



Original Article

Actinobacteria from sediment samples of Arabian Sea and Bay of Bengal: Biochemical and physiological characterization

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Abstract

Actinobacteria isolated from the sediment samples of Arabian Sea and Bay of Bengal were used for the study. Morphological and biochemical characterization of the isolates revealed *Streptomyces* (76%) as the dominant genus followed by *Nocardiopsis* (24%). Among the carbon sources tested, the hexose sugar glucose and pentose sugar rhamnose were utilized by all the isolates and the least preferred carbon sources were sorbitol (85.22%), lactose (81.3%) and xylose (76.96%). Acid production from carbohydrates varied significantly among the isolates. Hydrolytic enzyme profile revealed that most of the marine actinomycete isolates were capable of gelatinase production (99.13%) followed by DNase (96.09%), lipase (86.96%), phosphatase (84.78%), chitinase (63.48%), pectinase (22.17%) and ligninase (15.22%) producing forms. Melanin production was exhibited by 4% of the *Streptomyces* isolates. The present study has unraveled the metabolic potential of marine *Streptomyces* and *Nocardiopsis*, expanding the scope for the discovery of novel metabolites of marine origin.

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Key words: Actinomycetes; Marine sediments; Hydrolytic enzymes; *Streptomyces*; *Nocardiopsis*.

1. Introduction

Actinobacteria belonging to the order *Actinomycetales* of the domain bacteria have been of major scientific interest in the past few decades with the discovery of large number of commercially important metabolites. The contribution of actinomycetes to the field of biotechnology is immense, ranging from antibiotics to enzyme inhibitors and anti-cancer agents to various alkaloids. They are ubiquitously distributed in terrestrial, freshwater and marine environments and are versatile agents of biodegradation. Marine microorganisms have evolved greatest metabolic and genetic diversity in an attempt to adapt to extremes of environmental conditions and there is growing awareness on bioprospecting of this unique environment. Although the ubiquitous presence of actinomycetes in marine sediments has been well documented [1- 4] they have not been investigated extensively for bioactive compounds. In recent years, actinomycetes isolated from marine environment (sediments, sponges, tunicates, neuston etc.) have received considerable attention [5, 6]. Actinomycete diversity in Arabian Sea and Bay of Bengal and their metabolites still remain less understood. Redundancy in isolation of known compounds is a major obstacle in biodiscovery process. Understanding of the actinomycete diversity in the vast ocean realm is indispensable for maximizing the novel

biomolecules. Microbial systematics, physiology and natural product chemistry are underpinning disciplines in unraveling biodiscovery of marine natural products [7]. In this regard, characterization of actinobacteria from the vast ocean realm is gaining much importance. The present study is aimed at characterization of the actinobacteria isolated from shelf sediments of Arabian Sea and Bay of Bengal.

2. Materials and Methods

2.1. Samples used for the study

Actinobacteria (220 Nos.) isolated from the continental shelf and slope sediments of South West and South East coast of India and maintained in the Microbiology Laboratory of School of Marine Sciences, Cochin University of Science and Technology were used for the present study. These isolates were obtained from the sediment samples collected during the various cruises of Fisheries and Oceanographic Research Vessel (FORV) *SagarSampada* of Centre for Marine Living Resources and Ecology, Ministry of Earth Sciences, Govt. of India i.e., Cruise No. 162 (South West coast of India up to 200m depth in the Exclusive Economic Zone: stations extended off Cape Comorin to Dwaraka (8°03' 96" N to 21°56' 99" N latitude and 77°21' 90" E to 67°57' 69" E longitude), Cruise No. 228 (West coast of India: Cape Comorin to Porbander (7°10' 00" N to 21°29' 00" N latitude and 77°20' 00" E to

67°46' 00" E longitude), Cruise No. 233 (South West Coast of India: Cape Comorin to Coondapore (7°10' 00" N to 13°29' 00" N latitude and 77°20' 00" E to 73°17' 00" E longitude), Cruise No. 245 (East coast of India: Karaikal to Paradip (10°35' 77" N to 19°59' 46" N latitude and 80°27' 27" E to 87°19' 75" E longitude), Cruise No. 254 (West coast of India from Cape Comerin to Porbander (7°01' 00" N to 21°30' 00" N latitude and 77°15' 00" E to 67°28' 00" E longitude), Cruise No. 236 (East coast of India: Karaikal to Paradip (10°34' 00" N to 20°01' 00" N latitude and 80°26' 00" E to 87°30' 00" E longitude), Cruise No. 266 (South East coast of India: Karaikal to Singarayakonda (10°36' 00" N to 15°14' 82" N latitude and 80°07' 06" E to 81°35' 09" E longitude). Besides, sediment samples collected during Cruise No. 255 (South West coast of India: Trivandrum to Kannur (8°29' 28" N to 11°59' 08" N latitude and 76°43' 00" E to 74°25' 76" E longitude) of FORV *Sagar Sampada* were subjected to the isolation of actinomycetes.

2.2. Isolation of actinomycetes

The sediment samples of shelf region collected during Cruise No. 255 were subjected to the isolation of actinomycetes. Samples were pretreated to dry heat at 50-60°C for one hour. The pre-treated samples (15g) were suspended in sterile sea water (35ml), vortexed and kept undisturbed for 30 min. The supernatant was used as inoculum and pour plate method was employed for isolation using actinomycete isolation agar (Himedia) and starch casein agar supplemented with anti-fungal agent bavistin (BASF India Limited, Bombay) (13.75 mg/100 ml) and antibiotic gentamicin (Himedia) (100 µg/100 ml). The plates were incubated at 28°C for 2-4 weeks. Powdery colonies with characteristic appearance of actinomycetes were isolated and purified by streaking on to marine actinomycete growth medium (starch – 10 g, yeast extract – 4 g, peptone – 2 g, sea water (15 ppt) -1 L, agar -20 g, pH – 7). These actinomycete isolates (10 No.) were also used for the study and stocked in nutrient agar vials.

