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Antibacterial activity of the sponge *Suberites domuncula* and its primmorphs: potential basis for epibacterial chemical defense

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ABSTRACT: The epibacterial chemical defense of the marine sponge *Suberites domuncula* was explored by screening sponge extract, sponge primmorph (3-D aggregates containing proliferating cells) extract and sponge-associated as well as primmorph-associated bacteria for antibacterial activity. 16S rDNA sequencing revealed that the antimicrobially active bacteria belonged to the α- and γ-subdivisions of *Proteobacteria* (α-Proteobacterium MBIC 3368, *Idiomarina* sp. and *Pseudomonas* sp., respectively). Moreover, a recombinant perforin-like protein was cloned from *S. domuncula* that displayed strong antibacterial activity. Based on these observations, it is proposed that the sponge may be provided with a direct (by producing antibacterial metabolites) as well as an indirect (with the help of associated bacteria) epibacterial defense.

KEY WORDS: Sponges · Primmorphs · Chemical defense · Antibacterial · Perforin-like protein

INTRODUCTION

Sponges (Porifera), being evolutionarily ancient, multicellular, sessile organisms, inhabit every type of marine benthic environment. Particularly certain demosponges contain large numbers of bacteria that can amount to 40% of the biomass of the animal (Vacelet 1975), which may exceed the bacterial concentration of the seawater by 2 to 3 orders of magnitude (Friedrich et al. 2001). Sponges are thought to live in symbiosis with bacteria (Rützler 1985, Wilkinson 1992, Althoff et al. 1998, Haygood et al. 1999); however, the molecular basis for the proposed relationship is not well understood. Sponges are filter-feeders that pump large volumes of water through a unique and highly vascularized canal system (Bergquist 1978). Nutrients are acquired by phagocytosis of bacteria that are removed from the water column. Since typical seawater contains on average 1 to 5×10^6 bacteria ml^{-1}, sponges potentially face problems such as clogging of the canal system, formation of biofims and fouling of their surfaces.

The surfaces of the marine sponge *Suberites domuncula* (Demospongiae, Hadromerida, Suberitidae) are noticeably free from epibionts. To investigate the epibacterial chemical defense of *S. domuncula*, 3 different approaches were pursued: (1) a classical chemistry approach by testing organic extract of the sponge and sponge primmorphs for antibacterial activity; (2) a classical microbiological approach by screening sponge- and primmorph-associated bacteria for antibacterial activity; and (3) a molecular biological approach by cloning and recombinant expression of a perforin-like protein. The findings are discussed in light of the epibacterial chemical defense strategies of *S. domuncula.*

MATERIALS AND METHODS

Sponge collection. *Suberites domuncula* sponges were collected from depths of between 30 and 40 m, from the northern Adriatic, near Rovinj, Croatia

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(45° 07′ N, 13° 39′ E), by SCUBA diving. They were transported in water-cooled containers and kept in recirculating seawater aquaria in Mainz, Germany, at a temperature of 17°C under continuous aeration.

Sponge primmorph culture. Primmorphs were obtained from dissociated cells of *Suberites domuncula* as described before (Müller et al. 1999). To remove epibacterial flora, the single cells were treated with antibiotics (100 IU ml^{-1} of penicillin and 100 µg ml^{-1} of streptomycin) as described earlier (Müller et al. 1999). The primmorphs were cultivated in seawater (Sigma), supplemented with 0.2% of RPMI1640-medium and 60 µM silicate in the presence of antibiotics (Krasko et al. 2000). Primmorphs were used for the experiments 7 d (for bacteria isolation) or 26 d (for the preparation of organic extracts) after transfer of the cells into seawater.

Bacterial isolation. The surfaces of 3 aquarium-maintained sponges were swabbed with a sterile cotton tip applicator which was then placed into 10 ml of sterile seawater (Wahl et al. 1994). Following mixing and serial dilution, 3 × 100 µl of each dilution were plated on B1 medium (0.25% peptone, 0.15% yeast extract, 0.15% glycerol, 1.6% agar, 100% seawater) (Newbold et al. 1999). Six bacterial isolates were obtained that were subsequently named SB1 to SB6.

