Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article doi: 10.12980/jclm.4.2016J6-17

Antagonistic activity of marine sponges associated Actinobacteria

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ARTICLE INFO

Article history:
Received 18 Jan 2016
Received in revised form 10 Feb 2016
Accepted 10 Apr 2016
Available online 13 Jun 2016

Keywords:
Marine sponges
Spicules
Actinobacteria
Streptomyces

ABSTRACT

Objective: To focus on the isolation and preliminary characterization of marine sponges associated Actinobacteria particularly *Streptomyces* species and also their antagonistic activities against bacterial and fungal pathogens.

Methods: The sponges were collected from Kovalam and Vizhinjam port of south-west coast of Kerala, India. Isolation of strains was carried out from sponge extracts using international *Streptomyces* project media. For preliminary identification of the strains, morphological (mycelial colouration, soluble pigments, melanoid pigmentation, spore morphology), nutritional uptake (carbon utilisation, amonoacids influence, sodium chloride tolerance), physiological (pH, temperature) and chemotaxonomical characterization were done. Antimicrobial studies were also carried out for the selected strains.

Results: With the help of the spicule structures, the collected marine sponges were identified as *Callyspongia diffusa, Mycale mytilorum, Tedania anhelans* and *Dysidea fragilis*. Nearly 94 strains were primarily isolated from these sponges and further they were sub-cultured using international *Streptomyces* project media. The strains exhibited different mycelial colouration (aerial and substrate), soluble and melanoid pigmentations. The strains possessed three types of sporophore morphology namely rectus flexibilis, spiral and retinaculiaperti. Among the 94 isolates, seven exhibited antibacterial and antifungal activities with maximal zone of inhibition of 30 mm. The nutritional, physiological and chemotaxonomical characteristic study helped in the conventional identification of the seven strains and they all suggest that the strains to be grouped under the genus *Streptomyces*.

Conclusions: The present study clearly helps in the preliminary identification of the isolates associated with marine sponges. Antagonistic activities prove the production of antimicrobial metabolites against the pathogens. Marine sponges associated *Streptomyces* are universally well known for their synthesis of many bioactive compounds such as antibiotics, enzymes, enzyme inhibitors and food grade pigments. They also have certain biotechnological applications like probiotics and single cell proteins. These marine *Streptomyces* bioactive metabolites can be the futuristic solution for the dreadful diseases.

1. Introduction

Marine sponges have wealthy sources of structurally distinct natural compounds and several of them possess wide variety of biological activities[1]. Although marine sponges are excellent sources of various drugs, much has not been developed because some types of sponges occur rare in nature and other problem is that they were difficult to collect from the oceans[2]. Sponges harbour a rich diversity of marine microorganisms in their tissues. Metabolites synthesized by the marine sponge's exhibits many similarities to that of the microbes of that origin, signifying that microbes may be the actual source of these products or sometimes they might involve in their biosynthesis[3]. The symbiotic microorganisms isolated from the host species can be credited to the secondary metabolite production and also there are possibilities for the host to synthesize these compounds simultaneously[4].

Classification of sponges is based on their different forms of

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Foundation Project: Supported by Department of Science and Technology of the Indian Ministry of Science and Technology (Grant No. SR/S0/AS-57/2004).
The journal implements double-blind peer review practiced by specially invited international editorial board members.

spicules it possessed and these spicules are highly distinct for each species[5,6]. In recent times, marine sponges has been the central attraction in the research due to the following factors namely (i) they associate closely with diverse range of microbes and (ii) in nature they are the wealthy source of many secondary metabolites[7]. Association of microbes with sponges occurs in many ways. In some reports, it has been explained that marine sponges are filter feeders which allows the microbes to enter inside the body from the surroundings and these may result in the accumulation of many secondary metabolites of microbial origin[8-10]. It has been estimated that nearly 40% of sponge tissue volume contains associated microbes, with the population of 10^9 microbial cells per mL of sponge tissue, which is quite large numbers when compared to seawater density[11,12]. Many microorganisms have been associated with sponges, but very limited studies have been carried out in Indian coastal waters, that too particularly about marine *Streptomyces*[13]. Furthermore, a review describes about the marine microbes and their nature of association with fish, shellfish, seaweeds and sponges[14]. Subsequently, a report on sponge-derived *Kocuria* and *Micrococcus* spp. as sources of the new antibiotic kocurin was also studied[15].

The present investigation is the continuity on the isolation and preliminary characterisation of marine sponges associated Actinobacteria, specifically *Streptomyces*.

2. Materials and methods

2.1. Collection of sponges

By self-contained underwater breathing apparatus diving around the depth of 5–10 m in Kovalam coast and Vizhinjam port of southwest coast of India, the sponge samples were collected (Figures 1 and 2). They were transported to the laboratory within short time so that maximal microbial contamination can be prevented. The sponges were pre-washed with sterilised seawater to remove the adherent fauna.

2.2. Isolation of Actinobacteria from marine sponges

The extracts were prepared by squeezing the marine sponge with a glass stick. Nearly 1 mL of sponge extract was serial diluted with 99 mL of sterilised seawater[16,17]. From that 1 mL of the dilutions were added with 20 mL of sterile glycerol asparagine agar medium and it was incubated at room temperature of (28 ± 2) ˚C for 7 days. The antibiotics like rifampicin (2.5 µg/mL) and amphotericin B (75 µg/mL) were added to the medium to avoid bacterial and fungal contamination. After 7 days, the total actinobacterial populations were counted.

2.3. Maintenance of cultures

The isolated streptomycetes were selected randomly and sub-cultured using glycerol asparagine agar slants at (28 ± 2) ˚C. After obtaining satisfactory growth, slants were transferred to refrigerator (4 ˚C) until further analysis. The 7-day-old cultures were used for further screenings[18].

2.4. Marine sponge's fixation and preservation

The gathered marine sponges were frozen promptly, which to a certain degree settles the shading or live material. The sponges were again settled in 10% formalin for 24 h, after which the specimens were exchanged instantly to 70% ethanol and put away in sealed shut glass holders[19].

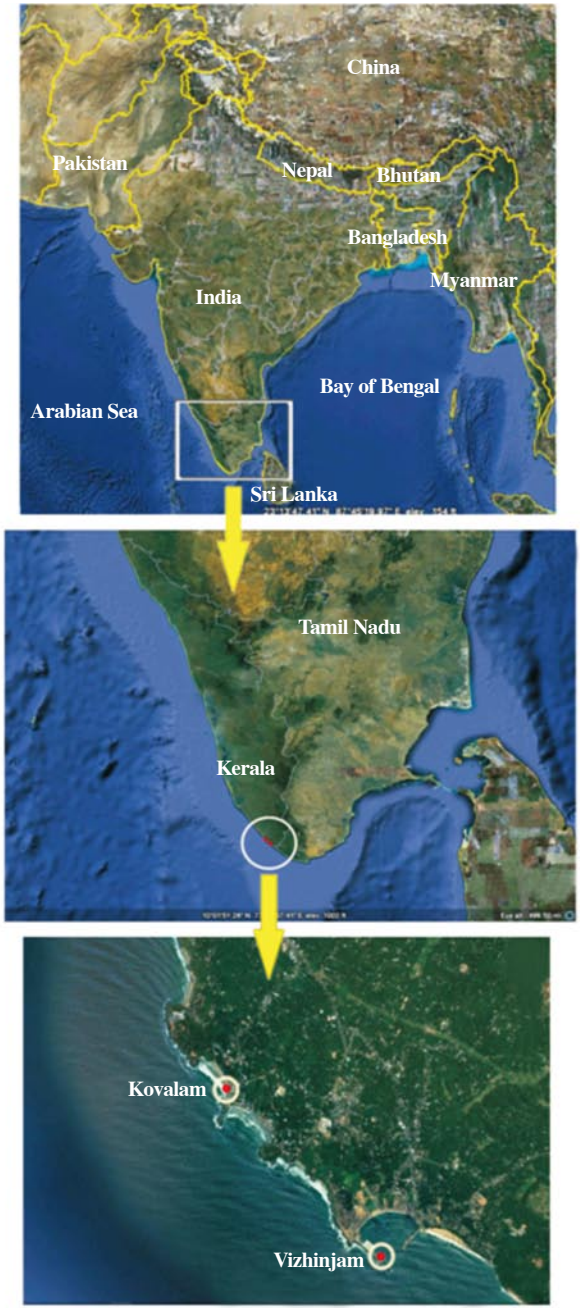

Figure 1. Collection site of marine sponges.

