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ORIGINAL ARTICLE

Isolation and characterization of bioactive metabolites producing marine *Streptomyces parvulus* strain sankarensis-A10

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Abstract The significance and frequency of marine microorganisms as producers of bioactive metabolites-a natural source of drug discovery had varied significantly during the last decades, making marine ecosystem a huge treasure trove of novel isolates and novel compounds. Among the twelve actinomycetes isolated from marine sediment sample (Lat. 17°41′962″N, Long. 83°19′633″E), amylase, protease, lipase and cellulase activities were exhibited by 8,7,4,3 isolates respectively. Five isolates exhibited L-asparaginase activity, while 5, 6, 2 isolates exhibited antibacterial, antifungal and antimicrobial activities respectively. One isolate VMS-A10 efficiently producing alpha-amylase (25.53 ± 0.50 U/mL), protease (19.26 ± 0.25 U/mL), lipase (36.25 ± 0.10 U/mL), cellulase (14.43 ± 0.513 U/mL), L-asparaginase (0.125 ± 0.004 U/mL), antimicrobial metabolites against *B. subtilis* (503.33 ± 5.77 U/mL), *S. aureus* (536.66 ± 5.77 U/mL), *E. coli* (533.33 ± 5.77 U/mL), *P. aeruginosa* (500.00 ± 10.0 U/mL), *MRSA* (538.33 ± 5.77 U/mL), *C. albicans* (353.33 ± 11.54 U/mL) and *A. niger* (443.33 ± 15.27 U/mL) was selected, identified on the basis of morphological, cultural, physiological, and biochemical properties together with 16S rDNA sequence, designated as ***Streptomyces parvulus* strain sankarensis-A10** and sequencing product (1490 bp) was deposited in the GenBank database under accession number **KT906299,** Culture Deposit No: **NCIM-5601**. Isolation and characterization of each potential actinobacteria having immense industrial and therapeutic value on an unprecedented scale from marine sediments of Visakhapatnam coast will have a burgeoning effect.

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1. Introduction

Natural products have played a pivotal role in drug discovery. The marine biosphere is a substantial sampling point, especially a huge treasure trove of actinomycetes resource for drug discovery. Within marine actinomycetes, *Streptomycetes* play a

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pivotal role in the soil environment because of their broad range of metabolic processes which include biotransformations, degradation of the insoluble remains of other organisms, such as lignocellulose and chitin one of the world's most abundant biopolymers [1]; relevantly, there is a growing interest in the bioconversion of cellulose into fermentable sugars, which would allow the production of biofuels and chemicals through industrial fermentation processes [2], degrading complex organic materials into compost, soil or sediments by producing several biocatalysts (enzymes) such as proteases, cellulases, amylase, gelatinase, pectinases, ureases, amidases, esterases and lipases making *Streptomycetes* central organisms in carbon recycling thus offering green and clean solutions to chemical processes that are emerging as a challenging and revered alternative to chemical technology. *Streptomycetes* produce over two-thirds of the clinically useful antibiotics of natural origin such as daptomycin and Lincomycin [3] by complex secondary metabolism. Nevertheless, it is well established that each actinobacteria strain apparently has the genetic potential ability to produce 10–20 secondary metabolites [4]. Consequently, specifically targeted isolation and screening of actinomycetes producing potential antibiotics or therapeutic enzymes or industrial enzymes have been a major part of the research i.e., isolation of new streptophenazines from marine *Streptomyces* sp. 182SMLY active against methicillin-resistant *S. aureus* [5], L-asparaginase from *Streptomycetes parvulus* KUAP106 [6], α-glucosidase inhibitors from *Streptomyces* sp. OUCMDZ-3434 [7], and Cellulase and xylanase production by *Streptomyces albidoflavus* strain SAMRC-UFH5 [8]. The large genetic potential for primary and secondary metabolism present in the genome of most actinomycetes appears to be in contrast with the limited number of metabolites that are actually detected in a single isolate [9]. Exploitation of either unexplored or less explored ecosystems for such actinomycetes is highly necessary. Despite Indian marine ecosystem is considered as an important source for the microbial expedition, the studies on the diversity of actinomycetes and bioactive metabolite's production from Indian peninsula are scanty and need to be explored [10].

