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Current Genomics, 2020, *21*, 271-282

RESEARCH ARTICLE

A Comprehensive Investigation of Potential Novel Marine Psychrotolerant Actinomycetes sp. Isolated from the Bay-of-Bengal

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Abstract: ***Background*:** This study was carried out to classify the diversity of the deep marine psychrotolerant actinomycetes sp. nov., in the Bay of Bengal and exploit the production of cold-active industrial and pharmaceutical biomolecules.

***Objective*:** 1) Characterization, optimum the growth conditions and classify the diversity of the novel isolated deep marine psychrotolerant actinomycetes sp from the Bay-of-Bengal. 2) Screening for industrially important biocatalysts and determine the antimicrobial activities against the five dreadful pathogens. 3) The differential expression profiling of the candidate genes to regulate the biosynthesis of selected enzymes.

***Methods*:** The cold-adapted actinomycetes were isolated from the deep marine water collections at 1200 mts below the surface in Bay-of-Bengal. The phenotypic and genotypic characterizations have been carried out to understand the persistent diversity of this novel marine psychrotolerant actinomycetes species. The production of cold-active enzymes, such as amylase, cellulase, lipase, pectinase, and L-asparaginase, were screened and the expression profiling genes were determined by using qRT PCR. The antibacterial and antifungal activities have also been investigated.

***Results*:** A total number of 37 novel actinomycetes were isolated and the phenotypic and genotypic characterizations identified the genus, dominated by *Streptomyces* (17 distinct sub-groups) as the major group, followed by *Micromonospora, Actinopolyspora, Actinosynnema, Streptoverticillium, Saccharopolyspora, Nocardiopsis,* and *Nocardia*. The optimum growth and abundant mycelium formation are observed at 15°C to 20°C and also capability for thriving at 4°C. All the isolates exhibited a significant role in the production of biocatalysts, and the antagonistic activities were also noted against five major selected pathogens.

***Conclusion*:** The *Streptomyces* from the Bay-of-Bengal have high biosynthetic potential and can serve as a good resource for the exploration of bioactive natural products.

ARTICLE HISTORY

Received: December 14, 2019
Revised: March 05, 2020
Accepted: March 12, 2020

DOI:
10.2174/1389202921666200330150642

Keywords: Antimicrobial activity, psychrotolerant, actinomycetes, metabolites, psychrozyme, streptomyces.

1. INTRODUCTION

Approximately 71% of the earth's surface is covered by oceans, containing a wide range (approximately 3.67×10^{30}) of distinct microbes that are found no-where in the terrestrial environment [1, 2]. The marine environment acts as a large reservoir of the natural compounds and thus, offers a colossal asset for novel compounds to be assessed for pharmaceutical and industrial applications.

The rapid emergence of drug-resistant pathogens has led to a demand for novel antimicrobial compounds. However, despite intensive drug discovery efforts, for decades, no new classes of antibiotics were developed. Thus, requisites for effective antibiotic drugs, the research interest is quite shifting towards the extremophile habitant for a potentially rich source of new antimicrobial compounds. Consequently, unexplored profound marine microbial territories represent a colossal pool of possibly imperative microbial biodiversity and can be a potential source of numerous bioactive molecules [3-5].

The occurrence of deep-sea microbial diversity of actinomycetes genera and their metabolite potential at Bay of Bengal peninsular is rarely documented. In these perspectives, the present study aimed to characterize the cold adap-

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1389-2029/20 $65.00+.00

tive actinomycetes from deep waters collected from four different stations of Bay of Bengal. The effort has also been made to screen the bioactive cold-active enzymes from the deep marine actinomycetes and evaluate the antibiosis against the dreadful pathogens. *Streptomyces* is the largest genus of actinobacteria, and an important source of bioactive compounds, thus the additional study was also conducted on the genotypic characterization and homologous distribution in the genus *Streptomyces* and their economic importance. This study explores the diversity of deep marine psychrotolerant actinomycetes and reports the commercial biomolecules produced from these terrestrial sources.

2. METHODS

2.1. Collection of Deep Marine Water Samples

The "Sagar Kanya Expedition" was organized by the National Centre for Antarctic and Ocean Research (NCAOR), presently known as the National Centre for Polar and Ocean Research, Government of India, to collect the water samples at the depth of 1200 meters (4000 feet deep from the surface). The water samples were collected at the depths of 1200 meters from four different locations at the Bay of Bengal *i.e*., (1) 8° 23' N; 84° 54' E (2) 10° 54' N; 89° 94' E (3) 9ºN; 81°5' E (4) 8° 76' N; 81°15' E, during (NCAOR) expedition. All the water samples were stored in a dark ice chest under controlled conditions for further processing in the laboratory.

2.2. Isolation of Actinomycetes from Marine Water Samples

The isolation of actinomycetes were carried out through filtration of 5 ml deep marine water samples using Sartorius cellulose nitrate filter (0.45-µm-pore-size) and then inverted on starch casein (SC) agar plates containing starch-10 g, casein- 0.3 g, $CaCO_3$- 0.02 g, $FeSO_4.7H_2O$- 0.01 g, KNO_3- 2 g, NaCl- 2 g, K_2HPO_4- 2 g, $MgSO_4.7H_2O$- 0.05 g, agar 15 g, seawater 500 ml, distilled water 500 ml and incubated at 20°C for 72 hr. The SC plates were supplemented with 50 µg/ml of cycloheximide to minimize the fungal contamination. Individual colonies were picked up and further streaked on SC agar and International Streptomyces Project medium 2 (ISP-2) agar plates containing (per liter) yeast extract- 4 g, malt extract- 10 g, dextrose- 4 g, agar- 20 g. Grown colonies of actinomycetes were observed under a light microscope for leathery or chalky appearance, colony size and shape, mycelial color, and filamentous nature. The subcultures were maintained on SC slants.

