

# Isolation and phylogenetic analysis of bacteria with antimicrobial activities from the Mediterranean sponges *Aplysina aerophoba* and *Aplysina cavernicola*

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## Abstract

The aim of this study was to isolate bacteria with antimicrobial activities from the marine sponges *Aplysina aerophoba* and *Aplysina cavernicola*. The obtained 27 isolates could be subdivided into eight phylogenetically different clusters based on comparative sequence analysis of their 16S rDNA genes. The sponge isolates were affiliated with the low (*Bacillus*) and high G+C Gram-positive bacteria (*Arthobacter*, *Micrococcus*), as well as the  $\alpha$ -Proteobacteria (unknown isolate) and  $\gamma$ -Proteobacteria (*Vibrio*, *Pseudoalteromonas*). One novel *Bacillus* species was identified and two species were closely related to previously uncharacterized strains. Isolates with antimicrobial activity were numerically most abundant in the genera *Pseudoalteromonas* and the  $\alpha$ -Proteobacteria. The sponge isolates show antimicrobial activities against Gram-positive and Gram-negative reference strains but not against the fungus *Candida albicans*. A general pattern was observed in that Gram-positive bacteria inhibited Gram-positive strains while Gram-negative bacteria inhibited Gram-negative isolates. Antimicrobial activities were also found against clinical isolates, i.e. multi-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* strains isolated from hospital patients. The high recovery of strains with antimicrobial activity suggests that marine sponges represent an ecological niche which harbors a hitherto largely uncharacterized microbial diversity and, concomitantly, a yet untapped metabolic potential. © 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

**Keywords:** 16S rDNA; Antimicrobial activity; Inhibition zone; Sponge-associated microorganism; Marine sponge; *Aplysina*

## 1. Introduction

Sponges have long been known as a source for natural products of pharmaceutical and medical relevance. More than 6000 metabolites have been recovered from marine sources, a large proportion of which originate from sponges. The bioactivity of the isolated substances includes antiviral, antitumor, antimicrobial or generally cytotoxic properties and is, therefore, of considerable biotechnological interest [1–4]. There is accumulating evidence that demonstrates the involvement of associated microorganisms in the secondary metabolism originally attributed to the sponge host [5–9]. Particularly when one animal species contains different classes of metabo-

lites, when taxonomically different species contain the same metabolite, or when the metabolite concentrations are exceedingly low, the involvement of microorganisms might be suspected.

While a contribution of microorganisms in the secondary metabolism of sponges is tempting to speculate, the actual proof is difficult to acquire. In order to provide unequivocal evidence, the microorganism has to produce the compound of interest under laboratory conditions. However, once outside its natural habitat, bacteria frequently change their metabolic profile because of altered growth conditions or the lack of selective pressure. Also, the question remains open whether a microorganism is truly symbiotic in the sense of being specific to and permanently associated with their host. As an alternative, the animal might simply provide a physical environment under which microbial secondary metabolism is induced. In either case, the search for microorganisms with bioactive compounds is a worthwhile endeavor, because upon

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successful culture, the metabolite of interest can be obtained in unlimited amounts.

Sponges of the Aplysinidae family are abundant in subtropical and tropical waters of the Mediterranean sea and the Pacific and Atlantic ocean. *Aplysina* sponges harbor large amounts of bacteria which can amount to 40% of the biomass of the animal and which exceed the bacterial concentrations of the seawater by two orders of magnitude (Friedrich and Hentschel, unpublished). Three types of associations of microorganisms with *Aplysina* sponges have been described [10]: (i) cosmopolitan bacteria which serve, most likely, as a food source, (ii) extracellular bacteria which are probably specific to the sponge mesohyl and (iii) intranuclear bacteria which permanently reside in the nuclei of specific host cells. The in situ application of fluorescently labeled 16S rRNA oligonucleotide probes directed against known bacterial groups (fluorescent in situ hybridization, FISH) revealed that the resident microbial consortium represents a specific adaptation to the sponge mesohyl [11].

*Aplysina* sponges also contain high concentrations of brominated metabolites (up to 13% of the dry weight) with antimicrobial activity [12,13], repellent properties against predators [14] and cytotoxic activity in human breast cancer cell lines [15]. In the natural environment, this process probably serves as a chemical defense against predators and biofouling [16–18]. The localization of the bioactive metabolite, aerothionin, within the spherulous cells of the sponge *Aplysina fistularis* provides evidence that these compounds are probably produced by the sponge itself [16]. However, the involvement of bacteria in the secondary metabolism or their precursors is still conceivable owing to the structural similarities to microbially produced analogs.