2.3. Purification of isolates

Actinomycetes (230 Nos.) were purified by repeated streaking on nutrient agar plates and stocked in soft nutrient agar vials overlaid with sterile liquid paraffin. The working cultures were maintained in nutrient agar slants and kept refrigerated at 4°C for further studies.

2.4. Morphological and cultural characterization

The isolates were streaked on to yeast extract malt extract agar (ISP 2), glycerol asparagine agar (ISP5), starch casein agar and the colony characteristics were noted; colours of mature sporulating aerial mycelium, substrate mycelium, macro morphology, diffusible pigment, colony reverse colour, colony texture etc. were recorded after observing the plates under stereomicroscope [8].

2.5. Coverslip culture technique

A loop full of spore suspension of actinomycete was dispensed at the intersection of the casein starch peptone yeast extract malt extract agar medium and the cover slip. The plates were incubated at 28°C for 4-8 days. The cover slips were removed at intervals of 2-4 days and were observed under oil immersion objective. Morphology of aerial mycelium, substrate mycelium, length of hyphae, arrangement of sporogenous hyphae and spore chain

morphology were recorded according to International Streptomyces Project [9,10]

2.6. Physiological and biochemical characterization

2.6.1. Decomposition of organic substrates

The physiological and biochemical tests for characterization of aerobic sporogenous actinomycetes were done according to Berd [11] with slight modifications. The decomposition of casein (1%), tyrosine (0.5%) xanthine (0.4%) and hypoxanthine (0.4%) were tested in nutrient agar plates supplemented with the respective compounds. Spot inoculation of isolates was done in the respective media and the plates were incubated at 28°C for 5-7 days. Clearing zone around the colony was recorded as positive.

2.6.2. Biochemical characterization

Melanin production ability of actinomycetes was tested on peptone yeast extract iron agar and tyrosine agar. Blackening of the media was noted as positive reaction. Nitrate reduction and citrate utilisation was tested in 0.1% potassium nitrate broth and Simmon's citrate agar medium respectively. Red colouration on adding the nitrate reagents were noted as positive. Change in the colour of medium from green to prussian blue in the case of citrate utilization test was recorded positive. Hydrogen sulphide production was detected using 0.5% lead acetate strips inserted into the nitrate broth. Blackening of lead acetate strips was recorded as positive. Hydrolysis of urea (2%), esculin (0.1%) and lysozyme resistance (0.05%) of the isolates were determined. A change of colour in the medium from yellow to pink was noted as urea hydrolysis, brownish black colouration in the medium as esculin hydrolysis and growth in the presence of lysozyme as lysozyme resistance.

2.6.3. Carbohydrate utilization test

Ability of the isolates to produce acid from various carbon sources like glucose, lactose, mannitol, sucrose, arabinose, trehalose, inositol, ribose, sorbitol and xylose were tested on carbon utilization agar (ISP 9) supplemented with 1% of the carbon sources [10] and bromocresol purple as the indicator. Basal medium without carbon source was used as the control. An acid reaction indicated by the change in colour of the carbohydrate medium from purple to yellow indicated a positive result.

2.7. Screening of marine actinomycetes for hydrolytic enzyme production

All the isolated marine actinomycetes were qualitatively screened for the production of seven important enzymes such as amylase (starch 1%), lipase (tributyrin 1%), protease (gelatin 1%), ligninase (methylene blue 0.02%), DNase (DNA 0.2%), pectinase (pectin 0.5%), phosphatase (phenolphthalein diphosphate 0.01%) and chitinase (colloidal chitin 5% w/v). Nutrient agar supplemented with the respective substrates was used for the enzyme assay. Each actinomycete strain was spot inoculated on the corresponding media and incubated for 5 days at 28°C. Clearing zone on the plates was regarded as positive except for phosphatase assay. For protease and pectinase assay, plates were flooded with 20% mercuric chloride and cetavlon respectively and the clearing zone was noted. In the case of phosphatase assay, a pink coloration of the medium around the colony on exposure to ammonia

vapour, due to the release of phenolphthalein from phenolphthalein phosphate was noted as positive.

3. Results

3.1. Morphological and cultural characterization

The actinomycete isolates exhibited good growth in ISP media and starch casein agar; majority of the strains grew within 3-5 days and the characteristic spores were noticed by 5-7 days. Approximately 12% of the isolates were slow growing, and distinct colony appearance was noticed only after 3 days. The colony texture of the isolates in the ISP medium varied from powdery, cottony, and velvety to leathery colonies. The appearance of colonies ranged from irregular cottony, concentric, convex, umbonate, and chrysanthemum (radial furrows) type (Fig.1 a-e). The spore mass colour of actinomycetes is considered taxonomic criteria for grouping of actinomycetes. Among the 230 marine actinomycete isolates, 134 produced white/off white spore mass, 48 isolates exhibited ash/ grey colour, 9 isolates pink/red series, 20 produced yellow spore mass colour and 15 isolates green spore mass (Fig. 2). Only six isolates produced diffusible pigment in almost all the culture media viz., starch casein agar, glycerol asparagine agar and nutrient agar. The colony reverse colour/colour of the substrate mycelium in starch casein agar, glycerol asparagine agar, and yeast extract malt extract agar were observed and varied from off white to pink, dark maroon, grey, green, etc.