2.3. Morphological and Biochemical Characterization of Marine Actinomycetes

The coverslip culture technique was used to study the morphological differentiation, such as the number of spores present in the chain, the color of aerial, and substrate mycelium. The gram staining, reduction of nitrate, catalase, starch hydrolysis test, production of exopolysaccharides (EPS), soluble and diffusible pigments have been done as per the standard protocols. The utilizations of varied carbon sources were investigated in order to reach a possible classification of the isolated species.

2.4. Physiological Characterization of Marine Actinomycetes

All the marine isolates were incubated at a temperature ranging from 4ºC to 40ºC, pH of 6.5 to 9 in SC broth media. To study the effect of NaCl on growth, the isolates were inoculated at 0 to 20% of NaCl concentrations. The growth of marine actinomycetes was differentiated into three categories, such as abundant, moderate, and slow growers. All the isolates of marine actinomycetes were grown on SC broth media at 20°C, 150 rpm and the growth was monitored up to 30 days.

The structures of micro-organisms, appearing on SC agar plates, were examined by a light microscope and then in a more detailed fashion by the Scanning Electron Microscope (SEM). The colonies and structures were fixed by placement in 2% (v/v) glutaraldehyde in 0.1 M sodium cacodylate [$Na(CH_3)_2.AsO_2$] buffer (pH 7.2-7.4) followed by treating with Triton wetting agent overnight for 5 days [6, 7].

2.5. Genotypic Characterization and Phylogenetic Classification of Streptomyces

Molecular characterization of *Streptomyces* was carried out by 16S rRNA analysis. The cells from the biomass were harvested by centrifugation, washed, and re-suspended in TE buffer (10 mM Tris/HCl, 1 mM EDTA; pH 8.0). Genomic DNA was extracted by a standard protocol and the PCR amplification was conducted in Eppendorf Master Cycle gradient AG22331 (Model No. 5331), using appropriate forward primer 27F (5' AGA GTT TGA TCC TGG CTC AG 3') and reverse primer 1525R (5' AGA AAG GAG GTG ATC CAG CC 3') in the *E. coli* numbering system [8, 9]. The amplified products were sequenced in Applied Biosystem Sequencer (ABI 3730XL DNA Analyzer) with appropriate primers.

This 16S rRNA gene sequence was used for phylogenetic analysis. The 16S rRNA gene sequence related taxa were acquired from the GenBank database and the primer was designed using the Primer 3 software. Multiple sequence alignment was conducted using the Clustal W program and the phylogenetic tree was constructed using the neighbor-joining method [10, 11] using the MEGA7 software (https://www.megasoftware.net/) and phylogeny program (http://www.phylogeny.fr/). The evolutionary distances for the neighbor-joining and maximum likelihood tree were calculated by Kimura's two-parameters method. The topologies of each tree have been evaluated using bootstrap resampling methods based on 1,000 replications.

2.6. Screening for Industrially Important Cold-active Enzymes

The isolates were screened for cold-active catalysts such as amylase, cellulase, lipase, pectinase, and L-asparaginase. An individual genus of actinomycete was spot inoculated and incubated at 20°C on the agar plate with related substrates.

Amylase assay was carried out on modified Bennett agar containing dextrin- 10 g, yeast extract- 1 g, N-Z Amine A- 2 g, beef extract- 1 g, $CoCl_2.6H_2O$- 0.01 g, agar- 15 g, supplemented with 1% (w/v) starch at pH 7.6. The organisms were spot inoculated and incubated for 5 days and afterwards

flooded with Gram's iodine. The development of a clear zone indicated the production of amylase by the test organisms.

Cellulase assay was carried out by spot inoculation on the Mendel and Reese medium composed of KH_2PO_4- 2 g, $(NH_4)_2SO_4$- 1.4 g, CO $(NH_2)_2$- 0.3 g, $MgSO_4.7H_2O$- 0.3 g, $CaCl_2$- 0.3 g, peptone- 1 g, tween80- 2 ml, $FeSO_4.7H_2O$- 0.005 g, $MnSO_4.H_2O$- 0.002 g, $ZnSO_4.7H_2O$- 0.001 g, $CoCl_2$- 0.002 g, agar- 15 g and 1% pre-treated birchwood xylan (pH 6). After 72 hours of incubation, the plates were flooded with iodine and potassium iodide solution for the confirmation of cellulase production.

Lipase assay was carried using nutrient agar medium with beef extract (0.3%), peptone (0.5%) and agar (1.5%), supplemented with 31.25 ml olive oil and 10 ml of Rhodamine B solution (0.001%, w/v). The isolates were inoculated for 72 hours at 20°C. The lipase activity of the isolates was identified by the production of orange fluorescence halo under UV irradiation.

A plate assay was performed to screen the pectinase activity. The SC agar media with 1% of pectin was used for the above-mentioned purpose. The test organisms were inoculated and incubated at 20ºC for 72 hours with a pH 6.5. Ruthenium red (w/v 0.005%) solution was then added to the plates and the presence of clearance zones indicated the production of pectinases.

The screening for L-asparaginase was done using the rapid plate assay technique described at earlier publications [12, 13]. Phenol red indicator and 0.1% asparagine was used for screening L-asparaginase production. After 48 hours of incubation, L-asparaginase producing actinomycetes exhibited pink color zones due to shifting in pH of the medium.

2.7. Expression Profile of Candidate Genes from Streptomyces

The expression profile of the candidate genes that regulate the biosynthesis of selected enzymes was carried out by quantitative real-time PCR (RT-qPCR). The qRT-PCR was performed using an Applied Biosystems Step-One PCR system in 10 µl reaction volume 2.5 µl of cDNA with 200 µM of each primer set using EvaGreen dye as a detector (Biotium, Hayward, CA, USA). The primers were designed using the online program Primer-3 (www.bioinfo.ut.ee/primer3-0.4.0/) (Table **1**). The experiment was quantified in triplicate under the following amplification conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 30 s and 60°C for 1 min.

