophytes, collectively the eelgrass symbionts, protect their plant host and are likely to contribute to pathogen suppression in seawater. The antifouling activity of both endo- and epibionts may suggest the exudation of their metabolites onto leaf surface and subsequently into seawater. Also, the seagrass host deploys its own chemical weapons onto surface (Papazian et al., 2019). Hence it is plausible that the pathogen removal effect is a joint effort of the seagrass holobiont.

4.4. The impact of pathogen suppression effect of seagrasses

Seagrass ecosystem service of filtering pathogens along the water reservoir implies decrease in exposure to pathogenic agents and global infection/disease outbreak risks, hence is a major benefit to health of ocean and human. This is particularly important within the context of climate change that may have grave impact on the fitness, virulence and dominance of certain pathogens in our coasts. A natural 'sanitation' service offers additional advantages, e.g., provision of sustainable fish and bivalve aquaculture that is cleaner and safer for coastal ecosystems and human consumers (de Los Santos et al., 2020). Improvement of water quality by seagrasses may assist maintenance of tourism services in coastal regions, and improve the health and life quality of low-income communities living in the coastal areas without sanitary systems (Lamb et al., 2017; Jamison et al., 2018). On the long term, it could make financial impact by decreasing the costs of global health care systems due to lower incidence of e.g., gastrointestinal and other infections (Ascioti et al., 2022). Alone in the United States, several virulent *Vibrio* spp. are cumulatively responsible for annual health costs over USD 250

M (Trevathan-Tackett et al., 2019).

Although seagrass meadows are declining worldwide, recent efforts for conservation and restoration of natural seagrass ecosystems are encouraging. Seagrass beds are widely used as indicators (and for monitoring) of the health status of coastal ecosystems (Sun et al., 2020; Soto, 2022). Recent investigations voice the need for a microbiome-driven perspective and strategies for conservation and protection of seagrass meadows (Trevathan-Tackett et al., 2019; Sun et al., 2020). Preserving healthy microbial assemblages through restoration efforts could create ecosystems that can control HABs and pathogen exposure globally.

5. Conclusion

We characterized the cultivable epiphytic and endopytic microbiome of the healthy and decaying leaves, and the roots of *Z. marina*, then assessed their inhibitory effects against a large panel of pathogens and finally mined their metabolomes for identifying putative compounds responsible for the detected antibiotic activities. Based on the potent inhibition of a number of aquatic/marine and introduced (fecal, human and plant) pathogens, we demonstrated the potential role of the microbiome, especially the healthy leaf epibiome, of the Baltic eelgrass *Z. marina* in pathogen removal, hence supporting the sanitary effect of seagrass meadows in coastal waters. The current study paves the way for in-depth studies to illuminate the multi-faceted roles (and the mechanism) of the seagrass microbiome in maintaining a healthy status of the seagrass meadows and coastal ecosystems. Further studies will improve our understanding in tripartite host–pathogen–microbiome interactions, and may assist to predict the outcomes of such interactions across variable marine environments, especially under future climate change scenarios. Finally, eelgrass microbiome can deliver novel molecules for development of anti-infectives for environmental or human use.

CRediT authorship contribution statement

DT: Conceptualization, methodology, formal analysis, investigation, writing-original draft preparation, review & editing, supervision. **SS:** Investigation, methodology, formal analysis, writing-original draft preparation. **CUT**: Methodology, formal analysis, writing-original draft preparation. **TJ**: Investigation, methodology. **MB**: Methodology, formal analysis. **CW**: Methodology, formal analysis**. AWS**: Methodology, formal analysis. **VAE**: Methodology, formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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