Sponge vicinity bacteria were isolated from microfilms and developed on aluminum, glass, copper and acrylic panels that had been positioned in the immediate vicinity of sponge in aquarium. In addition, bacteria were also isolated from aquarium seawater. Altogether, 8 bacterial isolates were obtained from the sponge vicinity and were subsequently named VB1 to VB8.

Bacteria were isolated from 7 d old primmorphs which were 3 to 5 mm in diameter. Following repeated washing in sterile seawater, the primmorphs were squeezed in 10 ml sterile seawater using sterile forceps. Of this suspension, 6 × 100 µl was plated on B1 agar and plates were incubated at 30°C for 24 to 72 h. As a control, the sterile seawater from the last washing step was plated. These control plates were incubated at 30°C for up to 8 d and no bacterial growth was observed. Two bacterial isolates (PB1 and PB2) were obtained from the primmorphs.

Preparation of organic extracts. Sponge specimens (50 g) were cut into small pieces and placed in 200 ml mixture of methanol:dichloromethane (1:1). After 24 h, extract was decanted and collected for analysis. Following triplicate extraction, the extracts were pooled, filtered and concentrated in a rotary evaporator. Sponge primmorphs (26 d old, 1 to 2 g) were washed with sterile seawater to remove antibiotics used in the media. Primmorphs were also extracted as described above.

Sponge- and primmorphs-associated bacteria were extracted in n-butanol following a method of Elyakov et al. (1996). Bacterial isolates were inoculated into conical flasks (1 l capacity) with 500 ml cultural broth. The culture broth contained peptone (2.5 g), K_2HPO_4 (0.1 g), yeast extract (1.25 g), glucose (0.5 g), $MgSO_4$ (0.1 g), seawater (250 ml) and distilled water (250 ml); the pH was adjusted to between 7.2 and 7.5. The flasks were incubated at 30°C for 3 d with shaking (100 rpm). After addition of 150 ml of n-butanol, the mixtures were kept at 40°C for 24 h, stirred for 20 min, centrifuged and the butanol layer was evaporated. Dry residue was stored below 5°C until further use.

Antibacterial assays. Antibacterial activities were tested in triplicate using the standard paper disc diffusion method. Concentrations of 500 µg $disc^{-1}$ of sponge extract, 250 µg $disc^{-1}$ of primmorphs extract and 500 µg $disc^{-1}$ of bacterial extracts were applied to sterile paper discs (6 mm in diameter). The solvent was evaporated before they were placed onto agar plates that had been seeded with reference bacterial strains. The diameter of the inhibition zones (diameter of inhibition zone minus diameter of disc) was measured in mm after incubation at 30°C for 24 h. Solvent control discs without extract were prepared in the same manner and were never observed to inhibit bacterial growth.

Phylogenetic identification of bacteria. PCR amplification, cloning, sequencing and phylogenetic analysis of the microbial isolates (having antibacterial activity) from *Suberites domuncula* and its primmorphs was carried out as described previously (Hentschel et al. 2001). The obtained sequences were aligned using the ABI prism Auto assembler v. 2.1 software (Perkin Elmer) and entered into the BLAST and ARB 16S rDNA sequence database (see www.arb-home.de).

Cloning and expression of a perforin-like protein from *Suberites domuncula*. The cDNA (*SDPFL*) encoding the perforin-like protein, PFL_SD, was identified by differential display of mRNA in 6 d old primmorphs. A 200 bp fragment was obtained which was used to screen the *S. domuncula* cDNA library (Kruse et al. 1997). One type of insert with a size of 839 nt (termed *SDPFL*) was sequenced using an automatic DNA sequencer (Li-Cor 4200). The sequences were analyzed using computer programs BLAST and FASTA. The prediction of domains was performed using the ISREC server. The potential transmembrane region was predicted according to Rao & Argos (1986).