2.8. Screening for Antibacterial and Antifungal Activities of Newly Isolated Actinomycetes

The antibacterial activities of marine actinomycetes were carried out by the agar diffusion method. The marine isolates were streaked near the periphery of the culture plate on Muller-Hinton agar medium composed of (g/l) beef extract- 2, casein- 17.5, starch- 1.5, Agar- 17 (pH 7.5) and were incubated at 20°C. After four days of incubation, *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 1688), *Bacillus subtilis* (MTCC 441), and *Shigella flexneri* (MTCC 1457) were subjected to streak at a right angle against the previously streaked actinomycetes culture. The inhibition zones against the pathogens were observed after 48 hours of incubation.

The antagonistic activity of the actinomycetes against the fungal pathogen was evaluated according to the early described method [14]. Isolated strains were streaked on potato dextrose agar plate and incubated for 48 h at 20°C. The mycelial disk of *Rhizoctonia solani* (MTCC 4633) was punched in the center of the plate. The zone of inhibition was measured after 72 hrs of incubation at 20°C.

2.9. Statistical Analysis

Data are analyzed as mean ± SEM with $P<0.05$ being considered significant. The data were analyzed by the analysis of variance (ANOVA) for the relative quantitative expressions of the genes by real-time qRT-PCR.

Table 1. Primers details candidate genes.

Gene Name	Primer Sequences	Annealing Temperature (Ta)	Product Size	GenBank ID
aml	F 5' CTGCACCTCGCAGATAAACA 3'	60°C	246	Y13601.1
	R 5'GCCTCGTGCTTCCAGTAGAC 3'	60°C		
CelStrep	F 5'TACAACGCCTCGTACGACAT 3'	59°C	241	HE862416.1
	R 5'AGTCCATCACGTCGAAGCTC 3'	60°C		
lipA	F5'GGTAGATGGCGAAGACCTTG 3'	59°C	238	U80063
	R 5'GGTAACCACGTCACCTTCGT 3'	59°C		
chb1	F 5'GGTCCGTGATTGGTATGGAC 3'	60°C	237	X78535.1
	R 5'CACATCTTCTGCCTGCTGAC 3'	59°C		
asparaginase	F 5'CCCGGAAAACCTGTATGATG 3'	60°C	167	WP_037798283.1
	R 5'CAATTTTGCCCAGCAGTTCT 3'	60°C		

3. RESULTS

3.1. Characterization and Phylogenic Distribution of Deep Marine Actinomycetes

A total number of 37 Gram-positive deep marine actinomycetes sp. nov., were isolated using SC agar medium which exhibited convex, powdery or elevated colonies on the SC agar plates. The abundant growth was observed in 13 isolates, moderate growth found in 11 isolates, and the remaining 13 isolates exhibited a slow proliferation rate (Table **S1**). The primary microscopic observations and morphological studies confirmed that a large proportion (45.94%) of isolates belonged to the genus *Streptomyces*, followed by the genera *Micromonospora, Actinopolyspora, Actinosynnema, Streptoverticillium, Saccharopolyspora, Nocardiopsis,* and *Nocardia* (Table **S2**). The SEM image showed that the wild-type strains contained many chains of spores with very few apparent sporogenous aerial hyphae (Fig. **1A**).

3.2. Optimum Growth Temperature and NaCl Tolerance

The abundant growth and abundant mycelium formation were observed between 15°C to 20°C as well as the capability for thriving at 4°C. All the isolated actinomycetes required high salt concentration for their growth. The optimum growth was observed with 2 to 5% NaCl concentration and salinity tolerance was recorded at 16% (Table **2**), which is generally considered as extreme halophilic nature. Among 37 isolates, grey and white pigmented marine actinomycetes were more prominent inthe deep sea of Bay of Bengal. Further, it was also observed that out of all the isolates, 18 isolates produced diffusible pigments and 30 isolates exhibited EPS on ISP-2 agar.

3.3. Phenotypic Characterization of the Marine Streptomyces

The *Streptomyces* exhibited different morphological characters in spore chain morphology, mycelial color, pigmentation, and production of EPS. The phenotypic characterization was conducted to identify the distribution of 17 distinct sub-groups of *Streptomyces*. The spore surfaces were smooth, spiny, and rugose in nature, with each chain containing 3 to 14 spores. Examination under a light microscope sheds morphological characterization, such as spores in the branched-chain with the formation of rectiflexibiles (n= 9), retinaculipetri (n= 4) and spiral (n=4) spores, were observed.

In a chain, more than 50 spores were observed at *Streptomyces* sp. S1L-72 and *Streptomyces* sp. S3L-4 isolate. Among all the isolated strains, the white aerial and light beige color substrate mycelium was found most prominent. It was observed that out of 17 isolates, 11 strains produced diffusible pigments, 14 strains showed EPS, and 4 strains exhibited soluble pigments in ISP-2 medium (Table **S3**).

3.4. Genotypic Characterization of Streptomyces

The 16S rRNA gene analysis of 17 isolated strains exhibited greater than 94% homology with *Streptomyces* (Fig. **1B**) and the homologous relationship among the 17 *Streptomyces* was identified (Table **3**). The closest match (99.51%) was observed between *Streptomyces* S1L-39 and *Streptomyces*-YIMM10400, followed by 98.69% homology with *Streptomyces* sp. S1L- 89, *Streptomyces* sp. S4L- 25 and *Streptomyces* sp. S4L- 68.