Fig. 1(a-e) Colony appearance of actinomycete isolates under Stereomicroscope

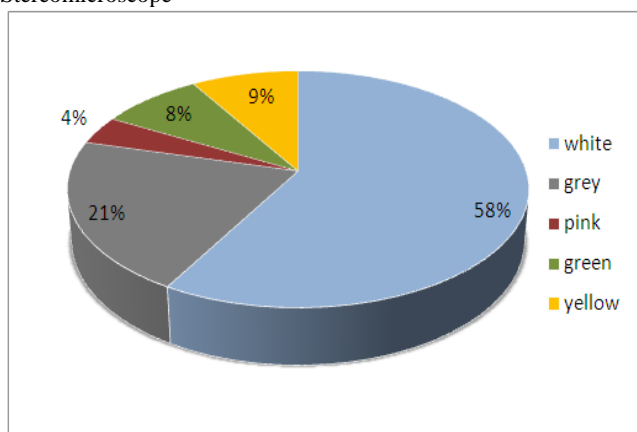


Fig. 2 Percentage of actinomycete isolates with different spore mass colour

3.2. Coverslip culture

The spore chain morphology of actinomycetes grown on coverslip and observed under oil immersion objectives revealed four types of spore chain morphology. The most prominent spore chain morphology was the spiral one; 34% of the strains exhibited spiral spore chain (mostly verticillate type), followed by 28% exhibiting rectiflexibles, and 14% with retinaculiaperti spore chain morphology (Fig. 3a, 3b). Remaining 24% of the isolates exhibited long chain of spores with zig zag fragmenting aerial hyphae, characteristic of non streptomycete genera.

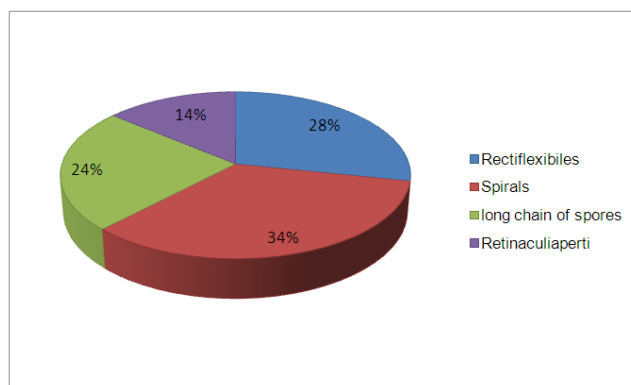


Fig. 3a Relative spore chain morphology of actinomycete isolates

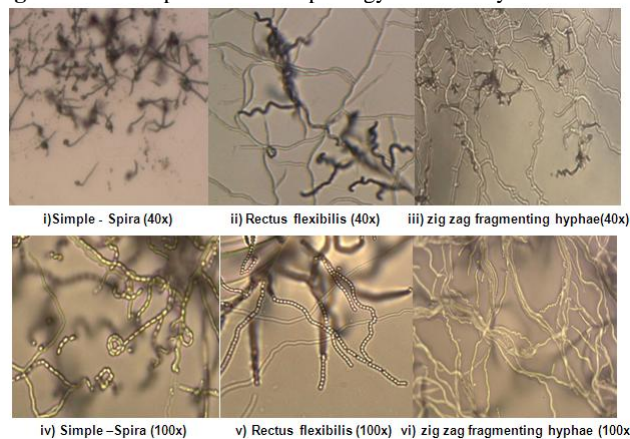


Fig. 3b (i-vi) Microscopic appearance of spore chain morphology of actinomycete isolates

3.3. Generic composition

Based on the morphological and biochemical criteria, the marine actinomycete isolates from Arabian Sea and Bay of Bengal were classified mainly into two genera, i.e., 175 isolates with characteristic spore chain morphology as belonging to genus *Streptomyces* and nearly 55 isolates with long chain of spores with zig zag fragmenting aerial hyphae as *Nocardiopsis* sp. (Fig. 4)

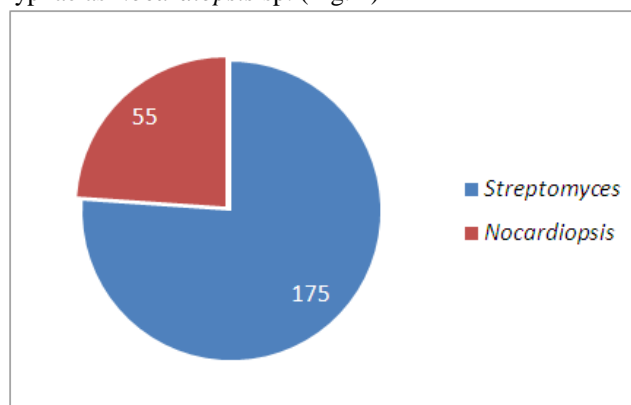


Fig. 4 Occurrence of actinomycetes in marine sediments of Arabian Sea and Bay of Bengal

3.4. Decomposition property

Decomposition of various substrates such as xanthine, hypoxanthine and tyrosine carried out in respective agar media revealed that, of the isolated 230 strains of marine actinomycetes, 86.47% had the ability to decompose tyrosine, 77.39% hypoxanthine and 67.42% xanthine. Decomposition of casein by actinomycete isolates were

tested on skim milk agar. Of the total, 73.91% were able to decompose casein indicated by a clearing zone around the colonies. Esculin decomposition was carried out by 90.43% of the actinomycete isolates. Among the 175 *Streptomyces* isolates identified, 154 strains (88%) hydrolysed esculin, 117 (67%) tyrosine, 125 (71%) hypoxanthine, and 90 (51%) xanthine. Out of the 55 *Nocardiosis* isolates, esculin was decomposed by 54 (98%), followed by casein 39 (71%), tyrosine 30 (55%), hypoxanthine 53 (96%) and 30 (55%) isolates decomposed xanthine (Fig. 5, 6).

