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BIOACTIVITY AND TAXONOMIC STUDIES OF *NOCARDIOPSIS DASSONVILLEI* AND NOCARDIOPSIS SPECIES FROM MARINE SEDIMENTS OF BAY OF BENGAL, INDIA

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ABSTRACT: Globally, the outbreak of antibiotic-resistant pathogens derived infections urged an intensifying necessity for the survey of underexplored marine zone microorganisms for novel metabolites. Our current study emphasized on isolation, polyphasic characterization and bioactivity of the novel actinomycetes from the Bay of Bengal near Kakinada and Nizampatnam, Andhra Pradesh, India, which could provide lead bioactive compounds of therapeutic efficacy to repress the of pervasiveness multidrug-resistant pathogens. A total of fifteen actinobacterial strains (VJRM-1 to VJRM-15) were isolated and tested. Micro-morphological characteristics of the isolates were perceived by SEM (scanning electron microscope) analysis. Phylogenetic analyses of isolates were appraised with 16S rRNA gene sequence homology. The dendrogram was contrived by deploying the parsimony method in MEGA software version 6. Based on polyphasic studies, strains VJRM-7 and VJRM-8 identified as *Nocardiopsis dassonvillei* (KP299158) and Nocardiopsis sp (KP299159). *In-vitro* antimicrobial activity of crude ethyl acetate extracts derived from these strains revealed strong antagonistic activity against Gram-positive, Gram-negative bacteria and fungi. Both strains displayed positive results for the production of enzymatic activities. The morpho-physio-molecular and biological studies on moderate halophilic strains VJRM-7&VJRM-8 indicated as pre-eminent resources of bioactive compounds for medicinal and industrial uses in an eco-friendly manner for mankind.

INTRODUCTION: Marine ecosystems are mostly self-reliant complexes of life forms. They serve as a massive stock for cogent and idiosyncratic actinomycetes with great versatility. Contemporary expansions in molecular ecology, metagenomics, proteomics, and ecological modeling elucidated that marine actinobacterium as one of the vital biotic groups in terms of anamorphosis and functional diversity on earth [1].

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Ecological or phylogenetically, the marine actinobacteria are distinct from their terrestrial relatives. There was an old intuition that the diversity of actinomycetes in the oceans was very small and restricted, but it has been completely dissipated by recent 16S phylogenetic diversity inventories, guesstimates, and culture cultivation approaches [2]. Marine actinobacteria are depicted as a predominant amenity for novel drugs.

They have been systematic makers for secondary metabolites with a diversified range of medicinal activities like anticancer, thrombolytic, anti-microbial, antiparasitic, hemolytic, antidiabetic, anti-inflammatory, neurological; antioxidant activity apart from industrial, agricultural, bioremediation and nanotechnological uses [3].

The genus name "Nocardiopsis" derived from the Greek name opsis, appearance, and from Edmond Nocard, who delineated in 1888 the type species of the genus Nocardia, family Nocardiopsaceae [4]. They are aerobic, Gram-positive with the G+C content in DNA (64-69). At first, this genus was isolated from mildewed grain in 1904 as *Streptothrix dassonvillei* by Brocq-Rousseau [5]. Based on the morphological and biochemical basis, in 1976, Meyer [6] segregated the genus Nocardiopsis from Actinomadura [7]. Later, several scientists strongly underpinned the origination of genus *Nocardiopsis dassonville* [8, 9, 10]. They are chiefly free-living entities in marine and hypersaline habitats on account of their diverse nature (salt-, alkali- and desiccation-resistant) and have a predominant role in reprocessing natural compounds. Under stress state, their survival is triggered by the yield of extracellular enzymes, antibiotics, surfactants, and toxins, which helps their hosts in evading pathogens and predators. Secondary metabolites from nocardiopsis members are mainly polyketides, cyclic peptides, macrolides, diketopiperazines, α-pyrones, γ-pyrones, alkaloids, naphthoquinones, phenazines and phenoxazine derivatives which are superintended for a large spectrum of medicinal and organic effects [11].

Present-day reports have shown that strains of genus Nocardiopsis are repeatedly sequestered from alkaline (pH 8 ± 5) soils with elevated salt concentrations or salterns [12-16].

Marine India is a unique asset of biodiversity. It provides us scope to inquest an overwhelming reservoir of potentially active secondary metabolites from actinomycetes by employing both modern biotechnological aspects and natural product chemistry. As the increasing rate of antimicrobial resistance had become a serious threat to global public health, now pharmaceutical companies are focusing on novel molecules whose specificity is resembled by decreased side effects. Till now, a substantial part of actinomycetes has been isolated chiefly from the soil. However, the rate of novel compound invention from the vast explored terrestrial strains has been declined remarkably in recent years [17]. To overpower this predicament, the isolation of actinomycetes has been switched to other resources such as marine sediments and desert ecosystem [18].