3.5. Screening for the Enzyme Activities of Cold-adaptive Actinomycetes

All of the genera *Micromonospora, Actinopolyspora, Actinosynnema, Streptoverticillium, Saccharopolyspora,* and *Nocardiopsis* showed cellulase activity (Table **2**). The amylase activity was prominently found in three genera including *Streptoverticillium*, *Micromonospora,* and *Actinopolyspora*. Interestingly, the members of the genera *Streptoverticillium* and *Nocardia* lacked lipase activity. Pectinase activity was well found in genera *Nocardiopsis, Actinosynnema,* and *Nocardia,* while L-asparaginase activity was revealed in *Saccharopolyspora*, *Streptoverticillium*, *Micromonospora,* and *Actinopolyspora*. The genera *Actinosynnema, Streptoverticillium,* and *Nocardia* thrived at an optimum pH of 8.5 and above, salinity up to 15% and optimum temperature of around 15°C.

3.6. Optimization of the Growth Conditions for Isolated Streptomyces

The utilization of carbohydrates by the *Streptomyces* strains showed identical variations in carbon source utilization (Table **S4**). The optimum growth was observed from 2 to 6% NaCl concentration and tolerance up to 10% (w/v), classified as moderate halophiles. *Streptomyces* strains sp. S1L-89 and sp. S3L-32 showed maximum tolerance of up to 16% (w/v).

The physiological characteristics of the *Streptomyces* strains were also studied and evaluated (Table **4**). All the *Streptomyces* strains exhibited starch hydrolysis ,while catalase activity was found mainly in nine strains. All the strains exhibited growth at 15°C to 35°C, at a pH of 7.5 to 8.5. Out of the 17 strains, 12 *Streptomyces* strains showed abundant growth in the ISP-2 medium.

3.7. Activities of Cold-active Enzymes from Streptomyces

All the 17 sub-group identified *Streptomyces* isolates exhibited lipase enzymatic activities (Fig. **2**). Maximum activities of amylase (34.89 U/ml), cellulase (36.89 U/ml), lipase (43.32 U/ml), pectinase (18.54 U/ml) and L- Asparaginase (23.67 U/ml) were identified in *Streptomyces* sp. S1L-89, S1L-72, S2L-41, S4L-54, and S3L-72, respectively. Contrarily, *Streptomyces* sp. S4L-68, sp. S2L-41, sp. S4L-24, sp. S2L-8 and sp. S4L-25 showed minimum amylase (15.89 U/ml), cellulose (10.78 U/ml), lipase (8.89 U/ml), pectinase (3.02 U/ml) and L- asparaginase (7.78 U/ml) activities, respectively (Fig. **2**). The enzymatic activities of the selected five enzymes in *Streptomyces* were confirmed by the relative genes expression levels (RQ). Quantitative RT-PCR was performed for validation of the candidate genes responsible for biosynthesis of amylase (*aml*; GenBank: Y13601.1), cellulase (*CelStrep*; GenBank: HE862416.1), lipase (*lipA*; GenBank: U80063), pectinase (*chb1*; GenBank: X78535.1), L-asparaginase (*asparaginase*; GenBank: WP_037798283.1) in *Streptomyces* (Fig. **3**).

The 10% to 30% enzymatic activities were observed at 5°C to 15°C, while 90-100% of the activity retained between