The high density as well as the taxonomic diversity of bacteria within the *Aplysina* mesohyl may create an environment which may be conducive for the production of antimicrobials and other defense compounds. Therefore, the aim of this study was to isolate bacteria with antimicrobial activities from the Mediterranean sponges *Aplysina aerophoba* and *Aplysina cavernicola*.

## 2. Materials and methods

### 2.1. Sponge collection

*A. cavernicola* (class Demospongiae, order Verongiida, family Aplysinidae) was collected by SCUBA in Marseille (April 1998) and Elba (May 1998). *A. aerophoba* was collected in Banyuls sur Mer (April 1998 and 1999). Upon transfer to the laboratory, a central core of sponge tissue was stanced out using an EtOH-sterilized 11-mm diameter corkborer and rinsed three times with 0.2 µm filtered seawater. The sponge piece was usually homogenized in 0.2 µm filtered seawater with a sterilized Dounce homogeniz-

er, but other methods of tissue disintegration (turrax, squeezing between sterile glass slides, cutting with scalpel) were performed as well. The resulting extract was further diluted ( $10^{-1}$ – $10^{-5}$ ) and each dilution was plated in quadruplicate on agar plates.

### 2.2. Cultivation media

Heterotrophic bacteria were isolated on the Zobell marine medium (Difco 2216) and on 3/4 strength local seawater-based medium containing 0.5% peptone and 0.01% yeast extract. Oligotrophic bacteria and Gram-positive bacteria were enriched for on media formulations accord-

Table 1  
Antimicrobial activities against reference strains

Sponge isolates	Indicator lawns			
	<i>E. coli</i>	<i>S. aureus</i>	SB1	<i>C. albicans</i>
<i>Bacillus</i>				
SB8	0	14	0	0
<b>SB17</b>	<b>0</b>	<b>14</b>	<b>0</b>	<b>0</b>
<i>Micrococcus</i>				
<b>SB58</b>	<b>(X)</b>	<b>0</b>	<b>0</b>	<b>0</b>
<i>Enterococcus</i>				
<b>SB91</b>	<b>0</b>	<b>16</b>	<b>0</b>	<b>0</b>
<i>Arthrobacter</i>				
<b>SB95</b>	<b>0</b>	<b>14</b>	<b>0</b>	<b>0</b>
Unidentified low G+C Gram-positive				
SB122	0	14	0	0
<b>SB144</b>	<b>0</b>	<b>18</b>	<b>0</b>	<b>0</b>
$\alpha$ -Proteobacteria				
SB6	0	16	0	0
SB55	0	13	0	0
SB63	0	13	0	0
<b>SB89</b>	<b>13</b>	<b>13</b>	<b>13</b>	<b>0</b>
SB156	0	12	0	0
SB197	0	0	12	0
SB202	12	13	18	0
SB207	14	16	13	0
SB214	12	13	13	0
<i>Vibrio</i>				
<b>SB177</b>	<b>0</b>	<b>0</b>	<b>19</b>	<b>0</b>
<i>Pseudoalteromonas</i>				
SB181	0	0	14	0
SB182	0	0	16	0
SB183	0	0	15	0
SB185	0	0	15	0
SB186	0	0	16	0
SB192	0	0	14	0
SB194	0	0	16	0
<b>SB200</b>	<b>0</b>	<b>0</b>	<b>18</b>	<b>0</b>
SB208	0	0	16	0
SB213	0	0	14	0

Antimicrobial activities were determined as inhibition zones around the agar plugs after overnight growth (mm,  $n=3$ ). One representative strain for each phylogenetic cluster is highlighted in bold (see Fig. 1). The genera *Bacillus*, *Micrococcus*, *Enterococcus* and *Arthrobacter* are Gram-positive and the  $\alpha$ -Proteobacteria, *Vibrio* and *Pseudoalteromonas* are Gram-negative. The reference strain *S. aureus* is Gram-positive and *E. coli* and the marine isolate SB1 are Gram-negative.

(x) = variable activity.

0 = no activity.