The recombinant perforin-like protein was prepared in *Escherichia coli* as a fusion protein. The coding part of the clone from aa_1 to aa_{198} (nt_{31} to nt_{624}) was used for expression in *E. coli*. The cDNA was inserted into the bacterial oligohistidine expression vector pQE-30 (Qiagen) via the *Bam*HI (5′-end) and the *Hind*III (3′-end). *E. coli* was transformed with this plasmid and expression of fusion protein was induced for 12 h at 37°C with 1 mM isopropyl 1-thio-β-D-galactopyra-

noside (IPTG) (Cariello et al. 1982). The fusion protein was extracted and purified using the BugBuster Protein Extraction Reagent (Novagen). The purity of the material was checked on 12% polyacrylamide gels containing 0.1% $NaDodSO_4$ ($NaDodSO_4$/PAGE) according to Laemmli (1970). The M_r of the recombinant perforin-like protein (termed r-Perforin) was 24 kDa, as expected. The protein was dialyzed against artificial seawater.

Table 2. *Suberites domuncula.* Antibacterial activities of sponge- (SB) and primmorph-associated bacteria (PB). Inhibition zones are in mm ± SE (n = 3). –: no inhibition zone. Isolates SB3, SB4, SB5 and PB2 did not show any activity. None of the isolates were active against the reference vicinity bacteria (VB) strains VB6, VB7 and VB8

Reference strains	α-Proteobacterium SB1	α-Proteobacterium SB2	*Idiomarina* SB6	*Pseudomonas* PB1
VB1	4.3 ± 0.5	2.6 ± 1.1	2.3 ± 0.5	3.3 ± 0.3
VB2	–	–	5.6 ± 0.5	–
VB3	–	–	2.6 ± 1.1	–
VB4	–	2.3 ± 0.5	4 ± 0	–
VB5	–	–	1 ± 1.0	–

RESULTS AND DISCUSSION

Antibacterial activity of sponge and its associated bacteria

Organic extracts obtained from the sponge displayed strong antibacterial activity (Table 1). *Suberites domuncula* produces a neurotoxin called suberitine which displays hemolytic/toxic activities (Böhm et al. 2001). It is conceivable that this protein toxin might also be responsible for the observed antimicrobial activities. The sponge extract inhibited growth of sponge vicinity bacteria. However, some of the bacterial strains isolated from sponge surface were sensitive to the sponge extract. Similar observation has also been made by Newbold et al. (1999). They opined that co-occurring bacteria might have influenced the evolution of secondary metabolites in some sponge taxa.

Table 1. *Suberites domuncula.* Antibacterial activity of sponge and primmorph extracts. Inhibition zones are in mm ± SE (n = 3). VB: vicinity bacteria that were isolated from the vicinity of the sponges; SB: sponge-associated bacteria that were isolated from the sponge surface; PB: primmorph-associated bacteria. –: no inhibition zone

Reference strains	Sponge extract	Primmorph extract
VB1	5.7 ± 0.6	12.5 ± 1.2
VB2	5.3 ± 1.2	13.0 ± 1.4
VB3	5.7 ± 0.6	14.0 ± 1.5
VB4	4.7 ± 1.2	–
VB5	–	9.5 ± 0.9
VB6	1.0 ± 0.4	4.5 ± 0.8
VB7	–	10.5 ± 1.1
VB8	–	–
SB1	–	11.0 ± 1.8
SB2	–	–
SB3	6.3 ± 0.6	–
SB4	–	–
SB5	5.7 ± 2.9	–
SB6	8.0 ± 1.7	4.0 ± 1.3
PB1	–	2.5 ± 0.8
PB2	–	5.0 ± 1.2

There is ample evidence documenting the existence of bacteria associated with sponges and their possible interaction with the host. Sponge-associated bacteria play an important role in the production of antibacterial metabolites (Kobayashi & Ishibashi 1993, Thakur & Anil 2000). However, most previous studies focused on the bacteria isolated from whole sponge or from the mesohyl (Kobayashi & Ishibashi 1993 and references cited herein, Hentschel et al. 2001). The present study was an attempt to investigate the hypothesis of an epibacterial chemical defense of the sponge *Suberites domuncula* by considering its surface-associated bacteria. Altogether, 3 isolates with antibacterial activity were identified from the sponge surface (Table 2). These results highlight the importance of these bacteria in the antibacterial activity of host.