In this present research, for the unique search of unrivaled actinobacteria, we have explored and reported two potent actinobacterial strains *Nocardiopsis dassonvillei* and Nocardiopsis sp. from some part of the underexploited marine habitats of Kakinada and Nizampatnam of Andhra Pradesh. Further, we have analyzed their complete phenotypic identification, genomic characterization, different enzyme screenings, and antimicrobial activity.

MATERIALS AND METHODS:

Location and Sample Collection: Marine samples were collected at a depth of about 20cm from the Kakinada sea coast, East Godavari district, and Nizampatnam, Guntur district of Andhra Pradesh, India. The geographic coordinates of sampling areas are 16° 57′ 58″ N, 82° 15′ 18″ E(Kakinada) and 15°54′ 0″ N, 80°40′ 0″ E(Nizampatnam). Collected samples were transported to the laboratory under sterile conditions followed by air drying for 3 to 5 days at room temperature. The marine samples were triturated and sieved prior to pretreatment [19, 20].

Enrichment and Selective Isolation Procedures: Pretreatment of marine samples with physical (dry heating in a hot air oven at 45 °C for 1 h) and chemical methods like calcium carbonate 1:1 w/w, phenols, SDS treatments were executed to reduce the contamination of bacteria and molds. Pretreatment of marine samples enhanced the chances of isolating rare and novel actinomycetes with morphologically unique structure and pigmentation [21, 22].

Five media were employed for the recovery of actinobacterial strains from samples. One gram of pretreated sample was suspended in 10 mL of sterile double distilled water. An aliquot of 0.1 ml of each dilution (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) of samples was spread evenly over the surfaces of ISP2 [22], Humic acid vitamin a gar [23], Starch casein agar, Gause`s no. 1 medium [24], Vegitone agar media in replicates of three supplemented with antibiotics cycloheximide (25 μg/ml) and streptomycin (25 μg/ml) in order to retard fungal and bacterial contamination, respectively. Plates were incubated for 14 to 28 days at 30 ± 2 °C in an incubator. Based on the colony morphology [25], unique actinomycete colonies were picked, streaked,

purified, and preserved on (ISP2) Yeast extract malt extract dextrose agar slants at 4 °C for further use and maintenance [26]. In our screening, a total of 15 actinomycetes were isolated and designated as VJRM-1 to VJRM-15. They were screened against microbial pathogens. Selected active isolates were further characterized by 16S rRNA sequencing technique [27].

Screening of Actinomycetes for Antimicrobial Efficacy: Screening of isolates for bioactivity potentiality was done by agar well diffusion method with culture crude extract [28].

Trial Pathogens Used: A series of trial organisms included for the screening of antimicrobial efficacy were Gram-positive bacteria: *Staphylococcus aureus* (MTCC 3160), *Bacillus megaterium* (NCIM 2187), *Bacillus cereus, Bacillus subtilis* (ATCC 6633). Gram-negative: *Xanthomonas campestris* (MTCC 2286), *Proteus vulgaris* (ATCC 6380), *Pseudomonas aeruginosa* (ATCC 9027), *Klebsiella pneumonia,* and *Escherichia coli* (ATCC 9027). Fungi: *Aspergillus niger* (ATCC 16404), *Penicillium citrinum* (MTCC 6849), and *Candida albicans* (MTCC 183).

Among the fifteen actinobacterial isolates tested, two isolates VJRM-7 and VJRM-8 displaying high antimicrobial activity were selected to determine their taxonomic position through cultural and molecular 16S rRNA gene fragment analysis.

Extraction Method for Secondary Metabolites & Bioactivity Assay: Production of bioactive compounds from the isolates was done by submerged fermentation, and yield can be increased by optimizing the conditions of culture broth during fermentation process [29]. For seed culture preparation, five-day-old actino culture was suspended in 50 mL (seed medium) yeast extract malt extract dextrose broth in 250 mL conical flask and incubated in a rotary shaker at 180 rpm at 35 °C for 48 h. 10% of this seed culture was transferred to a 500 mL YMD broth (Fermentation medium) flask. The fermentation was carried out at 35 °C under agitation at 180 rpm and incubated for 8 days. The antimicrobial metabolite was recovered from the filtrate by solvent extraction method [30]. After the incubation period, the broth was filtered by Whatman no. 1 filter paper.