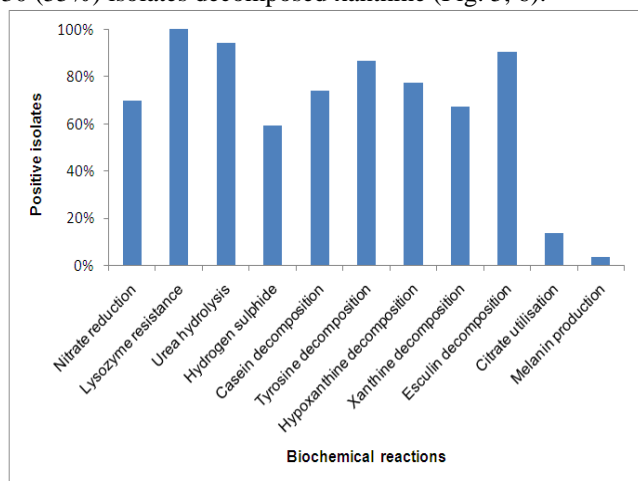


Fig. 5 Decomposition and biochemical profile of actinomycete isolates

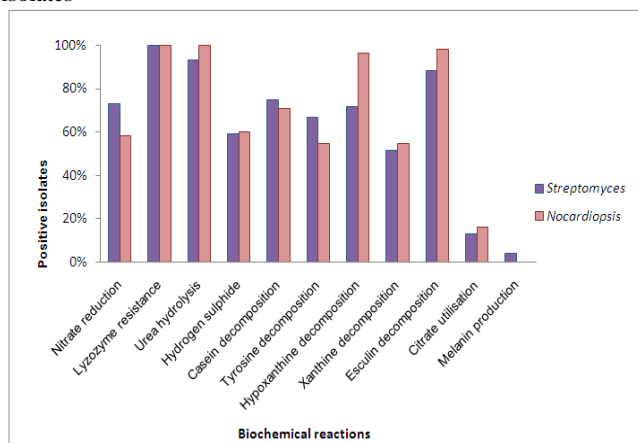


Fig.6 Decomposition and biochemical profile of *Streptomyces* and *Nocardiosis* isolates

3.5. Biochemical characterization

Melanin production ability was exhibited by only 4% of the isolates on peptone yeast extract iron agar and tyrosine agar. Only *Streptomyces* isolates (4%) were able to produce melanin. Almost all the isolates were able to grow in the presence of lysozyme, i.e. the strains were lysozyme resistant. 94.35% were able to hydrolyse urea. Nitrate reduction was exhibited by 69.57% of the actinomycete isolates, 59.13% was able to produce hydrogen sulphide indicated by blackening of lead acetate strips and only 13.48% were able to utilize citrate as the sole carbon source (Fig.5,6).

3.6. Carbohydrate utilization test

The entire carbohydrate source ranging from monosaccharides, dissaccharides and sugar alcohols were utilized by actinomycete isolates. The carbon source most

utilized by all isolates was the hexose sugar glucose and the pentose sugar rhamnose. Rhamnose was utilized by 98.7% isolates followed by glucose (97.4%), trehalose (94.35%), inositol and galactose (92.61%), arabinose (92.17%), mannitol (90.43%), sorbitol (85.22%), lactose (81.3%) and xylose (76.96%). Heavy growth and sporulation was observed for almost all strains in media supplemented with pentose sugar rhamnose. Acid production from carbohydrates varied among the isolates. Acid production was found to be significant among the isolates with carbon sources, glucose (81.3%), followed by mannitol (74.35%), galactose (63.04%), rhamnose (58.70%), xylose (58.26%), lactose (54.78%), trehalose (53.04%), arabinose (51.3%), sorbitol (20.0%) and inositol (19.13%) (Fig.7a). *Streptomyces* isolates were found to be better acid producers compared to *Nocardiosis* (Fig.7b).

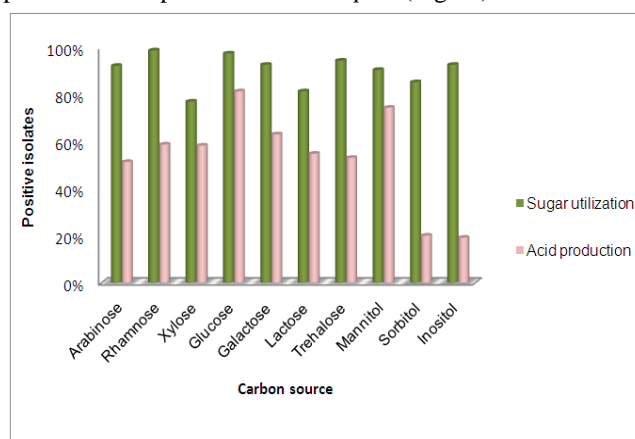


Fig.7a Carbohydrate utilization profile of actinomycete isolates

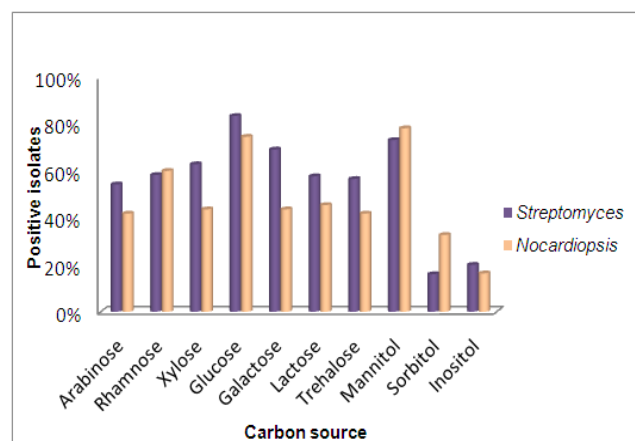


Fig.7b Acid production ability of *Streptomyces* and *Nocardiosis* isolates

3.7. Hydrolytic enzyme profile

Actinomycetes isolated from the shelf sediments invariably had the potential to elaborate a wide array of enzymes, ranging from gelatinase (99.13%) to ligninase (15.22%). DNase activity was exhibited by 96.09%, followed by lipase (86.96%), phosphatase (84.78%), amylase (76.96%) chitinase (63.48%), and pectinase (22.17%) (Fig.8a). Relative enzyme profile of *Streptomyces* and *Nocardiosis* revealed that both the genera from marine environment are potent producers of hydrolytic enzymes with *Streptomyces* displaying better ability to degrade recalcitrant compounds such as chitin, pectin and lignin (Fig. 8b).