2.5. Spicule separation

Marine sponges are classified according to the shape, ornamentation, size, origin and arrangement of these spicules. They are tiny spike-like structures made out of either silica or calcite. Small pieces of sponges were boiled in a tube containing fuming HNO_3 till the tissue components were completely dissolved. The tubes were three times washed with distilled water and the transparent spicules were settled at the bottom. Later it was rinsed three times with 95% alcohol. At last the spicules were immersed in 2 mL alcohol and placed onto a slide. The alcohol gets evaporated and the slide was dried at 60 ˚C. Then the slides were ready to get observed under microscope and later the spicules were drawn and

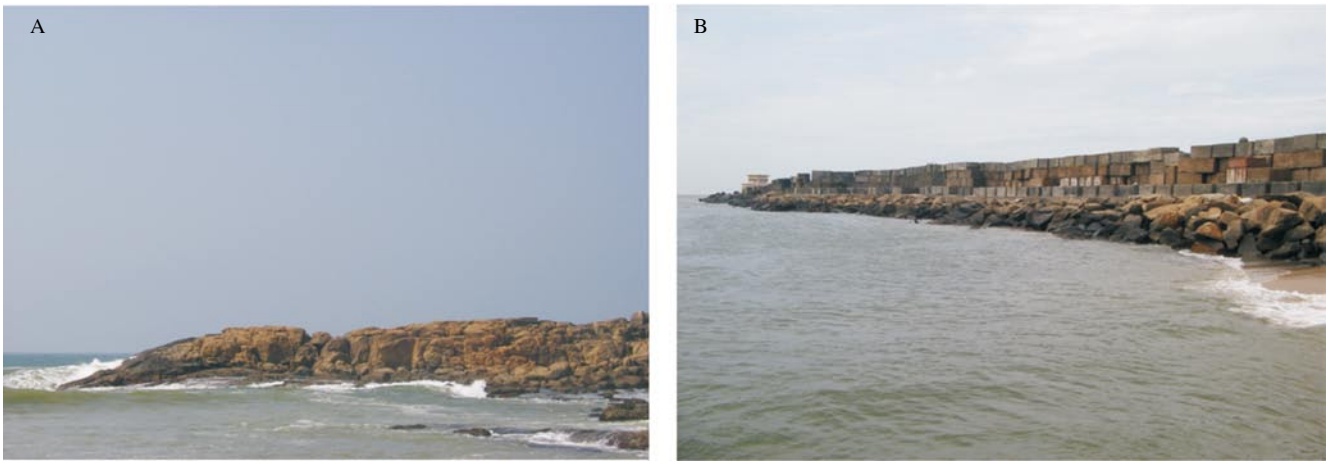

Figure 2. Collection site of marine sponges in Kovalam coast (A) and Vizhinjam port (B).

photographed through Camera Lucida microscope[20].

2.6. Streptomyces characterisation

The cultures were characterised morphologically, nutritionally and physiologically accordingly to the methods described earlier[21-23].

2.6.1. Colour determination

To determine the aerial and substrate mycelia colours (grey, white, red, yellow, orange, green, cream, *etc.*) of the cultures, they were streaked on glycerol asparagine agar and incubated at 28 ˚C for 7 days.

2.6.2. Melanoid production

For melanoid production, the selected cultures were grown in peptone yeast extract iron agar. They were incubated at 28 ˚C for 48 h. If the cultures start producing greenish brown colour which later get changed to brown and then to black, it indicates the presence of melanoid pigments.

2.6.3. Spore morphology

To observe spore chain morphology, the cultures were grown in casein-starch-peptone-yeast extract agar media and a cover slip inserted to the media at an angle of 45˚. After 7 days of incubation, the cover slip kept in the media was removed, dried and observed for spore morphology under the scanning electron microscope.

2.6.4. Antimicrobial assay

Preliminary screenings of antibacterial and antifungal activities of the strains were carried out. The strains were spot inoculated in Petri plates containing glycerol asparagine medium for 7 days. After 7 days, 1 mL of chloroform was added and made to stand for 40 min to arrest the growth of inoculated colonies and then they were overlaid with 5 mL of sloppy agar (0.6%) layer of potato dextrose medium (for fungi) and nutrient agar medium (for bacteria) previously seeded with one of the test organisms. They were then incubated for 24 h at 37 ˚C for bacteria and 48 h at 28 ˚C for fungi. Diameter of the inhibition zone was recorded in millimeters[24].

2.6.5. Nutritional grouping

2.6.5.1. Utilisation of carbon sources

The nutritional characteristics of the strains were studied using criteria like carbon utilisation, the influence of amino acids and sodium chloride tolerance[25]. The cultures were inoculated to test tubes containing 10 mL of basal mineral salt medium to which sterilised carbon sources (xylose, arabinose, rhamnose, fructose, galactose, raffinose, mannitol, inositol, sucrose, glucose) were added to a final concentration of 1%. The tubes were incubated at 28 ˚C and after 7 days the growth of the cultures were observed. Glucose was used as positive control.

2.6.5.2. Influence of amino acids

Various amino acids, namely glycine, cystine, alanine, tryptophan, and valine, were added at a concentration of 0.1% each to 5 mL of basal mineral salt medium. The medium was inoculated with the cultures and incubated at 28 ˚C for 7 days. The biomass thus obtained was separated from the broth, dried and weighed. The weight of the biomass was expressed in grams.

2.6.5.3. Sodium chloride tolerance

Sodium chloride at various concentrations (1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 9.0% and 10.0%) was added to 5 mL of the basal medium. The medium was inoculated with the cultures and incubated at 28 ˚C for 7 days. The biomass thus obtained was separated from the broth, dried and weighed. The weight of the biomass was expressed in grams.

2.6.6. Physiological characteristics

The strains growths were optimized using physiological parameters like pH and temperature. The strains were inoculated on starch casein agar and growth performance at different temperatures ranging from 4 ˚C to 65 ˚C for 7 days was observed. Similarly strains growths were observed at various pH ranged from 4 to 10[21,22].

2.6.7. Chemotaxonomy

The isomers of diaminopimelic acid (DAP) (LL-DAP or *meso*-DAP) and whole cell sugar patterns can be identified by thin layer chromatography using silica gel 60 F254. The cultures used for identification were grown in casein-starch-peptone-yeast medium, which were later scrapped and processed. To identify DAP, the scrapped cultures (50 mg) were treated with 6 mol/L HCl (1 mL), transferred to glass screw-capped and kept in hot air oven at 100 ˚C for 12 h. Then it was cooled, washed twice with distilled water (2 mL) and the final dried material was dissolved again with 0.2 mL of distilled water. Then 5 μL of the sample, 3 μL of standard DAP were separately spotted in the silica gel plate and the mobile phase used was methanol (80): distilled water (26): 6 mol/L HCl

(4): pyridine (10). Then it was incubated for 2 h and 40 min and dried in room temperature for 30 min. Later, the plate was sprayed with ninhydrin (0.1%) in acetone, followed by heating at 110 ˚C for 10 min. The DAP spots were spotted as gray-green fading to yellow[23].