Ethyl acetate was added to the filtrate 1:1 (v/v) and shaken vigorously for complete extraction. The ethyl acetate phase that contains bioactive metabolites was separated from the aqueous phase using separating funnel. The ethyl acetate extract was evaporated to dryness in the water bath (40 °C - 45 °C), and the residue thus obtained was used to determine antimicrobial activity by agar well diffusion method and the diameter of the growth inhibition zones was measured. Ethyl acetate itself was used as a negative control. The wells were prepared in the Nutrient agar media plate by using sterile cork borer (6 mm in diameter). A volume of 100 µL of crude extract of culture was carefully dispensed into each well and allowed to diffuse for 2 h and incubated at 37 °C for 24 h. The standard antibiotic of volume 100 µl was placed into the agar well as a positive control. Plates were incubated at 37 °C for 24 h. After incubation, the zone of inhibition around each well (in mm) was measured and recorded. The experiment was carried out in triplicates for each test organism, and the mean values were calculated [31].

Taxonomical Identification and Characterization of VJRM-7 and VJRM-8:

Phenotypic Characterization: In preliminary detection, microscopic observation of strains VJRM-7 and VJRM-8 for spore chain morphology was implemented by coverslip culture technique [32] under a compound microscope (model Motic-BA410).

SEM Microscopy: Micromorphological traits of potent strains were examined with cultures grown at 32 °C for 10 days on yeast extract malt extract dextrose agar (YMD). Samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 h at 4 °C and postfixed 2% in aqueous osmium tetroxide for 4 h. Dehydrated in a series of graded alcohols and dried to critical point drying with CPD (EMS-850) unit by using liquid carbon dioxide. The processed samples were mounted over the stubs with double-sided carbon conductivity tape, and a thin layer of the gold coat over the samples was done by using an automated sputter coater (Model - JEOL JFC-160s0) for 3 min and scanned under Scanning Electron Microscope (SEM - Model: JOEL-JSM 5600), and images were recorded at various magnifications [33].

The strains were subcultured on different media such as seven International Streptomyces Project (ISP) media and eight non-ISP media to observe the cultural characteristics such as the color of aerial mycelium, substrate mycelium, pigment production and spore formation [34].

Biochemical Analysis: Various biochemical tests were performed for the identification of the potent isolates VJRM-7 and VJRM-8 comprising the IMVIC test, hydrolysis of starch, casein hydrolysis, urea hydrolysis, gelatin hydrolysis, and arginine dihydrolase [35]. Utilization of different sources of carbohydrates such as Adonitol, dextrose, fructose, inositol, lactose, maltose, sucrose, sorbitol, raffinose, rhamnose, and xylose was tested by inoculating the isolates in ISP 2 broth supplemented with a final concentration of 0.4% respective carbon sources and incubated for 7 days at 30 °C. Physiological characteristics such as the effect of pH (6-11), temperature (35-45°C) and salinity on growth of the strains were analyzed [36, 37].

Preliminary Enzymatic Screening: The isolated actinomycetes strains VJRM-7 and VJRM-8 were screened for the production of different extracellular enzymes like L-Asparaginase, Glutaminase, Cellulase, Lipase, Esterase, Urease, Catalase, DNase, RNase, Phosphatase and Amylase were conducted. The strains which showed positive enzyme production were selected for further enzymatic assay [38, 39].

***In-vitro* Screening of Antibiotic Sensitivity Testing:** The antibiotic sensitivity test was conducted to assess the susceptibility of the strains VJRM-7 & VJRM-8 to divergent antibiotics by following the Kirby-Bauer disc diffusion method. Ten antibiotic discs *viz*., Chloramphenicol (30 µg), Gentamicin (10 µg), Kanamycin (30 µg), Vancomycin (30 µg) were used in this piece of study (from Hi-Media Pvt. Ltd., India). As per the specification, the concentration of each antibiotic was maintained, and each plate was incubated at 32 °C for 48 h during the study. After incubation, the occurrences and sizes of inhibition zones around the discs of the different antibiotics were tabulated. On the basis of forwarded specifications against each antibiotic by the Hi-Media Pvt. Ltd., India, the isolates were either considered as sensitive (S), intermediate (I), or resistant (R) to an antibiotic. Growth of VJRM-7 and VJRM-8 strains on medium containing antibiotics was then compared with that on control medium without antibiotics [40].