Table 2  
Antimicrobial activities of the sponge isolates tested against each other

Sponge isolates	Indicator lawns						
	SB17	SB58	SB89	SB95	SB144	SB177	SB200
<i>Bacillus</i> sp. SB17	0	22	0	13	15	0	0
<i>Micrococcus</i> sp. SB58	0	0	0	0	0	0	0
$\alpha$ -Proteobacterium SB89	0	0	0	0	0	14	0
<i>Arthrobacter</i> sp. SB95	14	16	0	0	15	0	0
<i>Bacillus</i> sp. SB144	18	28	0	35	0	0	0
<i>Vibrio</i> sp. SB177	0	0	0	0	0	0	0
<i>Pseudoalteromonas</i> sp. SB200	0	0	18	0	0	16	0

Antimicrobial activities were determined as inhibition zones around the agar plugs after overnight growth (mm,  $n=5$ ). 0 = no activity.

The genera *Bacillus*, *Micrococcus* and *Arthrobacter* are Gram-positive and the  $\alpha$ -Proteobacteria, *Vibrio* and *Pseudoalteromonas* are Gram-negative.

ing to Santavy et al. [19]. Cyanobacteria were isolated on 3/4 strength seawater according to Rippka [20]. Isolation of anaerobic or microaerobic bacteria was performed in anaerobic chambers using the AnaerocultA system (Merck, Germany). The plates were incubated at room temperature (20–25°C) and colonies were picked at 2, 5, 10 and 20 days. Plates were retained an additional month at 4°C to allow for the isolation of slow-growing bacteria. Zobell plates without bacteria were maintained under the same conditions and were inspected routinely to control for contamination. All strains originally isolated on different media or under different physiological conditions could also grow aerobically on Zobell plates. Therefore, Zobell was chosen as a standard medium. Strains were cryopreserved in Zobell medium containing 25% glycerol at –70°C. The strains were recovered from the frozen collection and the colony phenotype was noted.

### 2.3. Antimicrobial screening

A strain collection of 238 sponge isolates was tested for their antimicrobial potential against the following indicator strains: *Escherichia coli* DH5 $\alpha$  (Gram-negative), *Staphylococcus aureus* (Gram-positive), SB1 (Gram-negative, marine isolate) and *Candida albicans* (eukaryotic fun-

gus) (Table 1). 150  $\mu$ l of an overnight culture was grown as a lawn on Zobell media. Plugs of 11 mm in diameter were stanced out with a corkborer and placed with the bacterial side down onto agar plates, which had been seeded with a 1:100 dilution of a stationary phase culture of the above indicator strains. Following overnight incubation at 37°C (the marine isolate SB1 was incubated at room temperature), the plates were inspected for the formation of inhibition zones around the agar plugs. Of the 238 strains tested, 27 isolates showed inhibition zones and were chosen for further analysis. Nearly complete sequencing of the respective 27 16S rDNA genes revealed eight unique phylogenetic clusters of which seven representative isolates were chosen for further antimicrobial testing. These seven candidates were assayed against each other (Table 2). Furthermore, the four isolates which displayed activity against the Gram-positive wild-type strain *S. aureus* in the initial experiment (Table 1) were further screened against three different multi-resistant *S. aureus* and six different *Staphylococcus epidermidis* strains (Table 3).

### 2.4. Polymerase chain reaction (PCR) amplification

DNA was extracted from the 27 stationary phase cul-

Table 3  
Antimicrobial activities against clinical *S. aureus* and *S. epidermidis* strains

Indicator lawns	Antibiotic resistance profile	Sponge isolates			
		<i>Bacillus</i> SB17	$\alpha$ -Proteobacterium SB89	<i>Arthrobacter</i> SB95	<i>Bacillus</i> SB144
<i>S. aureus</i> wt	none	0	13	13	16
<i>S. aureus</i> 118	oxa, pen, pip, a/c, e, gm, tc	0	0	13	16
<i>S. aureus</i> A134	oxa, pen, e, gm, cip, cli, mup	0	14	14	16
<i>S. epidermidis</i> U14	unknown	(+)	14	13	17
<i>S. epidermidis</i> 40	oxa, pen, e, te, gm, sxt, cip	0	0	(+)	18
<i>S. epidermidis</i> 75	pen, pip	(+)	15	12	16
<i>S. epidermidis</i> U78	unknown	0	14	13	17
<i>S. epidermidis</i> 84	e	0	0	0	0
<i>S. epidermidis</i> 150 (RP62A)	oxa, pen, pip, a/c, gm	(+)	15	12	17

Antimicrobial activities were determined as inhibition zones around the agar plugs after overnight growth (mm,  $n=5$ ).