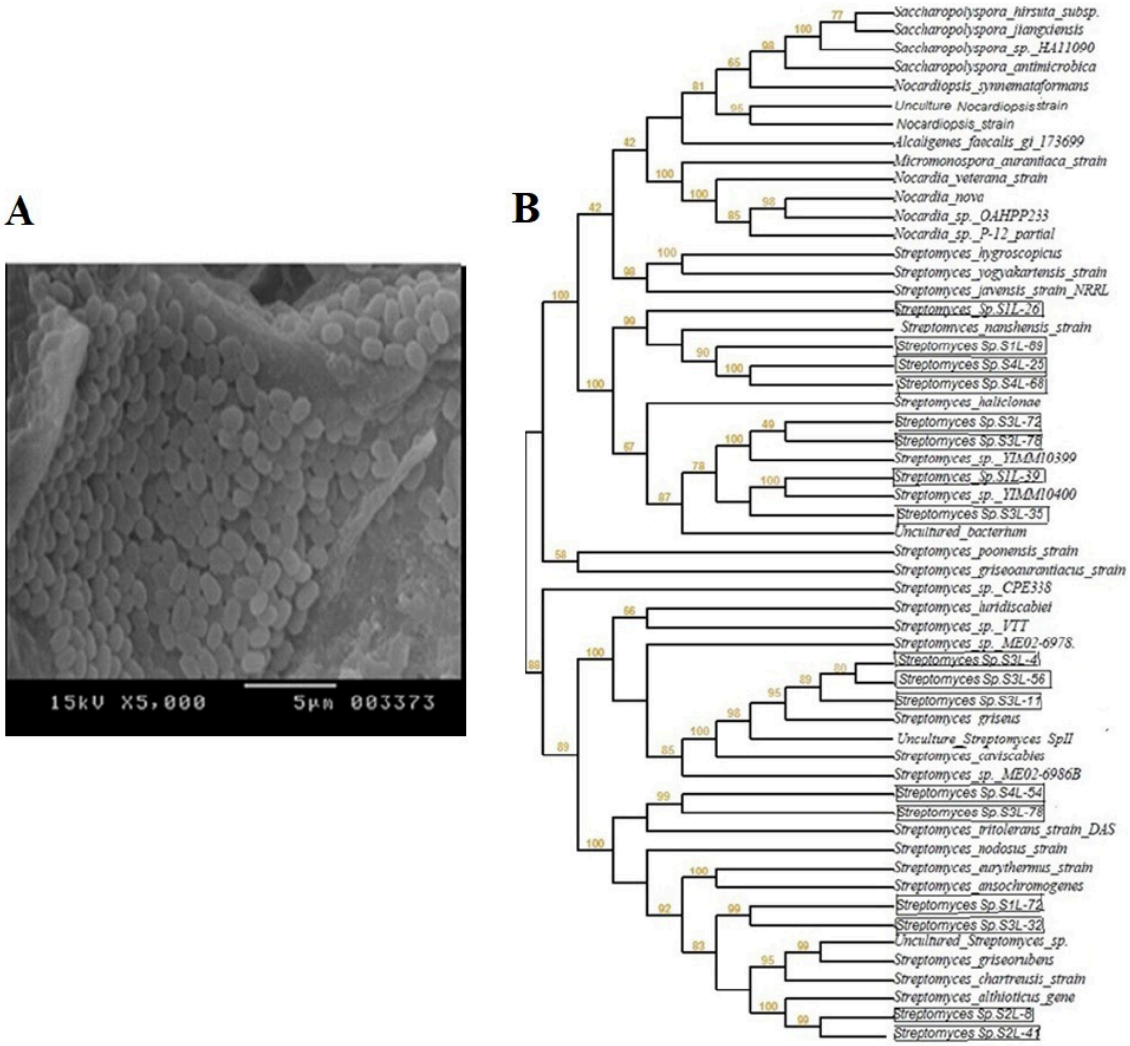

Fig. (1). (**A**) Scanning electron microscopic illustration of streptomycetes. The electronic photograph of isolated streptomycetes, the wild-type strain that contains many chains of spores with very few sporogenous aerial hyphae; (**B**) The neighbor-joining method depicting the diversity and phylogenetic relationship among the *Streptomyces* based on 16S rRNA gene sequences (bootstrap values higher than 50 % indicates the main nodes). Sequencing of the 16S rRNA gene of 17 isolates revealed that all the strains exhibited greater than 94% homology with the genus *Streptomyces*. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

20°C to 30°C. The thermal stability of the enzymes was found between 0°C to 35°C. However, above 40°C, all the enzymes lost their activity rapidly.

3.8. Antibacterial and Antifungal Activities of all Isolated Actinomycetes

The antimicrobial activities of these isolates against the selected pathogens were evaluated. The genera *Saccharopolyspora, Actinosynnema,* and *Micromonospora* did not exhibit any antifungal activities (Fig. **4**). Out of all the species, activity against the dreadful pathogen *Shigella flexneri* was only found in *Streptoverticillium* and *Nocardiopsis*.

The anti-bacterial activities were found in all the sub-grouped *Streptomyces* strains. Among the 17 isolates, a total of 15 showed promising antimicrobial activity against *Staphylococcus aureus*, 14 isolates exhibited activity against *Bacillus subtilis* and *Pseudomonas aeruginosa* and 13 strains showed activity against *Shigella flexneri.* However, five sub-groups from *Streptomyces* namely sp. S1L-72, sp. S3L-4, sp. S3L-35, sp. S4L-24 and sp. S4L-68 did not show activity against *Rhizoctonia solani* (Fig. **4**). In addition, a total of 6 sub-grouped *Streptomyces* strains have exhibited high activity against both the above mentioned pathogenic bacteria and fungus.

4. DISCUSSION

According to Bruun's ecological zonation of the marine temperature, the average annual temperature across 90% of the marine environment ranges between 10°C to 5°C [15, 16] and it contains a wide range of unique microbes that cannot be found in the terrestrial environment [17]. The bioactive compounds from deep marine microbial communities have unrivaled and unmatched significances. Thus, collectively, all these microbes have revolutionized the area of cold marine biology and industrial biotechnology [3]. Herein, we described the new dimensions that have driven the diversified groups of actinomycetes that thrive in deep waters of Bay of Bengal, uncovered the evidence that these

Table 2. Physiological distributions of deep marine actinomycetes.

Characteristics	*Saccharopolyspora*	*Streptoverticillium*	*Nocardiopsis*	*Actinosynnema*	*Micromonospora*	*Nocardia*	*Actinopolyspora*
Total isolates	2	3	1	3	6	1	4
pH range	7.0-8.5	8.0-8.5	8.0- 8.5	7.0- 8.5	6.5- 7.0	7.0- 8.5	7.5- 8.5
Growth at							
4°C	-	+	+	+	-	+	+
41°C	W	-	w	-	w	-	-
Optimum growth temperature (°C)	38±2.0	15±2.0	35±2.0	20±2.0	35±2.0	15±2.0	15±2.0
NaCl concentration (%)	0.5-16	0.5- 14	0.5- 4.0	1.0- 13	0.5- 14	2.0- 10.0	0.5- 15.0
Optimum NaCl concentration (%)	2.0	2.0	2.5	3.0	2.5	3.0	5.0
Antimicrobial activity (zone of inhibition in mm)							
S. aureus	15±0.2	11±0.4	0±0	14±0.2	9.0±0.5	0±0	11±0.2
B. subtilis	14±0.6	0±0	0±0	10±0.3	11±0.4	14±0.2	14±0.3
P. aeruginosa	0±0	0±0	0±0	11±0.2	17±0.4	12±0.4	10±0.4
S. flexneri	0±0	18±0.2	13±0.4	0±0	0±0	0±0	0±0
R. solani	0±0	15±0.2	14±0.2	0±0	0±0	22±0.3	14±0.2
Enzyme activity							
Amylase	-	+	-	-	+	-	+
Cellulase	+	+	+	+	+	+	+
Lipase	+	-	+	+	+	-	+
Pectinase	-	-	+	+	-	+	-
L-asperaginase	+	+	-	-	+	-	+

Key: +, Positive; -, Negative; w, weak; Mean ± SD

marine-derived species act as a Pandora's Box which can produce a variety of bioactive metabolites that accelerate the possibility of their applications in pharmaceutical and bio-tech industries [1, 2].

Most of the actinomycetes have received little attention from researchers in genomic information and associated bio-synthetic potential. Eight different genera of actinomycetes, from the deep waters (1.2 km), were isolated in a modified seawater amended SC agar media which allows only marine actinomycetes to proliferate. The primary identification of various genera of actinomycetes was conducted based on colony morphology at the surface of agar plates. The colony appearance, spore morphology, color of mycelia, and diffusible pigments were considered as important characteristic properties to identify and differentiate *Streptomyces* from other relevant actinomycetes species [18]. The preliminary observation suggested that the *Streptomyces* genus are the most reported genus in the Bay of Bengal. It has been well reported that the majority of marine isolates exhibit grey and white-colored, aerial coiled mycelia. The spores in the chain were spiral, rectiflexibile or retinaculipetri as well as produced diffusible and EPS pigments. Unlike others, this diffusible pigment and EPS is a supporting mechanism for the marine actinomycetes to survive in the marine environment [19].