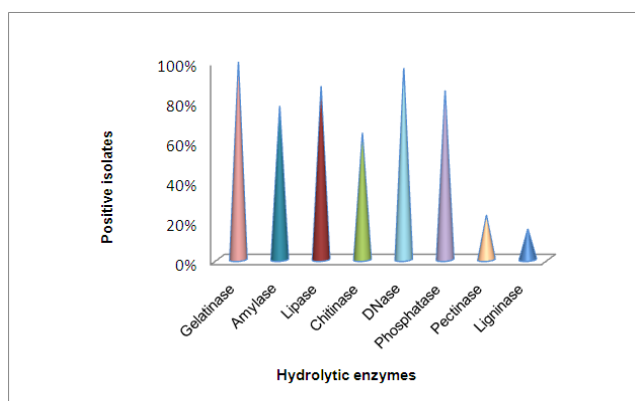


Fig. 8a Hydrolytic enzyme profile of marine actinomycetes

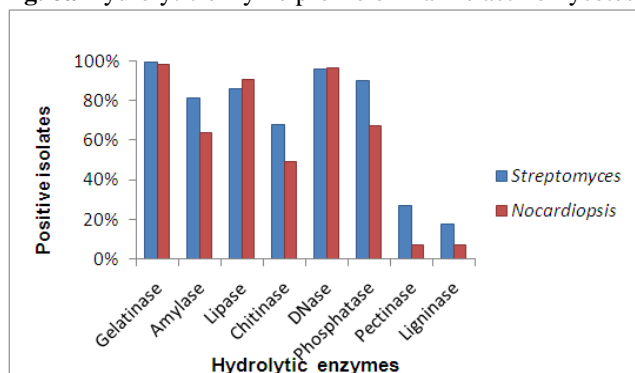


Fig. 8b Relative hydrolytic enzyme profile of different genera of marine actinomycetes

4. Discussion

Bioprospecting focusing on the isolation and screening of actinobacteria from ocean habitats [12,13] have added new records to the order *actinomycetales* and revealed a range of novel natural products of pharmacological value [14]. As marine environment is extremely different from terrestrial habitat, it is well documented that actinomycetes adapted to marine environment exhibited a unique metabolic diversity and enzymatic potential [15]. Most of the studies conducted in Indian Peninsula, along south east coast have been restricted to isolation, identification and maintenance of actinobacteria and their antagonistic properties [16-19].

The actinomycete isolates were morphologically distinct on the basis of spore mass colour, aerial, substrate mycelium, pigmentation etc. Based on the results of various morphological and biochemical criteria, as outlined in the Bergey's Manual of Determinative Bacteriology, 9th edition [20] and the International Streptomyces Project [9], the marine isolates were characterized and identified. Among the 230 actinomycete isolates from the Arabian Sea and Bay of Bengal, 175 (76%) were *Streptomyces* spp. and the remaining 55 isolates (24%) belonged to the rare actinomycete genus, *Nocardiopsis*.

Reports on actinobacteria from sediments have shown that *Streptomyces* is the most dominant group [21-23,4]. Contrary to these results, *Micromonospora* were reported as the dominant genus in marine sediments by various workers [24,13,25]. *Streptomyces*, *Micromonospora* and *Nocardia* are the three most common genera in the marine environment [26,27]. Predominance of *Streptomyces* (60% to 99%) in all marine environmental samples around Nagasaki Prefecture, Japan, and *Nocardiopsis* as the

second most predominant organism in the environmental samples of Iki coast was reported [28]. This report is in agreement with our findings of *Nocardiopsis* as the prevalent culturable rare actinomycete genera next to *Streptomyces* in marine sediments of Indian coast. A total of sixty eight actinomycetes were isolated from near sea shore marine environment locations of Bigeum Island, South West coast of South Korea. The majority of these isolates were assigned to the genus *Streptomyces* (66%) and the remaining were identified as *Nocardiopsis* (18%), *Micromonospora* (11%) and *Actinopolyspora* (5%) on the basis of their morphological, physiological and biochemical properties [29]. Like wise, the isolation and characterization of actinomycetes from West coast of India revealed that majority (47%) of the isolates belonged to the genera *Streptomyces* and the rarer genera *Glycomyces*, *Nocardiopsis* (11%), *Nocardia* etc [30].

However, it has been realized that there are several actinomycetes present in the ecosystem which either occur in fewer numbers or grow comparatively slowly or do not produce spores in abundance like the genus *Streptomyces* [31]. This could be a reason for the difficulty in isolation of some of the rare actinomycete genera compared to *Streptomyces* and *Nocardiopsis*. The observation that *Streptomyces* decreased with depth in marine sediments suggested that most of the *Streptomyces* species in such sediments originate from terrestrial sources, and have been washed off shore [32,24,33]. The results of the present study are in agreement with earlier findings which states that *Streptomyces* species are mainly found in shelf and shallow areas when compared to other genera of actinomycetes [34,35].

Regarding the morphological characterization, it has been already reported that the majority of the marine Streptomycete isolates produced aerial mycelia with coiled spiral spore chains [36-38,23,4] followed by rectiflexibilis spores. The reports of the present study also agree with majority of marine actinomycete isolates having spiral spore chain. Chromogenicity of aerial mycelium is considered an important character for grouping of actinomycetes [39]. Actinomycete strains from South Pacific coast of Philippines revealed that most (54%) of the isolates belonged to white and grey color series [29]. Interestingly, grey and white mycelial pigmented marine actinomycetes were prominent in the Bay of Bengal as revealed by previous reports [40]. The predominance of the occurrence of grey, white spore mass colour of marine actinomycetes was observed in the present study also.

Actinomycetes have a reputation for marked nutritional versatility which is supported by the results of our analysis. In the present study, more than 75% of the isolates were able to utilize and grow in a variety of supplemented carbon sources. Rhamnose was utilized by almost 99% isolates and the carbon source was found to induce heavy growth and sporulation compared to other sugars. The least acid production was observed in sorbitol and inositol. *Streptomyces* isolated from the sea water samples of Visakhapatnam coast of Bay of Bengal could utilize dextrose, fructose, lactose, maltose, mannitol, rhamnose and sucrose as the carbon source along with acid

production; however, xylose, adonitol, sorbitol, inositol and raffinose were not utilized by the organism [41]. Carbohydrate utilization pattern suggests potential ability of the strains to assimilate different carbon sources. The difference in carbon utilization may be as a result of availability of the carbon source and adaptation of isolates to different niches in the marine environment. Carbohydrate utilization for species differentiation of actinomycetes reported was not found to be reliable in the present study due to inconsistent results.