Whole-cell sugars can be identified on addition of 1 mL of sulphuric acid (0.5 mol/L) to 50 mg of scrapped culture in a glass screw-cap tubes and kept in hot air oven for 2 h at 100 ˚C. Then they were washed three times with distilled water to remove the excess acid and redissolved in 0.2 mL of distilled water. Next the samples were of centrifuged at 3 000 r/min for 10 min and it was adjusted to pH 5 using saturated barium hydroxide. Again it was centrifuged at 8 000 r/min for 10 min and the supernatant was removed. The remaining filtrate was lyophilized and dissolved in distilled water (0.1 mL) Then 5 μL of the sample, 3 μL of 1% standard sugar solutions (D-glucose, D-mannose, L-arabinose, D-fructose, D-xylose and L-rhamnose) were separately spotted in the silica gel plate and the mobile phase used was *n*-butanol (10): distilled water (6): pyridine (6): toluene (1). Then it is incubated for 3 h and 20 min and dried in a room temperature for 30 min. Later, the plate was sprayed with aniline phthalate (3.25 g of phthalic acid dissolved in 100 mL of water-saturated butanol with 2 mL of aniline), followed by heating at 110 ˚C for 4 min. In the silica gel plates, hexoses exhibited yellow and pentoses are maroon colour spots[23,26].

3. Results

The present study was conducted about the association of *Streptomyces* with the marine sponges and its taxonomical characterisations. The spicules that were separated from marine sponges were fixed. On observation under the Camera Lucida microscope they resulted with monoaxonic and tetraaxonic silicious spicules. The sponges were identified as *Callyspongia diffusa* (*C. diffusa*), *Mycale mytilorum* (*M. mytilorum*), *Tedania anhelans* (*T. anhelans*) and *Dysidea fragilis* (*D. fragilis*).

Using the glycerol asparagine agar media (ISP 5), the enumeration of microbial population from marine sponges was studied. The total microbial population isolated from marine sponges collected from Kovalam coast and Vizhinjam port was shown in Table 1. Maximum population of 31 and minimum of 18 isolates were recorded from Station I – Kovalam. In Station II – Vizhinjam port, maximum population of 29 and minimum of 15 isolates were recorded. Maximum streptomycetes population was observed during southwest monsoon season. While the glycerol asparaginase agar (ISP 5) enabled the growth of streptomycetes, addition of antibiotics namely, rifampicin and amphotericin B to the medium prevented the growth of bacteria and fungi, respectively. Nearly 94 strains of streptomycetes were randomly selected for further studies.

By the methods adopted from ISP, the selected isolates were characterised. The cultures were designated as AQBCD – isolates of *C. diffusa*; AQBMM – isolates of *M. mytilorum*; AQBTA – isolates of *T. anhelans*; AQBDF – isolates of *D. fragilis*. The colonies were found to be growing slow, chalky in texture, mostly folded and they are aerobic in nature. The mycelial characters of the strains were shown in Table 2. Most of the strains exhibited the aerial mycelial colouration of white, grey and yellow series. Nearly 40 strains were categorised under grey series, 38 white series and 16 yellow series. Out of the total 94 cultures, 66 of them exhibited substrate mycelial colouration (Table 3). The pigmentation like brown, dark green, greenish grey, olive green, orange, pale brown, pale yellow, red, reddish brown, white and yellow were observed on the reverse side of the cultures and rest of them were colourless. Aerial and substrate mycelial colouration of some selected strains were displayed in Figure 3. Around 30 cultures produce soluble pigments which were shown in Table 4. Melanoid pigments were synthesized by 38 strains which indicated black colouration in the media after 48 h of incubation (Table 5). Spore morphological studies resulted with 43 strains were of type rectus flexibilis, 35 of spiral sporophores and 16 of retinaculiaperti sporophores (Table 6).

Out of 94 isolates, only seven exhibited antagonistic activity against bacterial and fungal pathogens which are shown in Table 7. These strains exhibited inhibition zone in the range of 10–30 mm. The strains AQBMM35 and AQBDF81 did not show any inhibitory activities against fungal pathogens. AQBTA66 was the only strain that exhibited an inhibition against *Aspergillus niger* (*A. niger*) with an zonation of 15 mm. Five strains namely AQBCD03, AQBCD11, AQBCD24, AQBMM49 and AQBTA66 showed both antibacterial and antifungal activities. The seven strains which showed better antagonistic activity were nutritionally and physiologically characterised. The nutritional parameters included carbon utilisation, affinity towards amino acids influence and sodium chloride tolerance. Invariably the strains grew well in presence of glucose and xylose but did not assimilate inositol. All the strains showed poor

Table 1

Total microbial population isolated from marine sponges from Station I (Kovalam coast) and Station II (Vizhinjam port).

Season	No. of *Streptomycetes* (CFU $\times 10^2$/g)							
	Station I (Kovalam)				Station II (Vizhinjam)			
	S1	S2	S3	S4	S1	S2	S3	S4
Non-monsoon	5.0 ± 2.0	8.0 ± 1.0	0.0 ± 0.0	1.0 ± 1.0	6.0 ± 1.0	5.0 ± 1.0	5.0 ± 1.2	7.0 ± 1.5
	10.0 ± 1.5	12.0 ± 1.5	5.0 ± 1.0	7.0 ± 0.6	8.0 ± 1.0	7.0 ± 1.0	7.0 ± 1.0	8.0 ± 0.6
	11.0 ± 1.2	13.0 ± 1.0	10.0 ± 2.0	10.0 ± 1.0	9.0 ± 1.0	10.0 ± 0.5	7.0 ± 0.5	9.0 ± 1.0
	20.0 ± 1.2	22.0 ± 2.0	12.0 ± 1.0	15.0 ± 0.6	15.0 ± 2.0	18.0 ± 1.0	8.0 ± 1.0	11.0 ± 2.0
South-West monsoon	24.0 ± 1.2	25.0 ± 1.0	15.0 ± 2.0	15.0 ± 1.0	18.0 ± 2.0	20.0 ± 2.0	11.0 ± 1.0	14.0 ± 2.0
	26.0 ± 1.7	28.0 ± 2.0	16.0 ± 2.0	19.0 ± 1.0	18.0 ± 1.0	24.0 ± 0.6	13.0 ± 1.5	15.0 ± 1.0
	28.0 ± 1.0	31.0 ± 2.0	18.0 ± 1.5	19.0 ± 1.0	25.0 ± 1.0	29.0 ± 0.5	15.0 ± 1.0	17.0 ± 1.0
	15.0 ± 0.6	19.0 ± 1.0	12.0 ± 2.0	13.0 ± 1.0	12.0 ± 2.0	13.0 ± 1.0	14.0 ± 1.0	16.0 ± 1.0
North-East monsoon	11.0 ± 0.6	15.0 ± 1.0	7.0 ± 1.5	10.0 ± 1.5	11.0 ± 0.5	11.0 ± 0.6	12.0 ± 2.0	15.0 ± 1.0
	9.0 ± 1.0	11.0 ± 2.0	6.0 ± 1.2	8.0 ± 1.0	10.0 ± 2.0	6.0 ± 1.5	12.0 ± 1.5	14.0 ± 2.0
	6.0 ± 1.0	10.0 ± 1.7	3.0 ± 1.0	5.0 ± 1.0	7.0 ± 1.0	4.0 ± 1.0	11.0 ± 1.2	10.0 ± 2.0
	5.0 ± 1.0	6.0 ± 1.5	2.0 ± 2.0	4.0 ± 1.0	7.0 ± 1.0	3.0 ± 1.0	8.0 ± 1.2	6.0 ± 0.5

Values are the average of three replicates. S1: *C. diffusa*; S2: *M. mytilorum*; S3: *T. anhelans*; S4: *D. fragilis*.