Antibacterial activity of primmorphs and their associated bacteria

As the sponge as well as its associated bacteria exhibit antibacterial activity, the determination of the origin of active metabolites (sponge and/or bacteria) became complicated. One possible way to ascertain the origin is to separate these 2 partners and test their activity individually. In the light of this, we developed sponge primmorphs *in vitro* to eliminate the associated bacteria. It was observed that primmorph extracts also exhibit antibacterial activity (Table 1). Interestingly, the activity of the primmorph extract was stronger than that of the sponge extract when used at a lower concentration (primmorph extracts were tested at a lower concentration because only a limited number of primmorphs were available for extraction).

Primary cell culture of sponge *Suberites domuncula* was developed in the laboratory under sterile condi-

tion. However, we did not succeed in eliminating some of the sponge-associated bacteria. The presence of bacteria in sponge and its primmorphs was traced by using bacterial rRNA, and the results showed that the bacterial population was present even after dissociation of sponge cells. As a further step, 2 bacterial strains were isolated and cultured from the primmorphs. Interestingly, both the bacterial isolates displayed antibacterial activity (Table 2).

Autoinhibition of sponge- and primmorph-associated bacteria

Apart from deterring sponge vicinity bacteria, some of the sponge- and primmorph-associated bacteria selectively inhibited the growth of their neighbors (Table 3). These results might indicate that antibacterial metabolites confer a selective advantage to the producer for competition with other bacteria, populating the same ecological niche. The isolates SB1, SB6, PB1 and PB2 displayed autoinhibition (Table 3). This phenomenon has been reported for marine *Pseudoalteromonas* and *Alteromonas* (Holmström & Kjelleberg 1999), pathogenic *Pseudomonas* (Xiong et al. 1994) and marine *Chromobacterium/ Pseudomonas* (Anderson 1974, Gauthier 1975). However, it does not appear to be widely distributed among genera of other marine microorganisms (Nair & Simidu 1987). Earlier researchers suggested that in some bacteria, the production of autotoxic compounds is important for inhibiting their further growth in dense culture.

Phylogenetic identification of bacteria

Partial 16S rDNA sequences were obtained from PCR products for phylogenetic identification (Table 4). Although precise phylogenetic identification from partial sequences is limited, they provide sufficient information for a first assessment. The isolates SB1 and SB2 show species-level similarity (>98.0%) to the α-*Proteobacterium* MBIC3368. Interestingly, this bacterium has been isolated from several different sponges. Webster & Hill (2001) reported that the α-*Proteobacterium* MBIC3368 strain NW001 dominates the culturable microbial community of the Australian sponge *Rhopaloeides odorabile*. The α-*Proteobacterium* MBIC3368 strain SB89 was also recovered from the Mediterranean sponge *Aplysina aerophoba*, which displayed antimicrobial activity against various Gram-positive and Gram-negative bacteria (Hentschel et al. 2001). Additionally, a gene bank entry reports on the isolation of the α-*Proteobacterium* MBIC3368 from an unidentified sponge (Genbank Accession Number AB012864). Apparently, the α-*Proteobacterium* MBIC3368 is frequently associated with diverse marine sponges irrespective of their taxonomic identity, geographic location or natural products profile. 16S rDNA sequence analysis of the isolate SB6 revealed species-level similarity to *Idiomarina loihiensis* (Alteromonadaceae) (98.8%). Marine alteromonads are well known to be abundant producers of antimicrobial compounds (Holmström & Kjelleberg 1999). Therefore, these findings are not unexpected.