The diversity in deep marine actinomycetes has not been extensively investigated earlier [20]. The abundant growth in alkaline pH, thriving at low temperatures and high salinity

Table 3. The homologous relationship among the 17 isolates of the Streptomyces. The degrees of homology are mentioned in percentages.

-	**Streptomyces sp. S1L-26**	**Streptomyces sp. S1L-89**	**Streptomyces sp. S4L-25**	**Streptomyces sp. S4L-68**	**Streptomyces sp. S3L-72**	**Streptomyces sp. S3L-78**	**Streptomyces sp. S1L-39**	**Streptomyces sp. S3L-35**	**Streptomyces sp. S3L-4**	**Streptomyces sp. S3L-56**	**Streptomyces sp. S3L-11**	**Streptomyces sp. S4L-54**	**Streptomyces sp. S3L-78**	**Streptomyces sp. S1L-72**	**Streptomyces sp. S3L-32**	**Streptomyces sp. S2L-8**	**Streptomyces sp. S2L-41**
Streptomyces sp. S1L-26	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Streptomyces sp. S1L-89	98.8	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Streptomyces sp. S4L-25	98.2	98.7	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Streptomyces sp. S4L-68	98	98.5	99.2	100	-	-	-	-	-	-	-	-	-	-	-	-	-
Streptomyces sp. S3L-72	97.89	97.3	98	98.5	100	-	-	-	-	-	-	-	-	-	-	-	-
Streptomyces sp. S3L-78	97.01	97.7	98	98.3	99	100	-	-	-	-	-	-	-	-	-	-	-
Streptomyces sp. S1L-39	96	96.8	97	97.4	98	98.5	100	-	-	-	-	-	-	-	-	-	-
Streptomyces sp. S3L-35	96	96.2	96.9	97	97	98	98.7	100	-	-	-	-	-	-	-	-	-
Streptomyces sp. S3L-4	94.1	94.7	95	95	95	95.4	96	96.8	100	-	-	-	-	-	-	-	-
Streptomyces sp. S3L-56	94	94.4	94.8	95	95	95.2	95.8	96	99	100	-	-	-	-	-	-	-
Streptomyces sp. S3L-11	94	94.2	94.5	94.89	94.7	95	95	96	99	99.6	100	-	-	-	-	-	-
Streptomyces sp. S4L-54	93	94	94	94.1	94	94.34	94.2	95	95	95.8	98.5	100	-	-	-	-	-
Streptomyces sp. S3L-78	93	94	94	93.3	93.8	94	94	94.65	95.2	95	98	99	100	-	-	-	-
Streptomyces sp. S1L-72	93	93.89	93.94	93.1	93	93.4	93.9	94	95	95	97	98	98	100	-	-	-
Streptomyces sp. S3L-32	93	93.32	93.54	93	93.4	93	93.67	93.98	94	94.23	95	97	97	99	100	-	-
Streptomyces sp. S2L-8	92	92.18	93	93	93	93	93	93.58	93.89	94	94.38	95	96	97	97	100	-
Streptomyces sp. S2L-41	89	89.54	90.43	90.86	91	91.07	91.68	92	92.13	92.8	93.13	96	96.2	96.8	97	99	100

Table 4. Physiological characteristics of isolates *Streptomyces* strains.

Isolates *Streptomyces* strains	pH	Growth at		Optimum temperature (°C)	Nitrate reduction	NaCl Concentration (%)	Optimum NaCl (%)	Growth Rate	Catalase activity	Starch hydrolysis
		4°C	41°C							
Streptomyces sp. S1L-26	7.5±0.2	+	-	20±2.0	+	0.5- 6.0	2	Abundant	-	+
Streptomyces sp. S1L-39	8.5±0.2	+	-	20±2.0	+	0.5- 10.0	2	Abundant	-	+
Streptomyces sp. S1L-72	8.0±0.2	+	-	20±2.0	+	0.5- 6.0	2	Slow	+	+
Streptomyces sp. S1L-89	8.5±0.2	-	+	35±2.0	-	2.0- 16.0	5	Abundant	+	+
Streptomyces sp. S2L-8	7.0±0.2	+	-	20±2.0	+	2.0- 10.0	3	Abundant	+	w
Streptomyces sp. S2L-41	7.5±0.2	+	-	20±2.0	+	1.0- 8.0	2	Abundant	-	+
Streptomyces sp. S3L-4	7.5±0.2	w	-	30±2.0	-	2.0- 10.0	2	Abundant	+	+
Streptomyces sp. S3L-11	8.0±0.2	+	-	15±2.0	-	0.5- 8.0	2	Moderate	-	+
Streptomyces sp. S3L-32	7.5±0.2	+	-	20±2.0	-	2.0- 16.0	5	Moderate	+	+
Streptomyces sp. S3L-35	7.5±0.2	-	w	35±2.0	+	0.5- 6.0	1	Abundant	+	w
Streptomyces sp. S3L-56	8.5±0.2	+	-	20±2.0	+	1.0- 8.0	2	Abundant	+	+
Streptomyces sp. S3L-72	8.5±0.2	+	-	20±2.0	-	1.0- 8.0	2	Abundant	+	+
Streptomyces sp. S3L-78	8.0±0.2	+	-	25±2.0	+	0.5- 6.0	1	Abundant	-	+
Streptomyces sp. S4L-24	7.5±0.2	+	-	15±2.0	-	0.5- 6.0	2	Slow	-	+
Streptomyces sp. S4L-25	7.5±0.2	-	+	35±2.0	-	2.0- 10.0	3	Abundant	-	w
Streptomyces sp. S4L-54	7.5±0.2	+	-	20±2.0	-	0.5- 10.0	3	Abundant	+	+
Streptomyces sp. S4L-68	8.0±0.2	+	-	20±2.0	-	2.0- 10.0	4	Slow	-	+