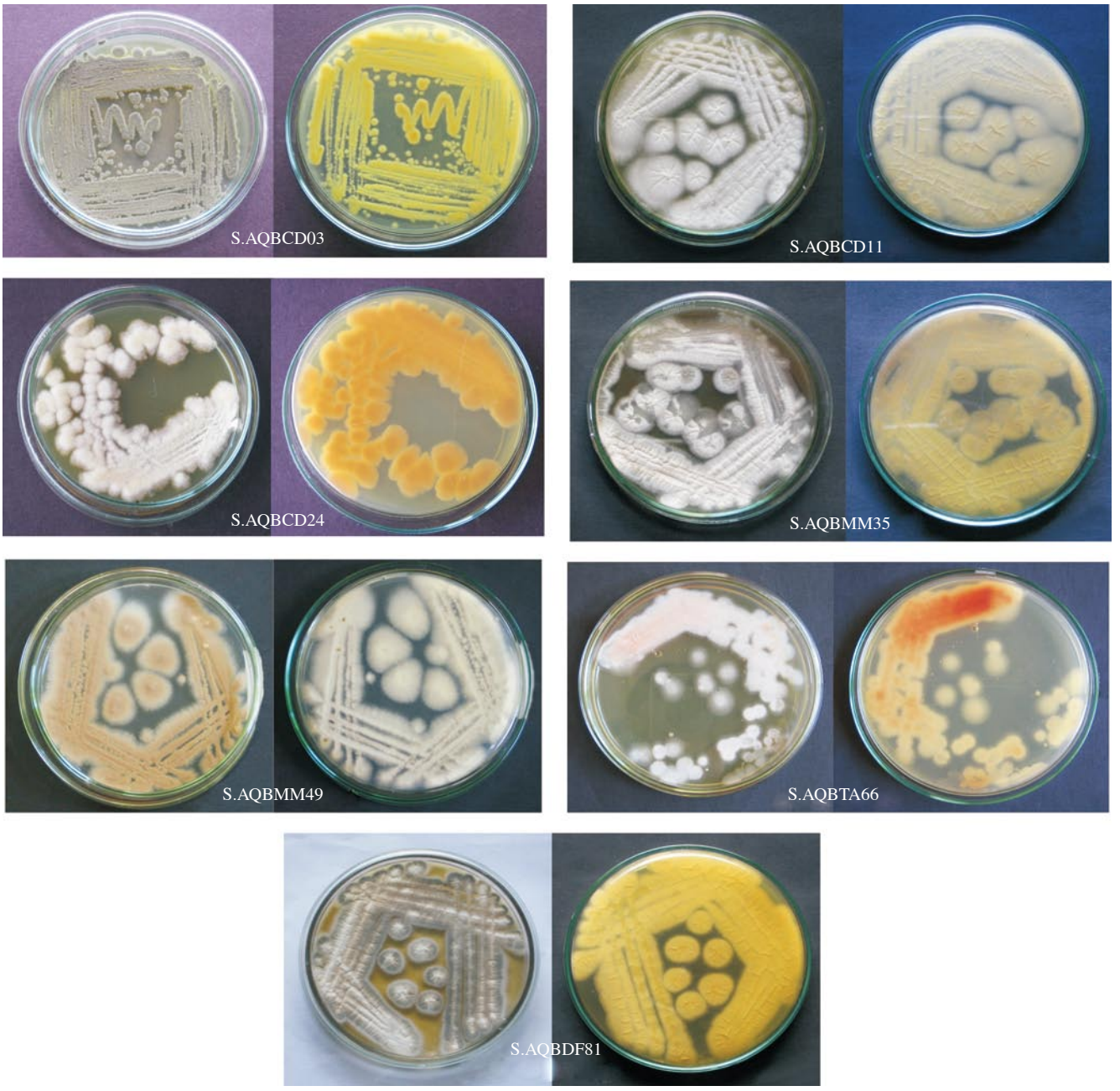

Figure 3. Aerial and substrate mycelial colourations of selected *Streptomyces* spp. inhabiting marine sponges.
S.AQBCD03, S.AQBCD11, and S.AQBCD24 are isolated from *C. diffusa*; S.AQBMM35 and S.AQBMM49 are isolated from *M. mytilorum*; S.AQBTA66 is isolated from *T. anhelans*; S.AQBDF81 is isolated from *D. fragilis.*

growth in presence of sucrose. Precursor amino acids like glycine and tryptophan helped in excellent growth of the strains other than the strain AQBCD11 which grew well in the presence of sulphur containing amino acid cystine. The strains AQBCD11, AQBMM35 and AQBMM49 exhibited excellent growth at 6% and 7% sodium chloride concentrations. All the strains were able to grow between 22 and 45 ˚C and pH 4–10. Chemotaxonomical studies revealed the presence LL-DAP and all the strains possessed cell wall type I, which is the specific characteristic of the *Streptomyces* genus. The cultures don't possess specific sugars in the whole cell hydrolysates. The systematic of the seven strains are given as below.

3.1. Streptomyces AQBCD03

The strain exhibited true mycelium with positive aerial and negative mycelial spore production. The number of spores on aerial mycelium ranged from 13 to 20. The shape of spores was globose and the spore surfaces were smooth. The spore chain morphology was rectiflexibilis and had spore diameter of 1.0 µm. The cell wall was of type 1 containing LL-DAP. The aerial mycelia colour was grey and substrate mycelia were yellow. The strain did not produce melanoid pigment. The strain exhibited carbon utilization positive towards sugars like glucose, xylose, fructose, arabinose, rhamnose, mannitol and negative to galactose, raffinose, inositol and sucrose. Profused growth of the strain was observed with the usage of amino acid like tryptophan, glycine, valine, cystine, alanine and poor growth with the control. The strain was able to grow at sodium chloride tolerance level of 5% to 8%. Moderate growth (3%, 4%, 9%) and poor growth (0%, 1%, 1.5%, 2%, 2.5%, 10%) were also observed. The strain exhibited antibacterial activity against

Table 2

Mycelial colour characteristics of the selected *Streptomyces* isolates.

S. No.	White	Grey	Yellow
1	S.AQBCD01	S.AQBCD03	S.AQBCD04
2	S.AQBCD02	S.AQBCD06	S.AQBCD07
3	S.AQBCD05	S.AQBCD12	S.AQBCD16
4	S.AQBCD08	S.AQBCD13	S.AQBCD19
5	S.AQBCD09	S.AQBCD14	S.AQBCD27
6	S.AQBCD10	S.AQBCD20	S.AQBMM38
7	S.AQBCD11	S.AQBCD23	S.AQBMM39
8	S.AQBCD15	S.AQBMM29	S.AQBMM49
9	S.AQBCD17	S.AQBMM30	S.AQBTA67
10	S.AQBCD18	S.AQBMM36	S.AQBTA72
11	S.AQBCD21	S.AQBMM37	S.AQBTA73
12	S.AQBCD22	S.AQBMM40	S.AQBTA79
13	S.AQBCD24	S.AQBMM41	S.AQBDF85
14	S.AQBCD25	S.AQBMM47	S.AQBDF86
15	S.AQBCD26	S.AQBMM48	S.AQBDF93
16	S.AQBCD28	S.AQBMM50	SAQBDF94
17	S.AQBMM31	S.AQBMM51	
18	S.AQBMM32	S.AQBMM52	
19	S.AQBMM33	S.AQBMM53	
20	S.AQBMM34	S.AQBMM54	
21	S.AQBMM35	S.AQBMM55	
22	S.AQBMM42	S.AQBMM56	
23	S.AQBMM43	S.AQBMM59	
24	S.AQBMM44	S.AQBMM60	
25	S.AQBMM45	S.AQBMM61	
26	S.AQBMM46	S.AQBMM62	
27	S.AQBMM57	S.AQBMM63	
28	S.AQBMM58	S.AQBMM64	
29	S.AQBTA65	S.AQBTA68	
30	S.AQBTA66	S.AQBTA69	
31	S.AQBTA71	S.AQBTA70	
32	S.AQBTA74	S.AQBTA75	
33	S.AQBDF83	S.AQBTA76	
34	S.AQBDF84	S.AQBTA77	
35	S.AQBDF88	S.AQBTA78	
36	S.AQBDF89	S.AQBTA80	
37	S.AQBDF90	S.AQBDF81	
38	S.AQBDF91	S.AQBDF82	
39		S.AQBDF87	
40		S.AQBDF92	
Total	38	40	16
Net total		94	

Table 4

Soluble pigment characterisation of the selected isolates.