(+) = smaller colonies around agar plugs; 0 = no activity.

Antibiotics: oxa = oxacillin; pen = penicillin; pip = piperacillin; a/c = amoxicillin/clavulanic acid; e = erythromycin; gm = gentamicin; tc = tetracycline; cip = ciprofloxacin; cli = clindamycin; mup = mupirocin; te = teicoplanin; sxt = co-trimoxazole.

tures using standard phenol–chloroform extraction procedures [21]. PCR amplification was performed in a total volume of 50  $\mu$ l containing the appropriate reaction buffer and reagents, 4 U of DAP polymerase (Goldstar, Eurogentec, Seraing, Belgium) and the universal primers 27f (5'-GAGTTTGATCCTGGCTCA-3') and 1385r (5'-CGGTGTGT(A/G)CAAGGCC-3') corresponding to *E. coli* 16S rDNA numbering [22]. The conditions were as follows: initial denaturation (2 min at 95°C), followed by 30 cycles of denaturation (1 min at 95°C), primer annealing (1 min at 50–54°C adjusted for the individual strains), and primer extension (1.5 min at 72°C).

### 2.5. Cloning, sequencing and phylogenetic analysis

The PCR amplification product was purified using the GeneClean kit (Bio101, Vista, CA, USA). The recovered fragment was ligated into the pGEM-Teasy vector and transformed in CaCl-competent *E. coli* DH5 $\alpha$  (Promega, Madison, WI, USA). Detection of successful transformants was performed on the appropriate indicator plates. Plasmid DNA of the 27 representative clones was isolated and sequenced on a Li-Cor 4200 automated sequencer (Li-Cor Inc., Lincoln, NE, USA) using the M13 universal (5'-ACGACGTTGTAACGACGGCCAG-3') and M13 reverse (5'-TTCACACAGGAAACAGCTATGACC-3') se-

quencing primers. The obtained sequences were aligned using the ABI Prism Autoassembler v. 2.1 software (Perkin Elmer, Foster City, CA, USA) and entered into the ARB 16S rDNA sequence database [23]. The 27 analyzed 16S rDNA sequences were assigned to eight unique sequence clusters. An isolate of each cluster (highlighted in bold in Table 1 and Fig. 1) which displayed a representative antimicrobial activity for the entire group was chosen for further analysis. The isolate SB91 (*Enterococcus faecalis*) was omitted from future experiments because it was considered to be a laboratory contamination. Each one of the highlighted strains and, in addition, SB182 was sequenced another two times both forward and reverse to control for sequencing errors. Because SB182 differs from SB200 only by 19/1385 sequenced bases, it was omitted from future bioactivity screens. Phylogenetic analyses were performed by applying the ARB parsimony tool and maximum likelihood as well as neighborhood-joining analysis to different data sets.

Sequences with > 98% sequence similarity to their nearest phylogenetic neighbor over an average of 1372–1400 bases were identified to the species level. Sequences with < 98.0% sequence similarity were identified to the genus level and the percentage sequence similarity is reported in brackets. The 16S rRNA gene sequences have been deposited into GenBank with the following accession numbers:

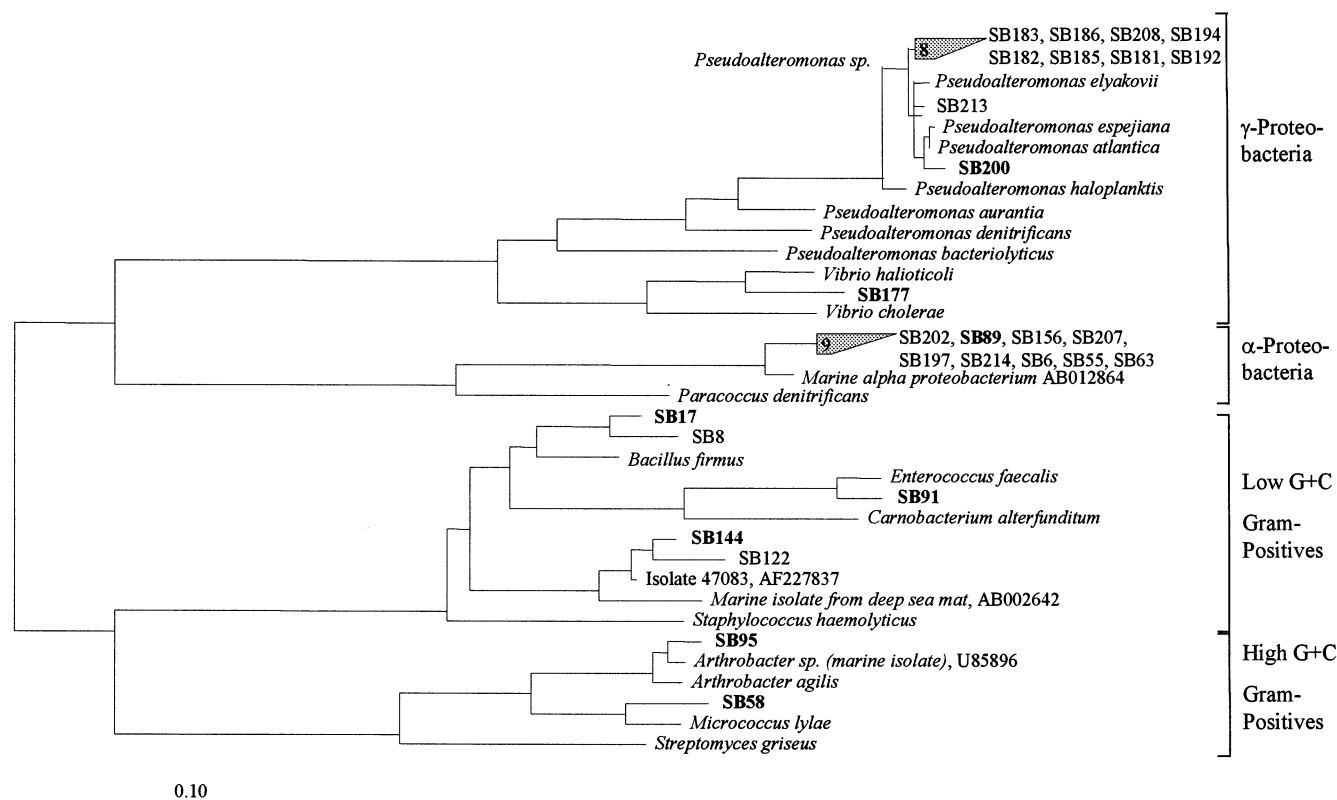


Fig. 1. Phylogenetic dendrogram calculated with almost full length 16S rRNA sequences of the isolates obtained using maximum likelihood approach included in the ARB software package (TU München). The scale bar indicates 10% sequence divergence. One representative strain for each phylogenetic cluster is highlighted in bold (see Table 1).

SB17 (AF218239), SB58 (AF218240), SB89 (AF218241), SB95 (AF218242), SB144 (AF218243), SB177 (AF218244), SB182 (AF218245) and SB200 (AF218246).

### 3. Results and discussion

In the natural environment, antibiotics and other secondary metabolites serve multiple functions related to the survival of the microorganisms [24]. Their production is subject to complex regulatory networks and is generally induced in stationary phase under conditions of nutrient limitation [25]. Antimicrobials are thought to confer a selective advantage when in competition with other bacteria populating the same ecological niche. Other functions of bacterially produced secondary metabolites include the prevention of phagocytosis by predatory amoeba [26], the establishment of symbiotic interactions with invertebrate hosts [27] and their role as virulence factors (phytotoxins) in microbe–plant interactions [28].

In the *Aplysina* sponge mesohyl the production of antimicrobial substances may serve several different functions to a given microorganism inhabiting this particular niche. Bacterial–bacterial antagonism will likely play a role as high numbers of phylogenetically diverse microorganisms are present [11]. A second function may relate to the ability of a microorganism to escape digestion by the sponge archaeocytes, a property which will be crucial for its survival. Archaeocytes are single, amoeboid host cells which ingest bacteria via phagocytosis for nutrient uptake. One might envision that sponge-associated microbes produce secondary metabolites in order to repel the archaeocytes and to avoid digestion.