Moreover, reports on the potential of each/every single actinomycetes strain for primary as well as secondary metabolites production in a single study are scanty. Present study was intended to isolate, screen the marine actinobacteria from the sediment sample for their potential to produce hydrolytic enzymes (amylase, protease, Lipase, cellulase), L-asparaginase, antimicrobial metabolites and characterize them up to the genetic level.

2. Materials and methods

2.1. Sampling site

Sediment sample from Visakhapatnam coast at deeper region (10 m depth) of Bay of Bengal (Lat. 17°41′962″N, Long. 83°19′633″E) was collected in the month of January 2014 at the time of low tide in sterile polythene bags containing filtered and sterilized seawater (50% v/v (seawater: distilled water: 50:50)) in order to maintain moisture condition, refrigerated at 4 °C until further use for isolation of marine actinobacteria.

2.2. Measurement of physicochemical parameters of the sediment sample

The pH of the sediment sample, temperature and salinity were documented with PCSTestr 35 (Eutech PCSTEST35-01X441506/Oakton 35425-10) [11]. Dissolved Oxygen (DO) and Biological oxygen demand (BOD) of the sampling site were documented as described by Wangersky [12].

2.3. Isolation and maintenance of actinobacteria

One gram of sediment sample was aseptically transferred to 50 mL of sterile seawater (50% v/v) contained in 250 mL flask and kept on the orbital shaker (150 rpm) for 30 min at 28 ± 2 °C, keeping aside for 15–30 min to settle down the particulate matter. The suspension was serially diluted with sterilized seawater (50% v/v) up to 10^{-7} level. 0.1 mL of each of these dilutions was pour plated [13] in triplicates on starch casein agar medium (SCA) [composition (g/L): soluble starch 10, potassium nitrate 2, vitamin free casein 0.3, sodium chloride 2, di-potassium hydrogen phosphate 2, magnesium sulfate 0.05, calcium carbonate 0.02, ferrous sulfate 0.01, seawater (50% v/v) 1,000 mL, pH 7.0 ± 0.2] and Zobell marine agar medium (ZMA, Himedia) with the addition of 50 μg/mL of cycloheximide, 5 μg/mL of rifampicin to prevent the fungal and fastidious bacteria growth respectively, incubated for 10–21 days at 28 ± 2 °C. Individual actinobacterial colonies with chalky to leathery appearance were subcultured on SCA, ZMA, (ISP-2) slants (50% v/v seawater), incubated for 5–7 days at 28 ± 2°C for good sporulation to check the purity and then preserved at 4 °C.

2.4. Preliminary screening for bioactive metabolites

2.4.1. Amylase activity

Isolated actinomycetes were spot inoculated on starch agar media (SAM) [composition, g/L: soluble starch 10.0, Meat extract 3.0, seawater (50% v/v) 1000 mL, pH 7.0 ± 0.2, agar 15.0] and incubated for 7 days at 28 ± 2 °C, flooded with povidone–iodine solution and left for 5 min. The organisms secreting amylase produce the zone of clearance or decolorization against the blue color background [14].

2.4.2. Protease activity

The proteolytic activity was studied using milk casein agar (g/L: Peptone 1.0, sterile skimmed milk (10%), Agar 15.0, pH 7.0 ± 0.2, seawater (50% v/v) 1000 mL). Test actinobacteria were streaked and incubated for 7 days at 28 ± 2 °C. Following incubation, organisms secreting protease enzyme will exhibit a zone of proteolysis, which is demonstrated by clear zone surrounding the actinomycetes growth. The width of the hydrolyzed zone around the growth versus the width of growth was measured and recorded [15].

2.4.3. Lipase activity

Qualitative lipolytic activity of all the isolated actinobacteria was determined by streaking them on Tributyrin Agar (TA) plates [composition, g/L:Tributyrin 15 mL (v/v), peptone 5.0, agar 15.0, beef extract 3.0, seawater (50% v/v) 1000 mL, pH 7.0 ± 0.2], incubated for 7 days at 28 ± 2 °C. Lipolytic

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