S. No.	White	Grey	Yellow
1	S.AQBCD01	S.AQBCD03	S.AQBCD07
2	S.AQBCD02	S.AQBCD06	S.AQBCD16
3	S.AQBCD11	S.AQBCD20	S.AQBMM39
4	S.AQBCD24	S.AQBCD23	S.AQBMM49
5	S.AQBMM31	S.AQBMM47	S.AQBDF86
6	S.AQBMM32	S.AQBMM48	S.AQBDF93
7	S.AQBMM35	S.AQBMM59	
8	S.AQBTA66	S.AQBMM60	
9	S.AQBTA74	S.AQBTA70	
10	S.AQBDF90	S.AQBTA75	
11		S.AQBTA76	
12		S.AQBDF81	
13		S.AQBDF87	
14		S.AQBDF92	
Total	10	14	6
Net total		30 (31.91%)	

Table 5

Melanoid pigment production of the selected isolates.

S. No.	White	Grey	Yellow
1	S.AQBCD01	S.AQBCD06	S.AQBCD04
2	S.AQBCD05	S.AQBCD12	S.AQBCD07
3	S.AQBCD08	S.AQBCD13	S.AQBCD27
4	S.AQBCD09	S.AQBMM30	S.AQBMM38
5	S.AQBCD25	S.AQBMM36	S.AQBMM39
6	S.AQBCD28	S.AQBMM37	S.AQBTA67
7	S.AQBMM33	S.AQBMM40	S.AQBTA79
8	S.AQBMM34	S.AQBMM55	S.AQBDF93
9	S.AQBMM45	S.AQBMM56	
10	S.AQBTA65	S.AQBMM59	
11	S.AQBDF84	S.AQBMM60	
12	S.AQBDF90	S.AQBMM61	
13		S.AQBMM62	
14		S.AQBTA70	
15		S.AQBTA72	
16		S.AQBTA80	
17		S.AQBDF87	
18		S.AQBDF92	
Total	12	18	8
Net total		38 (40.42%)	

Table 3

Substrate mycelial colour characteristics of the selected *Streptomyces* isolates.

S. No.	Brown	Dark green	Greenish grey	Olive green	Orange	Pale brown	Pale yellow	Red	Reddish brown	White	Yellow
1	S.AQBCD16	S.AQBCD06	S.AQBCD20	S.AQBCD10	S.AQBCD24	S.AQBMM38	S.AQBCD04	S.AQBTA66	S.AQBCD27	S.AQBCD01	S.AQBCD03
2	S.AQBCD19	S.AQBCD28	S.AQBCD23	S.AQBCD15	S.AQBMM53	S.AQBMM39	S.AQBCD05		S.AQBDF82	S.AQBCD02	S.AQBCD12
3	S.AQBMM37	S.AQBMM29	S.AQBMM31	S.AQBCD21		S.AQBTA79	S.AQBCD07		S.AQBDF83	S.AQBCD11	S.AQBCD13
4	S.AQBDF85	S.AQBMM30	S.AQBMM32	S.AQBCD22		S.AQBDF94	S.AQBMM35		S.AQBDF87	S.AQBMM42	S.AQBCD14
5	S.AQBDF86	S.AQBMM47	S.AQBMM33	S.AQBMM57			S.AQBMM50			S.AQBMM43	S.AQBMM36
6		S.AQBMM48	S.AQBMM34	S.AQBMM58			S.AQBMM51			S.AQBMM44	S.AQBMM40
7		S.AQBTA71	S.AQBTA67	S.AQBTA73			S.AQBMM52			S.AQBMM49	S.AQBMM41
8		S.AQBTA72		S.AQBTA74			S.AQBDF92			S.AQBTA65	S.AQBTA78
9							S.AQBDF93				S.AQBTA80
10											S.AQBDF81
Total	5	8	7	8	2	4	9	1	4	8	10
Net Total						66 (70.21%)					

pathogens like *Bacillus subtilis* (*B. subtilis*), *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*) and antifungal activity against *Saccharomyces cerevisiae* (*S. cerevisiae*). The strains grew well at pH 7 and temperature of 45 °C. The strain has been found to be associated with the sponges *C. diffusa* which has habitat from Kovalam coast, India.

Table 6
Sporophore morphology of the selected *Streptomyces* isolates.

Types of spores	White	Grey	Yellow	Total
Rectiflexible (RF)	S.AQBCD05	S.AQBCD03	S.AQBCD04	
	S.AQBCD08	S.AQBCD14	S.AQBCD16	
	S.AQBCD10	S.AQBMM29	S.AQBCD27	
	S.AQBCD11	S.AQBMM30	S.AQBMM49	
	S.AQBCD15	S.AQBMM50	S.AQBTA67	
	S.AQBCD17	S.AQBMM51	S.AQBTA79	
	S.AQBCD24	S.AQBMM52	S.AQBDF86	
	S.AQBCD25	S.AQBMM60	S.AQBDF93	
	S.AQBCD26	S.AQBMM61	S.AQBDF94	
	S.AQBCD28	S.AQBTA76		
	S.AQBMM33	S.AQBTA77		
	S.AQBMM34	S.AQBTA78		
	S.AQBMM35	S.AQBTA80		
	S.AQBMM46	S.AQBDF81		
	S.AQBMM57			
	S.AQBMM58			
	S.AQBTA66			
	S.AQBDF89			
	S.AQBDF90			
	S.AQBDF91			
Total	20	14	9	43 (45.74%)
Spirales (S)	S.AQBCD09	S.AQBCD06	S.AQBCD07	
	S.AQBCD18	S.AQBCD12	S.AQBMM38	
	S.AQBCD21	S.AQBCD23	S.AQBMM39	
	S.AQBCD22	S.AQBMM40	S.AQBTA72	
	S.AQBMM31	S.AQBMM41	S.AQBTA73	
	S.AQBMM32	S.AQBMM47		
	S.AQBMM42	S.AQBMM48		
	S.AQBMM43	S.AQBMM53		
	S.AQBTA65	S.AQBMM54		
	S.AQBDF83	S.AQBMM55		
	S.AQBDF84	S.AQBMM56		
	S.AQBDF88	S.AQBMM59		
		S.AQBMM62		
		S.AQBMM63		
		S.AQBMM64		
		S.AQBTA68		
		S.AQBTA69		
		S.AQBDF82		
Total	12	18	5	35 (37.23%)
Rectinaculiaperti (RA)	S.AQBCD01	S.AQBCD13	S.AQBCD19	
	S.AQBCD02	S.AQBCD20	S.AQBDF85	
	S.AQBMM44	S.AQBMM36		
	S.AQBMM45	S.AQBMM37		
	S.AQBTA71	S.AQBTA70		
	S.AQBTA74	S.AQBTA75		
		S.AQBDF87		
		S.AQBDF92		
Total	6	8	2	16 (17.02%)
Net total	38	40	16	94

Table 7
Antagonistic activities of *Streptomyces* strains against bacterial and fungal pathogens.

Strains	Bacterial pathogens				Fungal Pathogens		
	B. subtilis	*S. aureus*	*V. cholerae*	*E. coli*	*A. niger*	*S. cerevisiae*	*C. albicans*
AQBCD03	++	++	-	+++	-	+++	-
AQBCD11	+	-	++	+++	-	-	+++
AQBCD24	++	++	-	++	-	-	++
AQBMM35	-	+++	++	++	-	-	-
AQBMM49	++	++	-	-	-	+++	+++
AQBTA66	++	++	++	+	++	-	++
AQBDF81	++	+++	-	+++	-	-	-

+: < 10 mm; ++: < 20 mm; +++: < 30 mm; -: No inhibition zone.