In this study, 238 bacterial isolates retrieved from the sponge *A. aerophoba* were tested for their antimicrobial activity. Twenty-seven isolates showed a positive response in an inhibition zone assay against bacteria but not against the eukaryotic fungus *C. albicans*. Comparative 16S rDNA sequence analysis of the 27 candidates demonstrated that they could be assigned to eight phylogenetically different clusters (Fig. 1). The high recovery rate suggests that sponges are a rich source of novel microorganisms with potentially pharmacologically relevant bioactivity.

Four of the tested isolates belong to the Gram-positive bacteria. Many Gram-positive bacteria are known to generate spores under adverse conditions which might help to ensure their survival within the sponge tissue. In light of the isolated strains of this study, spore formation is known for *Bacilli* and *Micrococci*, but has not been documented for the genus *Arthrobacter*. Interestingly, spore formation is co-regulated with antibiotic production [29]. Gram-positive bacteria with a high G+C content have been observed in low, but consistent, amounts in the *Aplysina* mesohyl whereas those with a low G+C content could not be targeted due to the lack of a specific probe at the time of

experimentation [10]. Recently, a probe specific for the detection of Gram-positive bacteria with a low G+C content has been developed which awaits testing on sponge tissues [30]. Furthermore, the phylogenetic analysis of the marine sponge *Rhopaloeides odorabile* revealed a high abundance of Gram-positive *Actinobacteria* within the tissues of this sponge [31].

Two isolates belong to the low G+C Gram-positive bacteria. The isolate SB17 probably represents a new *Bacillus* species as its sequence similarity to previously described species of this genus is below 97% [32]. The other low G+C Gram-positive bacterium with antimicrobial activity (SB144) belongs to the same species as an unidentifiable bacterial isolate 47083 (GenBank accession number AF227837). Because these strains are physiologically uncharacterized, they may be candidates for the production of novel antimicrobial metabolites.

The isolate SB58 was identified as high G+C Gram-positive bacterium of the genus *Micrococcus* with the closest relative being *M. lylae* (97.0% sequence similarity over 1372 sequenced bases). In this context it is interesting to note that a *Micrococcus* strain has been isolated from the sponge *Xestospongia* sp. (New Caledonia) which produces a brominated compound similar to the bioactive metabolite, Verongiaquinol, from *A. cavernicola* [33]. Investigations are underway to determine whether, by inference, the bioactivity of the *Micrococcus* sp. SB58 is due to brominated compounds that are characteristic of *Aplysina* sponges. The isolate SB95 belongs to the same species as the marine *Arthrobacter* isolate MB8-13 that had been recovered from Antarctic sea ice [34]. These organisms have primarily been isolated from soil but have also been found in several marine habitats such as Antarctic sea brine [35] and once in a marine sponge [36]. To our knowledge, bacteria of the genus *Arthrobacter* are, with one exception [37], not generally known for the production of bioactive compounds.

The Gram-negative bacteria which displayed antimicrobial activity in the screen are largely representative of the typical population of the seawater. The isolate SB89 belongs to the same species as an abundant, yet uncharacterized marine  $\alpha$ -Proteobacterium MBIC3368 that has previously been isolated from a marine sponge (T. Hamada, unpublished). According to FISH analyses with an oligonucleotide directed against  $\alpha$ -Proteobacteria (alf1b), which reacted positive with the  $\alpha$ -Proteobacterium SB89, but negative with the sponge tissues [11], a permanent association of this strain with *Aplysina* appears unlikely. Because antimicrobial activities have, to our knowledge, not previously been reported for marine  $\alpha$ -Proteobacteria, the identification of novel bioactive metabolites may be possible in this taxonomic group. The isolate SB177 represents a strain of the species *Vibrio halioticoli*. Bacteria of the genus *Vibrio* are typically found in aquatic habitats where they are often attached to surfaces, but they may also be found as pelagic bacteria or at various depths at

sea [38,39]. Several species are pathogens to humans or fish while others are symbiotically associated with squids. Considering that  $\gamma$ -Proteobacteria are consistently found in sponges and that *Vibrios* are frequently animal-associated, it is conceivable that the *Vibrio* sp. SB177 is a common inhabitant of the sponge mesohyl.