Table 3. *Suberites domuncula.* Autoinhibition of sponge- (SB) and primmorph-associated bacteria (PB). Inhibition zones are in mm ± SE (n = 3). –: no inhibition zone. Isolates SB3, SB4 and SB5 did not show any activity

Reference strains	α-Proteobacterium SB1	α-Proteobacterium SB2	*Idiomarina* SB6	*Pseudomonas* PB1	*Pseudomonas* PB2
SB1	1.6 ± 0.6	–	3.7 ± 1.8	2.3 ± 0.6	–
SB2	–	–	–	2.3 ± 0.6	–
SB6	–	2 ± 1.0	3.0 ± 1.3	–	–
PB1	–	–	–	4.3 ± 0.6	4 ± 1.0
PB2	–	–	–	3.6 ± 0.6	4.3 ± 0.6

Table 4. *Suberites domuncula.* Phylogenetic identification of sponge- (SB) and primmorph-associated bacteria (PB)

Isolate	Method	Bases sequenced (bp)	Nearest phylogenetic neighbor	% similarity	Phylogenetic affiliation
SB1	PCR product	465	α-Proteobacterium MBIC 3368	98.9	α-*Proteobacteria*
SB2	PCR product	398	α-Proteobacterium MBIC 3368	98.5	α-*Proteobacteria*
SB6	PCR product	379	*Idiomarina loihiensis*	98.9	γ-*Proteobacteria*
PB1	Cloned	1453	Unidentified *Pseudomonas* sp.	94.5	γ-*Proteobacteria*
PB2	Cloned	1458	Unidentified *Pseudomonas* sp.	94.4	γ-*Proteobacteria*

The 16S rDNA genes of the primmorph Isolates PB1 and PB2 were cloned, sequenced nearly completely (>1350 bp) and deposited in GenBank with preliminary accession numbers AF482708 for PB1 and AF482707 for PB2. Considering that the genus *Pseudomonas* is phylogenetically well characterized, the sequence similarities to the nearest phylogenetic neighbor are remarkably low (94 to 95%). Also, both isolates cannot be grown on B1 agar plates after repeated subculture. It is conceivable that they require specific, possibly host-derived factors for sustained growth that are missing in standard laboratory media. Interestingly, the construction of 16S rDNA libraries from aquarium-maintained *Suberites domuncula* revealed only 1 sequence type, which also belonged to the genus *Pseudomonas* (Böhm et al. 2001). This study provides further evidence that *Pseudomonas* bacteria may be symbiotically associated with *S. domuncula* sponges. As the PB1 and PB2 isolates represent novel species, they are valid candidates for the production of novel antimicrobial compounds.

Thin-layer chromatography (TLC)

Previous efforts with *Suberites domuncula* indicated that the sponge as well as its primmorphs can produce a hemolytic protein 'suberitine'. In the present investigation, as the antibacterial activity of functional sponge was retained in the primmorphs, we performed TLC on both the extracts. The results showed similar profiles between sponge and primmorph extracts (data not shown). Four major spots were observed, which were characterized by the following Rf values: 0.22, 0.31, 0.63 and 0.83. It remains to be investigated whether the primmorph cells of *S. domuncula* are also capable of producing the active metabolites that are normally produced by the intact sponge. This study emphasizes the possibility of establishing primmorph cultures with its associated bacteria for the production of bioactive compounds *in vitro*. If sponge primmorph cultures can produce bioactive metabolites, similar or identical to those produced by functional sponge, they will be useful model systems to explore a wider application in the production of sponge secondary metabolites *in vitro*.

Production of an antibacterially active perforin-like protein

A cDNA clone was isolated that resembles human perforin and displayed antibacterial activity. Human perforin is found in lytic granules of cytotoxic natural killer and T-cells (Lowin et al. 1994). After release, perforin forms pores in the target cell membranes, initiating osmotic lysis. Hence, perforin is an effector molecule of the nonspecific cytotoxic T-lymphocyte-mediated defense system of mammalians. Perforin displays direct or indirect toxic effects during fungal infection in humans (Henkart et al. 1995), protozoan-mediated cytotoxicity (Zhou et al. 2001) as well as during bacterial infection (Nickell & Sharma 2000).