Genotypic Characterization of Potent Strains: Among molecular techniques, PCR and 16S rRNA analysis are the most widely used procedures for genomic identification of actinobacteria. For the Polymerase chain reaction (PCR), Genomic DNA was isolated from two potent antagonistic strains VJRM-7 and VJRM-8 pure colonies grown on YMD for 5 days. The total genomic DNA was extracted using InstaGene Matrix (Bio-Rad, USA) according to the manufacturer's specifications. PCR amplification reactions were conducted by using 1µL of genomic DNA in 20µL of PCR reaction solution by using 27F/1492R primers (27F 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R 5'- TACGGYTACCTTGTTACGACTT-3'). 35 cycles of amplification were performed at 94℃ for 45 sec, 55 ℃ for 60 sec, and 72 ℃ for 60 sec [41]. DNA fragments are amplified up to 1,400 bp with positive control (*E. coli* genomic DNA) and a negative control in the PCR. Purification of PCR products was executed by using Montage PCR Clean up kit (Millipore). PCR product was sent to Macrogen Company, South Korea for 16S rRNA sequencing by using the following primers (518F 5'- CCAGCAGCCGCGGTAATACG-3'and 800R 5'-TACCAGGGTATCTAATCC-3'). Sequencing was accomplished by using Big Dye terminator cycle sequencing kit (Applied BioSystems, USA). Sequencing products were resolved on an Applied BioSystems model 3730XL automated DNA sequencing system (Applied BioSystems, USA). The sequences thus obtained were analyzed for homology using BLASTN (Entrez Nucleotide database). The deduced 16S rDNA sequence was compared with the sequences in Genbank (http://www.ncbi.nlm.nih.gov/) using the basic local alignment search tool (BLAST) then aligned with the related reference sequences retrieved from NCBI Genbank databases using the Clustal W method [42].

Alignments of sequences of the strains were organized pair wisely using BLAST search against the gene library accessible for the prospective actinomycetes strains in the Genbank [43]. Evolutionary and molecular analyses were

computed by using MEGA (Molecular Evolutionary Genetics Analysis) version 6.0 software.

GenBank Accession Numbers: The16S rRNA gene sequences of the strains VJRM-7and VJRM-8 were submitted in NCBI (National Center for Biotechnology Information).

RESULTS:

Seclusion of Marine Actinobacteria: In the current research, a total of fifteen actinobacterial strains were isolated from the marine ecosystem of Kakinada and Nizampatnam on different media and designated as VJRM-1 to VJRM15. Combined Pre-treatment of the marine soils with calcium carbonate (1:1 w/w) and dry heat (45°C for 1 h) treatment reduced the growth of ubiquitous microbial species and facilitated the isolation of rare actinomycetes.

Altogether five culture media were used; the effectual media which recovered the highest number of marine actinobacteria was Humic acid Vitamin agar supplemented with antibiotics Nystatin (25 µg/ml) and Streptomycin (25 µg/ml). Purification was done by repeated streak method on HVagar medium and later preserved on yeast extract malt extract dextrose agar (ISP-2) slants at 4 °C. The isolates were evaluated for the coproduction of enzyme and antimicrobial activities. Two strains VJRM-7 and VJRM-8 exhibited high bioactivity among the tested isolates and were selected for in-depth taxonomic studies.

Polyphasic Taxonomy of VJRM-7 and VJRM-8:

Morphological, Physio-biochemical Traits: Micro-morphological view of the two strains was examined under Scanning electron microscopy. Both strains displayed massive sporulation with branched mycelium and abundant aerial hyphae. Spores of VJRM-7 strain were spherical to elongated form with smooth-surface. Another strain VJRM-8 spores appeared in a rod shape, smooth surface with aseptate branched mycelium. Paired spores on hyphae were observed (depicted in **Fig. 3** and **4**).

Cultural characteristics and growth properties of strains VJRM-7and VJRM-8 were studied on both ISP and non-ISP agar media (depicted in **Table 1** and **2**). Strain VJRM-7 exhibited excellent growth on ISP-1, ISP-2, ISP-3 agar media, while good growth on Bennett's, Nutrient agar, Starch peptone agar media, and moderate growth on ISP-4, ISP-7, ISP-9 agar media, and Actinomycetes isolation agar. Another strain VJRM-8 displayed excellent growth on ISP-2, ISP-3 and Starch peptone agar, good growth on ISP-1, nutrient agar, and Bennett's agar media while growth was moderate on actinomycetes isolation (AIA) agar and poor growth on ISP-7. Both strains showed nil growth on Czapek Dox agar. No growth was perceived on ISP-4 agar media in VJRM-8. Both are Gram-positive, moderately halophilic, and aerobic. Colonies of VJRM-7 are white, powdery, and VJRM-8 was slightly slimy.