3.2. Streptomyces AQBCD11

The strain exhibited true mycelium with positive aerial and negative mycelial spore production. The number of spores on aerial mycelium ranged from 21 to 50. The shape of spores was globose and the spore surfaces were smooth. The spore chain morphology was rectiflexibilis and had spore diameter of 2.0 µm. The cell wall is of type 1 containing LL-DAP. The aerial and substrate mycelial colour was white. The strain did not produce melanoid pigment. The strain exhibited carbon utilization positive towards sugars like glucose, xylose, fructose, raffinose and negative to galactose, rhamnose, inositol, sucrose and mannitol. Profused growth of the strain was observed with the usage of amino acid like cystine, tryptophan, glycine, valine, alanine and poor growth with the control. The strain was able to grow at sodium chloride tolerance level of 6% and 7%. Moderate growth (3%, 4%, 5%, 8%, 9%) and poor growth (0%, 1%, 1.5%, 2%, 2.5%, 10%) were also observed. The strain exhibited antibacterial activity against pathogens like *B. subtilis, E. coli, Vibrio cholerae* (*V. cholerae*) and antifungal activity against *Candida albicans* (*C. albicans*). The strains grew well at pH 5 and temperature of 30 °C. The strain has been found to be associated with the sponges *C. diffusa* which has habitat from Kovalam coast, India.

3.3. Streptomyces AQBCD24

The strain exhibited true mycelium with positive aerial and negative mycelial spore production. The number of spores on aerial mycelium ranged from 15 to 17. The shape of spores was globose and the spore surfaces were smooth. The spore chain morphology was rectiflexibilis and had spore diameter of 0.5 µm. The cell wall was of type 1 containing LL-DAP. The aerial mycelia colour was white and substrate mycelial colour was orange. The strain did not produce melanoid pigment. The strain exhibited carbon utilization positive towards sugars like glucose, xylose, galactose, fructose, rhamnose and negative to arabinose, raffinose, inositol and mannitol. Profused growth of the strain was observed with the usage of amino acid tryptophan, moderate growth observed in glycine, valine, cystine, alanine containing media and poor growth with the control. The strain was able to grow at sodium chloride tolerance level of 4%–8%. Moderate growth (2.5%, 3%, 9%, 10%) and poor growth (0%, 1%, 1.5%, 2%) were also observed. The strain exhibited antibacterial activity against pathogens like *B. subtilis*, *S. aureus*, *E. coli* and antifungal activity against *C. albicans*. The strains grew well at pH 7 and temperature of 37 °C. The strain has been found to be associated with the sponges *C. diffusa* which has habitat from Kovalam coast, India.

3.4. Streptomyces AQBMM35

The strain exhibited true mycelium with positive aerial and negative mycelial spore production. The number of spores on aerial mycelium ranged from 13 to 20. The shape of spores was globose and the spore surfaces were smooth. The spore chain morphology was rectiflexibilis and had spore diameter of 1.0 µm. The cell wall was of type 1 containing LL-DAP. The aerial mycelia colour was white and substrate mycelial colour was pale yellow. The strain did not produce melanoid pigment. The strain exhibited carbon utilization positive towards sugars like glucose, xylose, galactose, raffinose, arabinose and negative to inositol, fructose, rhamnose and mannitol. Profused growth of the strain was observed with the usage of amino acid like glycine, tryptophan, valine, cystine, alanine, and poor growth with the control. The strain was able to grow at sodium chloride tolerance level of 6%. Moderate growth (3%, 4%, 5%,

7%, 8%,) and poor growth (0%, 1%, 1.5%, 2%, 9%, 10%) were also observed. The strain exhibited antibacterial activity against pathogens like *S. aureus*, *E. coli* and *V. cholerae*. The strains grew well at pH 9 and temperature of 25 ℃. The strain has been found to be associated with the sponges *M. mytilorum* which has habitat from Vizhinjam coast, India.

3.5. Streptomyces AQBMM49

The strain exhibited true mycelium with positive aerial and negative mycelial spore production. The number of spores on aerial mycelium ranged from 30 to 50. The shape of spores was globose and the spore surfaces were smooth. The spore chain morphology was rectiflexibilis and had spore diameter of 1.5 μm. The cell wall is of type 1 containing LL-DAP. The aerial mycelia colour was yellow and substrate mycelial colour was white. The strain did not produce melanoid pigment. The strain exhibited carbon utilization positive towards sugars like glucose, xylose, arabinose, galactose, mannitol and negative to raffinose, inositol, sucrose, fructose, and rhamnose. Profused growth of the strain was observed with the usage of amino acid like tryptophan, glycine, valine, cystine, alanine and poor growth with the control. The strain was able to grow at sodium chloride tolerance level of 4%–7%, Moderate growth (2.5%, 3%, 8%, 9%, 10%) and poor growth (0%, 1%, 1.5%, 2%) were also observed. The strain exhibited antibacterial activity against pathogens like *B. subtilis*, *S. aureus* and antifungal activity against *S. cerevisiae*, *C. albicans*. The strains grew well at pH 4 and temperature of 37 ℃. The strain has been found to be associated with the sponges *M. mytilorum* which has habitat from Vizhinjam coast, India.

3.6. Streptomyces AQBTA66

The strain exhibited true mycelium with positive aerial and negative mycelial spore production. The number of spores on aerial mycelium ranges from 20 to 50. The shape of spores was globose and the spore surfaces were smooth. The spore chain morphology was rectiflexibilis and had spore diameter of 0.5 μm. The cell wall was of type 1 containing LL-DAP. The aerial mycelia colour was white and substrate mycelial colour was red. The strain did not produce melanoid pigment. The strain exhibited carbon utilization positive towards sugars like glucose, xylose, fructose, arabinose, mannitol, galactose and negative to raffinose, rhamnose, inositol. Profused growth of the strain was observed with the usage of amino acid like tryptophan, valine, alanine, moderate growth with glycine, cystine and poor growth with the control. The strain was able to grow at sodium chloride tolerance level of 4%–9%. Moderate growth (2.5%, 3%, 10%) and poor growth (0%, 1%, 1.5%, 2%) were also observed. The strain exhibited antibacterial activity against pathogens like *B. subtilis*, *S. aureus*, *E. coli*, *V. cholerae* and antifungal activity against *A. niger*, *C. albicans*. The strains grew well at pH 10 and temperature of 30 ℃. The strain has been found to be associated with the sponges *T. anhelans* which has habitat from Kovalam coast, India.

3.7. Streptomyces AQBDF81

The strain exhibited true mycelium with positive aerial and negative mycelial spore production. The number of spores on aerial mycelium ranged from 20 to 50. The shape of spores was globose and the spore surfaces were smooth. The spore chain morphology was rectiflexibilis and had spore diameter of 0.5 μm. The cell wall is of type 1 containing LL-DAP. The aerial mycelia colour was grey white and substrate mycelial colour was yellow. The strain did not produce melanoid pigment. The strain exhibited carbon utilization positive towards sugars like glucose, xylose, mannitol, fructose, galactose and negative to raffinose, inositol, arabinose, rhamnose. Profused growth of the strain was observed with the usage of amino acid like tryptophan, glycine, valine, cystine, alanine and poor growth with the control. The strain was able to grow at sodium chloride tolerance level of 5%–9%. Moderate growth (3%, 4%, 10%) and poor growth (0%, 1%, 1.5%, 2%, 2.5%) were also observed. The strain exhibited antibacterial activity against pathogens like *B. subtilis*, *S. aureus* and *E. coli*. The strains grew well at pH 7 and temperature of 22 ℃. The strain has been found to be associated with the sponges *D. fragilis* which has habitat from Vizhinjam coast, India.

4. Discussion

In the study on total microbial population associated with marine sponges, the maximum population occurred during southwest monsoon season. Ninety four strains of dominant streptomycetes were randomly selected and were characterized for the authentication of *Streptomyces* spp. Upon various parametric analyses for characterization, the cultures were identified up to genus level. All the cultures exhibited the similar morphology of streptomycetes which match well with the earlier descriptions of ISP[21,22]. Similar observation on diversity of streptomycetes from Chinnamuttam and Pallam coast, south west coast of India was reported[27]. Earlier observations by many investigators conferring that selected isolates were belonged to the genus *Streptomyces*[23]. All the 94 isolates showed different mycelial colourations and accordingly they were classified. Mainly because of different secondary metabolites production, the mycelial pigmentation of the strains showed variation. The secondary metabolites synthesized are vast source of components like amino acids, sugars, fatty acids, terpenes, *etc*.[14]. Earlier explanation on shape and spore chain morphology very well correlated with the present observations[21,22].