The cDNA was 839 nt long (accession number AJ307008). One open reading frame was present, which spans from nt_{31} to nt_{624} (Fig. 1A). The estimated size of the polypeptide is 22660 Da (PC/GENE 1995; Physchem). The instability index was computed with 44.39, suggesting that this polypeptide belongs to the

A

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SDPFL  GGCACGAGAAAAAACGAACCGCTTATTGAGATGGGTGTGCTTCGATTGGTCATATACGTT  60
PFL_SD                                  M  G  V  L  R  L  V  I  Y  V  10
SDPFL  GTCTTTTATGCAACGCTAGCCTCTGCTCATTCGCCATGTGAAACAAATTCTTGTTGTAAT 120
PFL_SD  V  F  Y  A  T  L  A  S  A  H  S  P  C  E  T  N  S  C  C  N  30
SDPFL  GGTGCAACATGTCGTGACACCTCTTCATACACATGTTCTTGTCGATCTGGTTACAGCGGA 180
PFL_SD  G  A  T  C  R  D  T  S  S  Y  T  C  S  C  R  S  G  Y  S  G  50
SDPFL  ACATACTGTCAGAACCCTTTTGGTCGACTTACCTTTTACGCTCGTTATGGCCATGGTCTT 240
PFL_SD  T  Y  C  Q  N  P  F  G  R  L  T  F  Y  A  R  Y  G  H  G  L  70
SDPFL  CCTGACGAAGATGGGTGGTGGAATGATAGTGACCCCTACATGGAGATAATTGCTTATTAT 300
PFL_SD  P  D  E  D  G  W  W  N  D  S  D  P  Y  M  E  I  I  A  Y  Y  90
SDPFL  CGAGGGGGTAGCATACGCCATACAACAAGCACTAGAAATGGAGATCATAGCCCAGTCTGG 360
PFL_SD  R  G  G  S  I  R  H  T  T  S  T  R  N  G  D  H  S  P  V  W 110
SDPFL  CACCAGAATATTGACTTTGGTAGGCGGGATTGGACACGTTTCACTGTTCGTGTGTGGGAT 420
PFL_SD  H  Q  N  I  D  F  G  R  R  D  W  T  R  F  T  V  R  V  W  D 130
SDPFL  AGTGACAATAATGCAGATGATGCGCTGTCAAGCACCGGTACATACGGTTTAAGTTCTCAC 480
PFL_SD  S  D  N  N  A  D  D  A  L  S  S  T  G  T  Y  G  L  S  S  H 150
SDPFL  ACTTCAAATACCATGGTGAAGATGAACTGCTACAGTGGATACGTTTATTTTGACTACCAC 540
PFL_SD  T  S  N  T  M  V  K  M  N  C  Y  S  G  Y  V  Y  F  D  Y  H 170
SDPFL  TTTCAGCCAGGATTTGCTTGGAGTAGTGGAGCCGTGCACTTGAACTATGATTACAAACTG 600
PFL_SD  F  Q  P  G  F  A  W  S  S  G  A  V  H  L  N  Y  D  Y  K  L 190
SDPFL  TATAATTATTACGATTCTTGTTATTAATGTGTGCAGAACCTTTCCAGTTGTATAAAACAT 660
PFL_SD  Y  N  Y  Y  D  S  C  Y  -                                   198
SDPFL  TAATATGCATGGTGTTATTGTTATTATGGAAGTTTTCATCTTGGAACATCGGTGTGTGTG 720
SDPFL  AGTGTAAGATTTCTTTTGTTGATCTATTAACATTGTATGAGTATTCAATTTGTATGCTTT 780
SDPFL  CCATTCATCTGTACAATTATTAGCTTTGTCTTGGAATGTTGTTCTTTATAACTCTATGA  839
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B

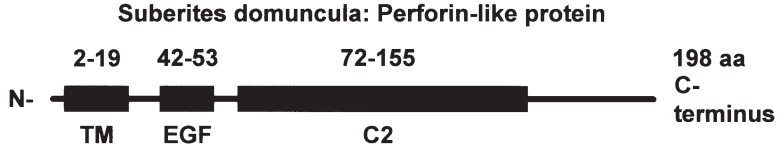

Fig. 1. *Suberites domuncula.* (A) The nucleotide sequence (*SDPFL*) and the deduced polypeptide of the putative perforin-like protein (termed PFL_SD). (B) Domain organization of the perforin-like protein. A transmembrane (TM) region is predicted close to the N-terminus of the protein, followed by the potential EGF-like domain cysteine pattern signature (EGF) and the C2 domain (C2). Total protein length is 198 aa