Our study highlights the metabolic potential of actinomycetes from marine habitats in terms of degradation capacity of organic compounds. The degradation of the substrates casein, tyrosine and xanthine was variable according to each *Streptomyces* isolate from Venezuelan soils [42]. Marine actinomycete isolates in the present study also showed variable reactions in the degradation of organic substrates. Compared to the degradation of casein (50%), tyrosine (79%) and xanthine (72%) of the Venezuelan soil isolates, the ability to degrade casein and tyrosine were higher for marine isolates whereas xanthine decomposition was slightly higher for Venezuelan soil isolates.

The physiological characteristics of actinomycetes varied depending on the available nutrients in the medium and the physiological conditions [43]. The ability to utilize a wide range of substrates suggest better survival in different environments and these views are better supported in the current study. A possible explanation for the positive reaction of different biochemical tests and extracellular enzymes is that the marine derived actinomycetes are metabolically active [2] and they are adapted physiologically to grow in seawater and sediments [24].

The ecological features of the habitat in which marine organisms thrive impact on their metabolic functions enabling their biomolecular machinery [44]. Marine actinobacteria elaborate a wide range of enzymes and secondary metabolites as an aid to their survival. Many researchers have reported the production and characterization of enzymes from *Streptomyces* and *Nocardiopsis*, mostly terrestrial, although few marine reports are available [45- 49]. In aquatic environments, microbial extracellular hydrolytic enzymes are the major biological mechanism for the decomposition of sedimentary particulate organic carbon and nitrogen [50,51]. There are reports on the multi enzyme activity of actinomycetes from marine sediments [52, 40]

Very few published reports are available regarding the enzyme profile of marine actinomycetes. Invariably, in the present screening, almost all the marine actinomycetes were gelatinase producers, while, previous reports from Bay of Bengal, reported only 116 out of 208 isolates as gelatinase producers [40]. Since proteins are one of the main components of sedimentary marine particulate organic matter (POM), proteases are the most abundant extracellular enzymes detected in marine bacteria isolated from the Antarctic coastal marine environment [53-55].

DNase was produced by both *Streptomyces* and *Nocardiopsis*, but the phosphatases were exhibited better by *Streptomyces*. Although lipase were produced by

Streptomyces and *Nocardiopsis*, it was slightly better by *Nocardiopsis*. Even though amylase, pectinase and chitinase were produced by both the groups, *Streptomyces* spp. was found to be more efficient producers. More than 95% of the isolates showed at least one of the extracellular enzymatic activities, and among the 230 isolates tested, 26 strains (11.3%) produced up to 7 extracellular enzymes; majority belonged to *Streptomyces* spp. In marine bacterial strains, it was observed that when the presence of one extracellular hydrolytic activity was detected, the production of other hydrolytic enzymes was frequently associated [56]. With growing awareness on environmental protection, microbial enzymes have been replacing chemical catalysts in various pharmaceutical, food, textile and agricultural industries. Although reisolation of known compounds is exploding, it has been predicted that only less than 10% of the streptomycete bioactive metabolites have been discovered [57,58].

5. Conclusion

There is better scope for screening and isolation of bioactive compounds of *Streptomyces* from the underexploited marine habitats. Currently, *Nocardiopsis* is gaining importance as producers of antibiotics and bioactive compounds. From this study, it is inferred that the rare genera *Nocardiopsis* can also be isolated along with *Streptomyces*, from marine environment without much selective procedures. It is clear from the above results that marine actinomycetes are biochemically and metabolically active and play significant role in the decomposition of organic matter in the marine habitat. Pentose sugar rhamnose induced sporulation in actinomycetes. The characterization studies proved the dominance of *Streptomyces* in the marine environment and their potential to contribute to the pool of novel metabolites and enzymes.

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References

1. M. Takizawa, R. R. Colwell, R. T. Hill, Isolation and diversity of actinomycetes in the Chesapeake Bay, Appl. Environ. Microbiol. 59 (1993) 997–1002.
2. M.A. Moran, L.T. Rutherford, R.E. Hodson, Evidence for indigenous *Streptomyces* populations in a marine environment determined with a 16SrRNA probe, Appl. Environ. Microbiol. 61 (1995) 3695-3700.
3. K.V. Bhaskar Rao, L. Karthik , Gaurav kumar, Diversity of marine actinomycetes from Nicobar marine sediments, Int. J. Pharm. Pharmaceutical Sci. 2 (2010) 199–203.
4. S.Chacko Vijai Sharma, Ernest David, A comparative study on selected marine actinomycetes from Pulicat, Muttukadu, and Ennore estuaries, Asian. Pac. J. Trop. Biomed. 2 (2012) S1827–S1834.
5. X. Liu, E. Ashforth, B. Ren, F. Song, H. Dai, M. Liu,