Streptomycetes are classified under the order of Actinomycetales and within the classes Actinobacteria. They are mostly aerobic, Gram-positive and have DNA guanine-cytosine content of 69–78 mol%[28]. Branching substrate and aerial mycelium are formed in streptomycetes. During growth phase, the substrate mycelia lack cross-walls and they possess hyphae of 0.5–1.0 μm in diameter. At the hyphal apex growth may be initiated followed by branching and thus produce tight complex woven hyphal matrix. As it matures, aerial mycelia (sporophores) are developed. Later chains of spores (conidia) are formed in the cross walls of multinucleate aerial filaments and lead to the separation of individual spores. The morphological changes like branching, spore chain configuration and spore surface arrangement are the most important characterisation of aerial mycelium. These characters were unique for each species and are used for *Streptomyces* species identification[23].

In the study, seven strains proved antagonism against bacterial pathogens like *B. subtilis, S. aureus, V. cholerae, E. coli* and fungal

pathogens like *A. niger*, *S. cerevisiae*, *C. albicans*. The strains showed inhibition zones in the ranges of 5–30 mm. Similarly in a previous report, the culture extracts of *Streptomyces* exhibited 10–30 mm diameter inhibition zone against fish pathogens[24]. In another report, *Streptomyces* associated with gut of marine ornamental fishes namely *Chaetodon collare* and *Archamia fucata* exhibited more than 10 mm of inhibition zone against *V. cholerae*[25]. Earlier it has been reported that four strains of *Streptomyces* showed inhibition zone of 10–35 mm diameter against fish pathogens like *Aeromonas hydrophila* and *Vibrio* species[29]. In another report, an inhibition zone of 10–15 mm was recorded against pathogens like *V. cholerae*, *E. coli* and *Pseudomonas* species[19].

The nutritional uptake (carbon utilisation, amino acids influence, sodium chloride tolerance), physiological (pH, temperature), and chemotaxonomic characteristics clearly proved that the seven strains to be classified under the genus *Streptomyces*[22,23]. In a previous report, *Streptomyces plicatus* isolated from the fish, *Gerres filamentoses* showed more affinity towards glucose[30]. Streptomycetes from the gut of ornamental fish, *Barbus schwanefeldi*, showed maximum growth in mannitol and xylose[31]. The amino acids are the precursors for the production of the secondary metabolites[18]. It has been reported that some sodium chloride-sensitive actinomycetes have increased tolerance to higher concentrations during successive cultivations and the filtrate of the tolerant strains had a significantly different absorbance in the visible spectrum suggesting the production of new metabolites[27,32]. Physiologically the strains grow well in the temperature range of 22–45 ℃ and pH of 4–10. Similar reports showed that the *Streptomyces* strains can grow in the range of 25–37 ℃, and at pH 8–10[33]. Presence of cell wall type I *sensu* is also one of the characteristic features which can distinguish *Streptomyces* species from other actinomycetes and this cell wall type shows the presence of LL-DAP, glycine and absence of characteristic sugars. The *Streptomyces* possess acetyl type of the muramyl residues in the cell-wall peptidoglycans which is character of chemo type I cell wall[34].

The conventional identification of the seven strains were carried out. The characters of the strain *Streptomyces* AQBCD03 can be compared to a reasonable degree with characters of the type strain *S. antibioticus*[35]. However the strain differs markedly from the *S. antibioticus* in the melanoid pigmentation production[36]. Hence the isolate *Streptomyces* AQBCD03 deviates from the *S. antibioticus* in this aspect probably is a different species. The strain *Streptomyces* AQBCD11 did not resemble any of the reported species of *Streptomyces* in the ISP and also the taxonomy classification and related genera[28]. The strain also differed from the species classified from the marine and estuarine sediment at Porto Novo along the east coast of India[13] and it may be a new taxon. The nutritional, physiological and biochemical characterization suggested that the strain *Streptomyces* AQBCD24 be classified under *Streptomyces* genus. The strain was further confirmed and identified as *Streptomyces noursei* (*S. noursei*) MTCC 10469 by Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India[33]. The strain produced similar antibiotic activity as *S. noursei*. It is well known universally that *S. noursei* produces polyene macrolide antibiotic, nystatin, which is an important antifungal agent[37]. Here the strain *Streptomyces* AQBCD24 showed antifungal activity against *C. albicans*. The strain *Streptomyces* AQBMM35 did not resemble any of the reported species of *Streptomyces* in the ISP and also the taxonomy classification and related genera[28]. The strain also differed from the species classified from the marine and estuarine sediment at Porto Novo along the east coast of India[13] and it may be a new taxon[18]. The strain *Streptomyces* AQBMM49 resembles *Streptomyces albidoflavus* (*S. albidoflavus*). It has been reported that *S. albidoflavus* utilises carbon sources like glucose, xylose, arabinose, fructose, mannitol but the strain *Streptomyces* AQBMM49 utilises the same carbon sources except fructose; instead galactose has been utilised[13]. Morphological characters like spore chain ornamentation and melanoid pigmentation showed similarity with *S. albidoflavus*. Antibacterial activity by *S. albidoflavus* correlated with the results of the strain *Streptomyces* AQBMM49[38]. The strain *Streptomyces* AQBTA66 resembles *Streptomyces aureocirculatus* (*S. aureocirculatus*). Carbon utilisation by *Streptomyces* AQBTA66 showed similarity with the strain *S. aureocirculatus*[13]. This strain produced antibiotic activity against bacteria which agreed with the earlier observation[27]. The strain *Streptomyces* AQBTA66 is tentatively identified as *S. aureocirculatus* or it may be a subspecies. The strain *Streptomyces* AQBDF81 resembles *Streptomyces olivaceus* (*S. olivaceus*). Carbon utilisation by *Streptomyces* AQBDF81 showed similarity with the strain *S. olivaceus*[13]. This strain produced antibiotic against bacteria which agreed with the earlier observation[39]. It has been observed that *S. olivaceus* produced β-lactam compounds. The strain *Streptomyces* AQBDF81 is tentatively identified as *S. olivaceus* or it may be a subspecies. Marine environment owns rich diversity of microbes and they are the source of large number of novel bioactive compounds. Because of filter feeding mechanism, the interaction of symbiotic microbes particularly Actinobacteria with the marine sponges were quite common. The present work was carried out on the diversity of Actinobacteria particularly *Streptomyces* associated with sponges, which were enormous and have been confirmed through spore morphology, mycelia colouration, various pigmentations, antimicrobial assay, nutritional uptake, physiological chemotaxonomic studies and the selected strains were identified till genus level. Although isolation strategies directed towards new marine-derived *Streptomyces* have been lacking, some progress has recently been made in this area. But the work on the recent development of DNA sequencing of marine streptomycetes, no pertinent work was carried out in Indian coastal regions. Recent investigations using enrichment techniques, new selection methods and media have led to the isolation of novel *Streptomyces* strains from various marine sources[19]. The oceans as the marine environment thus remain vast and untapped sources of a wide variety of microbial products which could be applied for commercial utility not only to the human beings benefits but also to the vast majority of animal's world.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This work was financially supported by Department of Science and Technology of the Indian Ministry of Science and Technology (Grant No. SR/S0/AS-57/2004).

References

[1] Kim SK, Dewapriya P. Bioactive compounds from marine sponges and their symbiotic microbes: a potential source of nutraceuticals. *Adv Food Nutr Res* 2012; **65**: 137-51.