class of unstable proteins. The perforin-like protein from *Suberites domuncula* does not contain a predictable eukaryotic secretory signal sequence. The domains present in the sponge perforin-like protein were predicted (ISREC 2001) (Fig. 1B). One EGF-like domain cysteine pattern signature is present from aa_{42} to aa_{53} in the deduced polypeptide. This domain is less conserved in a series of metazoan proteins and is predominantly found in membrane-bound proteins and in secreted molecules (Campbell & Bork 1993). Furthermore, a C2 domain, with lower similarity, between aa_{72} to aa_{155} is present; the C2 domain is thought to be involved in Ca^{2+}-dependent phospholipid binding (Davletov & Suedhof 1993). Highest sequence similarity of the sponge deduced polypeptide was found in the databases with the human preforming protein, perforin I (accession number NP_005031). The membrane attack complex components signature of perforin is not present in the sponge protein. The 'expect value' (*E*) between the sponge molecule and the human perforin is 7e-04, suggesting statistical significance. One potential transmembrane region was predicted, which spans from aa_2 to aa_{19}; the domain putative organization of the sponge perforin-like protein is shown in Fig. 1.

A Northern blot experiment revealed that the gene encoding the perforin-like protein was not expressed in dissociated cells from *Suberites domuncula*. However, after 3 and 6 d, the transcript became increasingly visible in the primmorphs (data not shown). The transcript was 0.9 kb, indicating that the full-length clone has been isolated. Additionally, the recombinant perforin protein displayed antimicrobial activity, while the control supplemented with a recombinant putative AF protein (rAF_GEOCY) did not. The sponge *Tethya lyncurium* synthesizes a pore-forming protein (Mangel et al. 1992), which is of similar size to other pore-forming toxins, e.g. the *Escherichia coli* hemolysin (Bhakdi et al. 1986). Future studies will likely elucidate the exact role of this potentially novel class of sponge protein toxins. These data provide additional evidence that sponges are provided with the capacity to synthesize antibacterially active proteins.

A model for epibacterial chemical defense of *Suberites domuncula*

The cumulative data of this contribution show that *Suberites domuncula* is provided with an array of protection strategies against bacteria (Fig. 2). This sponge produces antibacterial compounds for protection against bacterial epibiosis; this can be considered as a direct defense strategy. *S. domuncula* also harbors antimicrobially active bacteria on its surfaces, which might help the sponge indirectly in the epibacterial defense. Moreover, S*. domuncula* has a capacity to produce a perforin-like antibacterial protein. This investigation highlights the importance of the sponge *S. domuncula* and its associated bacteria as a valuable resource for the discovery of novel antimicrobials as well as the possible use of sponge primary cell culture (primmorphs) technique for the production of bioactive metabolites.

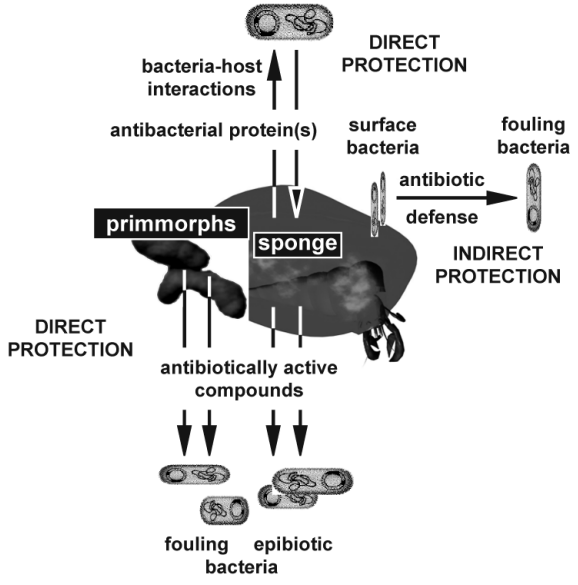

Fig. 2. *Suberites domuncula.* A direct and indirect epibacterial defense hypothesis

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