The isolate SB200 is affiliated with the genus *Pseudoalteromonas*, representatives of which are commonly found in the seawater. Altogether, nine independent bioactive *Pseudoalteromonas* strains were isolated thereby accounting for the numerically dominant phylogenetic cluster along with the unknown  $\alpha$ -Proteobacteria (eight independent bioactive isolates). The sequence similarity within this cluster and their closest relatives, *P. atlantica*, *P. espejiana* and *P. elyakovii* was very high (greater than 99.1% over approximately 1385 bases, Fig. 1). *Pseudoalteromonas* contain numerous species that synthesize biologically active metabolites with anti-bacterial, bacteriolytic and algicidal activities as well as toxins [40]. Moreover, marine *Pseudoalteromonas* are frequently associated with eukaryotic hosts such as sponges, tunicates, mussels, pufferfish as well as various algae. A closely related species, *P. haloplanktis*, is commonly found in the seawater at the collection site. It is noticeable that *P. haloplanktis* are prone to phage infections and that phage-infected bacteria are frequently observed in the nuclei of specific cells of *A. aerophoba* [41]. *P. haloplanktis* produces a bioactive siderophore, bisuscaberin, which renders tumor cells susceptible to cytolysis by murine macrophages [42]. Further experiments using specific 16S rDNA probes are necessary to determine whether the *Vibrio* sp. SB177 and the *Pseudomonas* sp. SB200 are permanently associated with *Aplysina* sponges.

#### 4. Ecological implications

Since the bacteria were isolated from the same environmental niche, the hypothesis was tested whether specific interactions may be observed among the isolates. When the isolates were tested against each other, a general pattern became apparent where the Gram-positive strains were active against Gram-positive bacteria and Gram-negative strains were active against Gram-negative bacteria (Tables 1 and 2). The noticeable exception to this pattern is the Gram-negative,  $\alpha$ -Proteobacterium SB89, which displays antimicrobial activity against both types of reference strains. Also, there is more variation among this different, yet phylogenetic highly conserved cluster when compared to the other genera (Table 1). Strain specific variation of antibiotic production is a well documented phenomenon, which partly relates to their frequent location of the respective genes on mobile genetic elements. Plasmids, transposons or phages may enable mobilization and transfer of these biosynthetic operons between different bacterial strains and even across the species barrier [43].

None of the strains was active against the eukaryotic fungus *C. albicans*. The lack of activity may be explained by the hypothesis that bacteria which reside within or on the surfaces of *A. aerophoba* may be less likely to produce cytotoxic activities targeted against eukaryotic host cells as this would harm the animal when produced under environmental conditions. Secondly, none of the strains displayed autoinhibition. This observed mode of action suggests that the activity could be due to bacteriocins. These are ribosomally synthesized polypeptides that serve to selectively kill closely related species while the producer strain remains unharmed [44]. Gram-negative hosts produce bacteriocins against Gram-negative organisms while Gram-positive bacteriocins are targeted against Gram-positive organisms. Their proposed function in the environment is to provide a selective advantage by eliminating a closely related competitor strain. The particular microbial ecology of the sponge mesohyl with respect to the high numbers of taxonomically diverse bacteria provides an environment which would be conducive for the production of bacteriocins.

#### 5. Clinical relevance

With the increased occurrence of multidrug resistant human pathogens the search for novel antibiotics has gained new urgency. Many clinically relevant microbes have developed resistances resulting from the exposure to sublethal concentrations of antibiotics in hospital environments but also in animal farms where antibiotics are used as growth enhancers [45]. The two bacteria *S. aureus* and *S. epidermidis* are the most common hospital-acquired infections and account for complications following surgical procedures (vascular grafts, catheters and other implants). Among those, the methicillin resistant *S. aureus* strains (MRSA) pose a particular threat as they are resistant against all known antibiotics except vancomycin [46–48]. We have chosen various multi-resistant *S. aureus* and *S. epidermidis* strains for our assays in order to screen for inhibitory mechanisms which may be different from the known antibiotics listed in Table 3. With the exception of the erythromycin resistant *S. epidermidis* strain 84, all other strains were sensitive to inhibition by the sponge isolates albeit to different degrees. The contrary findings with respect to the erythromycin resistance could be explained if two different resistance mechanisms would exist in the *S. aureus* and *S. epidermidis* strains. Whether the observed inhibitory effects against multi-resistant staphylococci are due to secondary metabolites and possibly to novel antibiotics will be the aim of future experiments.

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