Key: +, Positive; -, Negative; w, weak; Mean ± SD

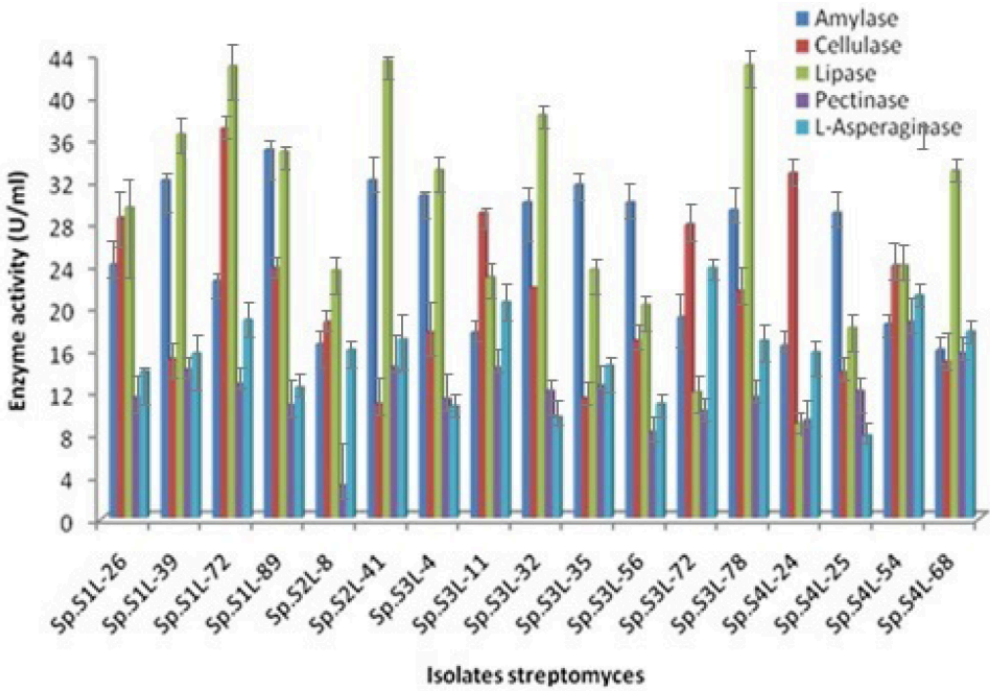

Fig. (2). The activities of five industrial important cold adaptive enzymes properties of *Streptomyces* strains. All the 17 identified strains from the *Streptomyces* exhibited the enzymatic activities. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

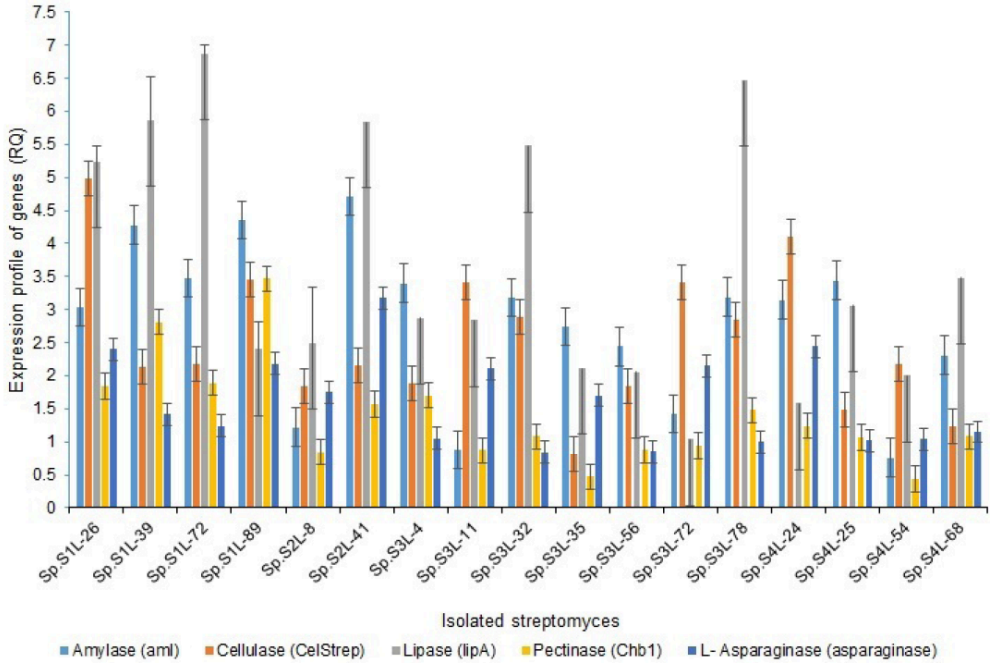

Fig. (3). Relative levels of expression. The bars showing the expression of five genes regulate the biosynthesis of selected enzymes in *Streptomyces*. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

environments, these isolates are categorized as psychrotolerant halophiles [21, 22]. With the paucity of investigation of deep marine psychrotolerant from the Bay of Bengal, this study can offer assistance in further examinations on genetic divergence of actinomycetes and unique potential in industrial approaches. Although high levels of homologous relationships were exhibited among the 17 sub-grouped *Streptomyces*, however, certain differences in the optimal growth temperature, salinity tolerance, and physiochemical properties indicate heterogeneity within the genus.