[2] Wijffels RH. Potential of sponges and microalgae for marine biotechnology. *Trends Biotechnol* 2008; **26**: 26-31.

[3] Thomas TRA, Kavlekar DP, LokaBharathi PA. Marine drugs from sponge-microbe association – a review. *Mar Drugs* 2010; **8**: 1417-68.

[4] Webster NS, Taylor MW. Marine sponges and their microbial symbionts: love and other relationships. *Environ Microbiol* 2012; **14**: 335-46.

[5] Hooper JNA, Van Soest RWM. *Systema porifera. A guide to the classification of sponges*. New York: Kluwer Academic/Plenum Publishers; 2002.

[6] Drozdov AL, Karpenko AA. Structural arrangement and properties of spicules in glass sponges. *ISRN Mater Sci* 2011; **2011**: 535872.

[7] Mehbub MF, Lei J, Franco C, Zhang W. Marine sponge derived natural products between 2001 and 2010: trends and opportunities for discovery of bioactives. *Mar Drugs* 2014; **12**: 4539-77.

[8] Sabdono A, Radjasa OK. Microbial symbionts in marine sponges: marine natural product factory. *J Coast Dev* 2008; **11**: 57-61.

[9] Vasanthabharathi V, Jayalakshmi S. Review on bioactive potential of marine microbes. *Afr J Microbiol Res* 2013; **7**: 4683-8.

[10] Hardoim CC, Costa R. Microbial communities and bioactive compounds in marine sponges of the family Irciniidae – a review. *Mar Drugs* 2014; **12**: 5089-122.

[11] Ribes M, Jiménez E, Yahel G, López-Sendino P, Diez B, Massana R, et al. Functional convergence of microbes associated with temperate marine sponges. *Environ Microbiol* 2012; **14**(5): 1224-39.

[12] Feby A, Nair S. Sponge-associated bacteria of Lakshadweep coral reefs, India: resource for extracellular hydrolytic enzymes. *Adv Biosci Biotechnol* 2010; **1**: 330-7.

[13] Sivakumar K, Sahu MK, Thangaradjou T, Kannan L. Research on marine actinobacteria in India. *Indian J Microbiol* 2007; **47**: 186-96.

[14] Dharmaraj S. Marine *Streptomyces* as a novel source of bioactive substances. *World J Microbiol Biotechnol* 2010; **26**: 2123-39.

[15] Palomo S, González I, de la Cruz M, Martín J, Tormo JR, Anderson M, et al. Sponge-derived *Kocuria* and *Micrococcus* spp. as sources of the new thiazolyl peptide antibiotic kocurin. *Mar Drugs* 2013; **11**: 1071-86.

[16] Dharmaraj S, Ashokkumar B, Dhevendaran K. Food-grade pigments from *Streptomyces* sp. isolated from the marine sponge *Callyspongia diffusa*. *Food Res Int* 2009; **42**: 487-92.

[17] Abdelmohsen UR, Pimentel-Elardo SM, Hanora A, Radwan M, Abou-El-Ela SH, Ahmed S, et al. Isolation, phylogenetic analysis and anti-infective activity screening of marine sponge-associated actinomycetes. *Mar Drugs* 2010; **8**: 399-412.

[18] Dharmaraj S, Ashokkumar B, Dhevendaran K. Fermentative production of carotenoids from marine actinomycetes. *Iran J Microbiol* 2009; **1**: 36-41.

[19] Nair AG, Selvakumar D, Dhevendaran K. Occurrence of sponges associated *Streptomyces* and its antimicrobial activity. *World J Fish Mar Sci* 2011; **3**: 151-8.

[20] Hooper JNA. *Sponguide: guide to sponge collection and identification*. South Brisbane: Queensland Museum; 2000.

[21] Reddy NG, Ramakrishna DPN, Raja Gopal SV. A morphological, physiological and biochemical studies on marine *Streptomyces rochei* (MTCC 10109) showing antagonistic activity against selective human pathogenic microorganisms. *Asian J Biol Sci* 2011; **4**: 1-14.

[22] Xu LH, Li WJ, Liu ZH, Jiang CL. *Actinomycetes systematic – principle, methods and practice*. Beijing: Science Press; 2006, p. 381-7.

[23] Kämpfer P. The family Streptomycetaceae, Part I: taxonomy. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E, editors. *The prokaryotes: a handbook on the biology of bacteria.* Berlin: Springer; 2006, p. 538-604.

[24] Dharmaraj S. Antagonistic potential of marine Actinobacteria against fish and shellfish pathogens. *Turk J Biol* 2011; **35**: 303-11.

[25] Sheeja MS, Dharmaraj S, Dhevendaran K. Bioactive potential of *Streptomyces* associated with gut of marine ornamental fishes. *Middle-East J Sci Res* 2011; **7**: 327-34.

[26] Dharmaraj S, Dhevendaran K. Production of indole acetic acid by marine Actinobacteria isolated from marine sponge *Mycale mytilorum*. *Indian J Appl Microbiol* 2013; **16**: 53-64.

[27] Ravikumar S, Krishnakumar S, Inbaneson SJ, Gnanadesigan M. Antagonistic activity of marine actinomycetes from Arabian Sea coast. *Arch Appl Sci Res* 2010; **2**: 273-80.

[28] Anderson SA, Wellington EM. The taxonomy of *Streptomyces* and related genera. *Int J Syst Evol Microbiol* 2001; **51**: 797-814.

[29] Dharmaraj S, Ashokkumar B, Dhevendaran K. Isolation of marine *Streptomyces* and the evaluation of its bioactive potential. *Afr J Microbiol Res* 2010; **4**: 240-8.

[30] Anithakumary YK. Studies on antagonistic streptomycetes associated with seaweeds of Kovalam coast, Kerala [dissertation]. Trivandrum: University of Kerala; 2008.

[31] Jagadamika Devi S. Studies on the occurrence of antagonistic *Streptomyces* species in the Hymavathy pond and their influence as a single cell protein feed on the growth of juvenile *Barbus schwanefeldi* (Blecker, 1853) [dissertation]. Trivandrum: University of Kerala; 2005.

[32] Suthindhiran K, Kannabiran K. Cytotoxic and antimicrobial potential of actinomycete species *Saccharopolyspora salina* VITSDK4 isolated from the Bay of Bengal coast of India. *Am J Infect Dis* 2009; **5**: 90-8.

[33] Dharmaraj S. Study of L-asparaginase production by *Streptomyces noursei* MTCC 10469, isolated from marine sponge *Callyspongia diffusa*. *Iran J Biotechnol* 2011; **9**: 102-8.

[34] Kim BH, Andersen C, Benz R. Identification of a cell wall channel of *Streptomyces griseus*: the channel contains a binding site for streptomycin. *Mol Microbiol* 2001; **41**: 665-73.

[35] Jones GH. Actinomycin production persists in a strain of *Streptomyces antibioticus* lacking phenoxazinone synthase. *Antimicrob Agents Chemother* 2000; **44**: 1322-7.

[36] Sujatha P, Bapi Raju KV, Ramana T. Studies on a new marine streptomycete BT-408 producing polyketide antibiotic SBR-22 effective against methicillin resistant *Staphylococcus aureus*. *Microbiol Res* 2005; **160**: 119-26.

[37] Brautaset T, Sekurova ON, Sletta H, Ellingsen TE, StrŁm AR, Valla S, et al. Biosynthesis of the polyene antifungal antibiotic nystatin in *Streptomyces noursei* ATCC 11455: analysis of the gene cluster and deduction of the biosynthetic pathway. *Chem Biol* 2000; **7**: 395-403.

[38] Roy RN, Laskar S, Sen SK. Dibutyl phthalate, the bioactive compound produced by *Streptomyces albidoflavus* 321.2. *Microbiol Res* 2006; **161**: 121-6.

[39] Prema P, Audline Suja S, Ranjani S, Immanuel G. UV/VIS, FTIR spectrum and anticandidial activity of *Streptomyces* strains. *Internet J Microbiol* 2008; **7**: 1-5.