- J.Wang, Q.Xie, L.Zhang, Bioprospecting microbial natural product libraries from the marine environment for drug discovery, *J. Antibiot.* 63 (2010) 415–422.
6. A.L. Lane, B.S. Moore, A sea of biosynthesis: marine natural products meet the molecular age, *Nat. Prod. Rep.* 28 (2011) 411–428.
7. A. T. Bull, J. E. M. Stach, Marine actinobacteria: new opportunities for natural product search and discovery, *Trends. Microbiol.* 15 (2007) 491–499.
8. H.D.Tresner, E.J. Backus, System of color wheels for streptomycete taxonomy, *Appl. Microbiol.* 11 (1963) 335–338.
9. E.B. Shirling, D.Gottlieb, Methods for characterization of Streptomyces species, *Int.J. Syst. Bacteriol.* 16 (1966) 313–340.
10. H. Nonomura, Key for classification and identification of 458 species of Streptomyces included in ISP project, *J. Ferm. Technol.* 52 (1974) 78– 92.
11. D. Berd, Laboratory Identification of Clinically Important Aerobic Actinomycetes, *Appl. Microbiol.* 25 (1973) 665–681.
12. N.A. Magarvey, J. M. Keller, V. Bernan, M. Dworkin and D. H. Sherman, Isolation and Characterization of Novel Marine-Derived Actinomycete Taxa Rich in Bioactive Metabolites, *Appl. Environ. Microbiol.* 70 (2004) 7520–7529.
13. T. J. Mincer, P. R. Jensen, C. A. Kauffman, and W. Fenical, Widespread and Persistent Populations of a Major New Marine Actinomycete Taxon in Ocean Sediments, *Appl. Environ. Microbiol.* 68 (2002) 5005–5011.
14. K. Engelhardt, K. F. Degnes, M. Kemmler, H. Bredholt, E. Fjaervik, G. Klinkenberg, H. Sletta, T. E. Ellingsen, S. B. Zotchev, Production of a new thiopeptide antibiotic, TP-1161, by a marine Nocardiosis species, *Appl. Environ. Microbiol.* 76 (2010) 4969–4976.
15. N. Nakashima, Y. Mitani, T. Tamura, Actinomycetes as host cells for production of recombinant proteins, *Microbial Cell Factories*, 4 (2005) 7.
16. K. Kathiresan, R. Balagurunathan, M. Masilamani Selvam, Fungicidal activity of marine actinomycetes against phytopathogenic fungi, *Indian. J. Biotechnol.* 4 (2005) 271–276.
17. D. Dhanasekaran, S. Selvamani, A. Panneerselvam, and N. Thajuddin, Isolation and characterization of actinomycetes in Vellar Estuary, Annagkoil, Tamil Nadu, *Afr. J. Biotechnol.* 8 (2009) 4159–4162.
18. R.Vijayakumar, C. Muthukumar, N.Thajuddin, A.Panneerselvam, Studies on the diversity of actinomycetes in the Palk Strait region of Bay of Bengal, India, *Actinomycetologica.* 21(2007) 59–65.
19. R. M. Gulve and A. M. Deshmukh, Antimicrobial activity of the marine actinomycetes, *Int. Multidiscipl. Res. J.* 2 (2012) 16–22.
20. S.T.Williams, M.E. Sharpe, J.G. Holt, *Bergey's Manual of Systematic Bacteriology*. Vol. 4 Williams and Wilkins, London, 1994.
21. P.A. Ellaiah, P.C.Reddy, Isolation of actinomycetes from marine sediments off Visakhapatnam, east coast of India, *Indian. J. Mar. Sci.* 16 (1987) 134–135.
22. J. Ravel, H. Schrempf, R.T. Hill, Mercury resistance is encoded by transferable giant linear plasmids in two Chesapeake Bay Streptomyces strains, *Appl. Environ. Microbiol.* 64 (1998) 3383–3388.
23. Surajit Das, P.S. Lyla, A.S. Khan, Distribution and generic composition of culturable marine actinomycetes from the sediments of Indian continental slope of Bay of Bengal, *Chin. J. Oceanol. Limn.* 26 (2008) 166–177.
24. P.R. Jensen, R. Dwight and W. Fenical, Distribution of actinomycetes in near-shore tropical marine sediments, *Appl. Environ. Microbiol.* 57(1991) 1102–1108.
25. L.A. Maldonado, J.E.M. Stach, W.Pathomaree, A.C.Ward, A.T.Bull, M.Goodfellow, Diversity of cultivable actinobacteria in geographically widespread marine sediments, *Anton. Van Leeuwenhoek.* 87 (2005) 11–18.
26. S.L. Sharma and A. Pant, Crude oil degradation by a marine actinomycete *Rhodococcus* sp., *Indian J. Mar. Sci.* 30 (2001) 146–150.
27. H.P. Fiedler, C. Bruntner, A. T. Bull, A. C. Ward, M. Goodfellow, O. Poterat, C. Puder, G. Mihm, Marine actinomycetes as a source of novel secondary metabolites, *Anton. Van. Leeuwenhoek.* 87 (2005) 37–42.
28. K. Anzai, T. Nakashima, N. Kuwahara, R. Suzuki, Y. Ohfuku, S. Takeshita and K. Ando, Actinomycete bacteria isolated from the sediments at coastal and offshore area of Nagasaki Prefecture, Japan: Diversity and biological activity, *J. Biosci. Bioeng.* 106 (2008) 215–217.
29. S. Parthasarathi, S. Sathya, G. Bupesh, R. Durai Samy, M. Ram Mohan, G. Selva Kumar, M. Manikandan, C.J. Kim, K. Balakrishnan, Isolation and Characterization of Antimicrobial Compound from Marine Streptomyces hygroscopicus BDUS 49, *World J. Fish. Mar. Sci.* 4 (2012) 268–277.
30. M. Remya, R.Vijayakumar, Isolation and characterization of marine antagonistic actinomycetes from West coast of India, *Facta Universitatis Med. Biol.* 15(2008) 13–19.
31. M.C. Srinivasan, R.S. Laxman, M.V. Deshpande, Physiology and nutritional aspects of actinomycetes : an overview, *World J. Microbiol. Biotechnol.* 7 (1991) 171–184.
32. H.Weyland, Distribution of actinomycetes on the sea floor, *Zentralbl. Bacteriol. supplement.* 11(1981)185–193.
33. H. Bredholdt, O. A. Galatenko, K. Engelhardt, E. Fjaervik, L. P. Terekhova, S. B. Zotchev, Rare actinomycete bacteria from the shallow water sediments of the Trondheim fjord, Norway: isolation, diversity and biological activity, *Environ. Microbiol.* 9 (2007) 2756–2764.
34. G. M. Thorne, J. Alder, Daptomycin: A novel lipopeptide antibiotic, *Clin. Microbiol. Newslett.* 24 (2002) 33–40.
35. B. Nithya, P. Ponnuragan, and M. Fredimoses, 16S