Actinomycetes are well known for the production of bioactive metabolites with a broad spectrum of parasitic, antitumor, insecticidal, and antimicrobial activities [23-25]. The majority of the isolated actinomycetes exhibited cellulase activity along with lipase, L-asparaginase, amylase, and pec-

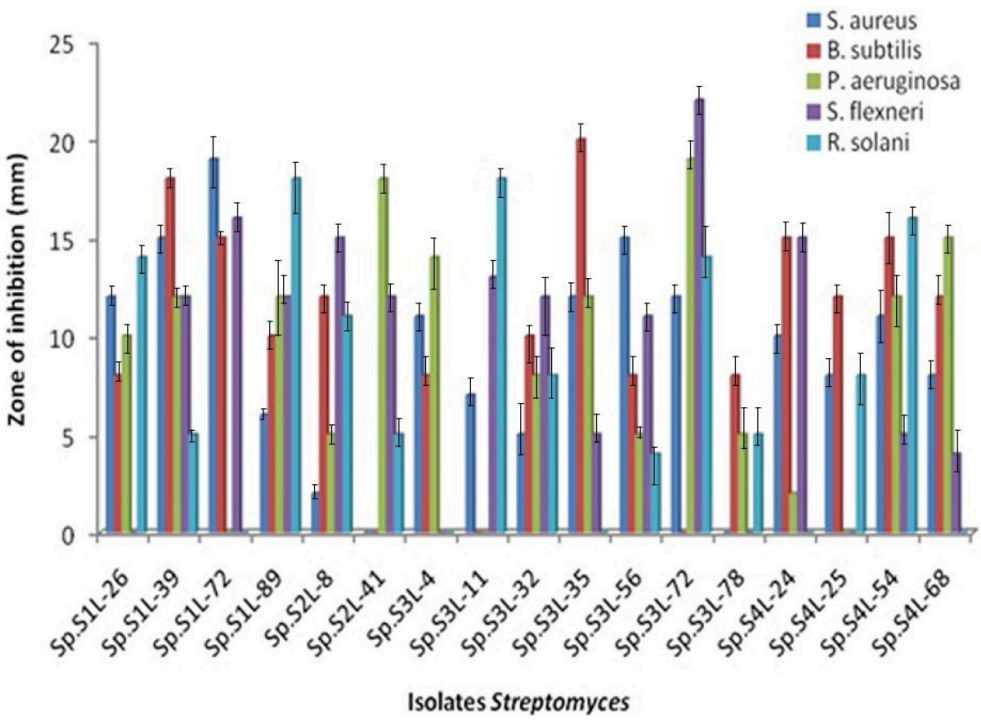

Fig. (4). The anti-microbial properties of *Streptomyces* strains against the selective pathogens. Among the 17 isolates *Streptomyces*, 15 of them have shown promising antimicrobial activity against *Streptomyces aureus*, 14 isolates exhibited activity against *Bacillus subtilis* and 204 *Pseudomonas aeruginosa* and 13 strains showed activity against *Shigella flexneri.* (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

tinase activity [26-29]. The improvements in the production and activity of the biocatalyst from these novel actinomycetes are further subjected to optimize the growth conditions.

The antibacterial and antifungal properties of these marine isolates against four dreadful human pathogenic bacteria and one agri-pathological fungus were evaluated. It was observed that our six best new strains of the genus *Streptomyces* have shown efficient activity parallelly against both bacteria and fungus [30]. We did not find antifungal activities in all of the non-streptomyces actinomycetes and five *Streptomyces* strains. Our observation quite resembled with the evidence which stated that a fraction of streptomyces and a majority of non-streptomyces from soil samples were found to be non-producers of pharmaceutical metabolites [31].

Actinomycetes, especially elusive *Streptomyces* sp. have grasped considerable attention worldwide due to the production of many useful bioactive metabolites [32]. These psychrozymes have a great demand due to short supply and availability [2]. The studies are being carried independently to optimize and enhance the production of cold-active enzymes and antagonistic metabolic exudates. *Streptomyces* are saprophytic and they contribute towards the turnover of complex biopolymers both in terrestrial and marine habitats as well as show a broad range of antimicrobial activity [33-35]. In the present day, *Streptomyces* sp. is the most important source of antibiotics for medical, veterinary, and agricultural use [4, 36-38]. Scientific research, bringing out the reports on deepwater halophiles phylogeny coupled with tolerance to extreme physio-chemical environment and growth to produce bio-enzymes and antimicrobial compounds all in a single attempt, is rare and invaluable.

CONCLUSION

In this study, we reported that deep marine actinomycetes of Bay of Bengal evolved with the greatest genomic and metabolic diversity that should be directed towards exploring sources of novel secondary metabolites. The success relies on the molecular studies on the secondary-metabolite produced by the deep marine actinomycetes, which can be useful for further developing pharmaceutical drugs and important agricultural compounds. All the isolated strains, reported in this study, exhibited multienzymes activity. The high specificity at low temperatures, thermal liability, and antagonistic activities against the fungal pathogens, indicate the versatility of deep marine actinomycetes isolated from the Bay of Bengal. The cold-active enzymes not only accelerate the intrigued by decreasing the economic burden in a process by shortening the method time but moreover, decrease misfortunes in unstable fermentative components [39-41]. However, the physiological, biochemical, and molecular properties of 95% marine actinomycetes are not still clearly understood. Therefore, the distribution of genetic divergence and population expansion of actinomycetes in Bay-of-Bengal will further need in-depth explorations and investigation.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Data curation, Formal analysis, and Investigation was carried out by Mrinmoy Ghosh and Krishna Kanth Pulicherla; Project administration by Krishna Kanth Pulicherla; scientific assistance and correction: Dr. Meeta Gera; Validation, Mrinmoy Ghosh; Writing - original draft, Mrinmoy Ghosh; Review and editing, Mrinmoy Ghosh, Krishna Kanth Pulicherla, Ram Prasad and Joginder Singh Panwar. All authors reviewed the manuscript. The authors are thankful to Prof. DongKee Jeong for his scientific support during the conduction of this experiment.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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