- rRNA phylogenetic analysis of actinomycetes isolated from Eastern Ghats and marine mangrove associated with antibacterial and anticancerous activities, *Afr. J. Biotechnol.* 11(2012) 12379–12388.
36. G. Mukherjee and S.K. Sen, Characterization and identification of chitinase producing *Streptomyces venezulae* P10, *Indian J. Exp. Biol.* 42 (2004) 541–544.
 37. L. M. Roes and P.R. Meyer, *Streptomyces pharetrae* sp. nov., isolated from soil from the semi-arid Karoo region, *Syst. Appl. Microbiol.* 28 (2005) 488–493.
 38. S. Peela, V. B. Kurada, R. Terli, Studies on antagonistic marine actinomycetes from the Bay of Bengal, *World J. Microbiol. Biotechnol.* 21(2005) 583–585.
 39. T.G. Pridham, H.D. Tresner, Genus I. *Streptomyces*, Waksman and Henrici 1943, 339. In: Buchanan RE, Gibbons NE (eds) *Bergey's Manual of Determinative Bacteriology*, 8th edn. Williams and Wilkins Company, Baltimore, 748–829, 1974.
 40. S. Ramesh, N. Mathivanan, Screening of marine actinomycetes isolated from the Bay of Bengal, India for antimicrobial activity and industrial enzymes, *World J. Microbiol. Biotechnol.* 25 (2009) 2103–2111.
 41. N.G. Reddy, D.P.N. Ramakrishna, and S.V. Raja Gopal, A morphological, physiological and biochemical studies of marine *Streptomyces rochei* (MTCC 10109) showing antagonistic activity against selective human pathogenic microorganisms, *Asian J. Biol. Sci.* 4 (2011) 1–14.
 42. A. Taddei, M. J. Rodríguez, E. Marquez-Vilchez, and C. Castelli, Isolation and identification of *Streptomyces* spp. from Venezuelan soils: morphological and biochemical studies, *Microbiol. Res.* 161 (2006) 222–231.
 43. R. Baskaran, R. Vijayakumar, P. M. Mohan, Enrichment method for the isolation of bioactive actinomycetes from mangrove sediments of Andaman Islands India, *Mal. J. Microbiol.* 7 (2011) 26–32.
 44. A. Trincone, Marine biocatalysts: enzymatic features and applications, *Mar. Drugs*. 9 (2011) 478–499.
 45. T.L.M. Stamford, N.P. Stamford, L.C.B.B. Coelho, J.M. Araujo, Production and characterization of a -thermostable glucoamylase from *Streptosporangium endophyte* of maize leaves, *Bioresour. Technol.* 83 (2002) 105–109.
 46. I. Goshev, A. Gousterova and E. Vasileva-Tonkova, Characterization of the enzyme complexed produced by two newly isolated thermophilic actinomycete strains during growth on collagen rich materials, *Process Biochem.* 40 (2005) 1627–1631.
 47. A. Kavitha, and M. Vijayalakshmi, Partial purification and antifungal profile of chitinase produced by *Streptomyces tendae* TK-VL 333, *Ann. Microbiol.* 61 (2011) 597–603.
 48. H.T. Sujibo, T. Kubota, M. Yamamoto, K. Miyamoto, Y. Inamori, Characterization of chitinase genes from an alkaliphilic actinomycete *Nocardia opacensis* OPC-131, *Appl. Environ. Microbiol.* 69 (2003) 894–900.
 49. S. Chakraborty, G. Raut, A. Khopade, K. Mahadik and C. Kokare, Study on calcium ion independent amylase from haloalkaliphilic marine *Streptomyces* strain A3, *Indian J. Biotechnol.* 11 (2012) 427–37.
 50. H. Dang, H. Zhu, J. Wang, L. Tiegang, Extracellular hydrolytic enzyme screening of culturable heterotrophic bacteria from deep-sea sediments of the Southern Okinawa Trough, *World J. Microbiol. Biotechnol.* 25 (2009) 71–79.
 51. J. Brunnegard, S. Grandel, H. Stahl, A. Tengberg and J. Hall, Nitrogen cycling in deep-sea sediments of the Porcupine Abyssal Plain NE Atlantic, *Prog. Oceanogr.* 63 (2004) 159–181.
 52. J. Leon, L. Liza, I. Soto, D. Cuadra, L. Patino, R. Zerpa, Bioactive actinomycetes of marine sediment from the central coast of Peru, *The Peru J. Biol.* 14 (2007) 259–270.
 53. M. Tropeano, S. Vazquez, S. Coria, A. Turjanski, Extracellular hydrolytic enzyme production by proteolytic bacteria from the Antarctic, *Polish Pol. Res.* 34 (2013) 1–15.
 54. T. N. Srinivas, N.S.S. Rao, V. Vardhan, P. Reddy, M.S. Pratibha, B. Sailaja, B. Kavya, K.H. Kishore, Z. Begum, S.M. Singh and S. Shivaji, Bacterial diversity and bioprospecting for cold active lipases, amylases and proteases, from culturable bacteria of Kongsfjorden and Ny Alesund, Svalbard, Arctic, *Current Microbiol.* 59 (2009) 537–547.
 55. Zhou, X.L. Chen, H.L. Zhao, H.Y. Dang, X.W. Luan, X.Y. Zhang, H.L. He, B.C. Zhou and Y.Z. Zhang, Diversity of both the culturable protease producing bacteria and their extracellular proteases in the sediments of the South China Sea, *Microbial Ecol.* 58 (2009) 582–590.
 56. M. Tropeano, S. Coria, A. Turjanski, D. Cicero and A. Bercovich, Culturable heterotrophic bacteria from Potter Cove, Antarctica, and their hydrolytic enzymes production, *Pol. Res.* 31 (2012) 18507.
 57. M. G. Watve, R. Tickoo, M. M. Jog, B.D. Bhole, How many antibiotics are produced by the genus *Streptomyces*? *Arch. Microbiol.* 176 (2001) 386–390.
 58. C. T. Clardy, J. Fischbach, M. A. and Walsh, New antibiotics from bacterial natural products, *Nat. Biotechnol.* 24 (2006) 1541–1550.

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