On enriched YMD agar media, the aerial spore mass color of strain VJRM-7 was white, and substrate mycelium was darkish brown whereas VJRM-8 strain exhibited white creamy aerial mycelia with green substrate mycelia. Color of substrate mycelium in VJRM-7 varied from yellow or dark brown to pale yellow on different media, while VJRM-8 substrate mycelia exhibited green color or yellow to pale yellow. Melanoid pigments are not produced on ISP 6 or tyrosine agar. In the carbon substrate assimilation test, the ability of two strains to assimilate (10) different carbon sources are presented in **Table 4.** Potent strain VJRM-7 displayed moderate utilization of mannose, sucrose, maltose rhamnose & mannitol, while strain VJRM-8 utilized mannitol & lactose moderately and utilization of arabinose and raffinose was very poor.

Potent strain VJRM-7 showed NaCl tolerance up to 10% and mildly alkaline conditions of pH 8. It showed optimum growth at a salinity of 6% NaCl and pH of 7. Another strain VJRM-8 showed tolerance of NaCl up to 9% & pH up to 10 and with an optimal growth rate at 5% NaCl and pH of 8. Moreover, both strains are moderately halophilic in nature. Both strains exhibited good growth at different NaCl concentrations of 6% and 7% with temperature ranging from 35° - 44 °C for strain VJRM-7 and 29° - 33 °C for strain VJRM-8. The optimum temperature for growth of strain VJRM-7 was 35 °C, whereas for strain VJRM-8 was 32 °C.

The details of the physiological and biochemical characteristics of the strains are stated in **Table 3**.

VJRM-7 evinced positive reaction to catalase production, citrate utilization, starch hydrolysis, gelatin liquefaction casein hydrolysis, urease test, lipid hydrolysis test, and negative for Indole, methyl red and vogues-proskauer tests. VJRM-8 indicated the positive reaction to the Indole test, catalase test, urease, casein hydrolysis test, and negative result observed for hydrogen sulphide production, nitrate reduction, methyl red, and Vogues-Proskauer tests.

On assessment for antibiotic sensitivity to divergent antibiotics, VJRM-7 strain exhibited resistance to Chloramphenicol, Metronidazole, Vancomycin, Clindamycin, Cephalexin, and sensitivity to Cefepime, Gentamicin, and Imipenem. Whereas VJRM-8 isolate displayed resistance towards antibiotic discs such as Imipenem, Cephalexin, Gentamicin, and Chloramphenicol.

Genomic Identification: The phylogenetic locus of puissant isolates VJRM-7 and VJRM-8 was determined by amplifying the 16S rRNA region. The acquired fragmental 16s rRNA gene sequences of strains were analogized with neighborly related sequences [44, 45] obtainable in GenBank using multisequence advanced BLAST analysis, which showed alignment up to 99% with those of Nocardiopsis reference species which confirmed the identification of isolates at the genus level Nocardiopsis. The phylogenetic analysis of the aligned sequence was wielded by the CLUSTAL W program. Evolutionary trees were constructed using the parsimony method along with the statistical analysis of bootstrap values by employing MEGA software version 6 **Fig. 7** and **8**.

The partial 16S rRNA sequences of the strains VJRM-7 and VJRM-8 were recorded in the National Center for Biotechnology Information (NCBI) with accession numbers of KP299158 & KP299159. Both strains evinced typical morpho-logical features of the genus Nocardiopsis.

In preliminary different enzyme activities screening, VJRM-7 displayed positive response for amylase, lipase, glutaminase, DNase, and lipase and negative response for asparaginase (depicted in **Fig. 5**. Another strain VJRM-8 evinced positive response for asparaginase, arginase, DNase, and lipase activities.

TABLE 1: CULTURAL CHARACTERISTICS OF STRAIN VJRM-7

Medium	Growth	Aerial mycelium	Substrate mycelium	Pigmentation
Tryptone Yeast-extract agar [ISP-1]	Excellent	White	Brown	Brown
YMD agar [ISP-2]	Excellent	White	Dark brown	Brown
Oat-meal agar [ISP-3]	Excellent	White	Brown	Brown
Inorganic salt starch agar [ISP-4]	Moderate	White	Pale yellow	No
Peptone yeast extract iron agar [ISP-6]	Poor	Pale white	Pale yellow	No
Tyrosine agar [ISP-7]	Poor	White	Pale Yellow	No
Nutrient agar medium	Good	White	Yellowish-brown	No
Bennett's agar	Good	White	Brown	No
Actinomycetes isolation agar	Moderate	White	Pale yellow	No
Starch peptone agar	Good	White	Yellowish-brown	No
Czapek Dox agar	Nil	No	No	No
ISP-9	Moderate	White	Pale brown	No

TABLE 2: CULTURAL CHARACTERISTICS OF STRAIN VJRM-8

Medium	Growth	Color of Aerial mycelium	Color of Substrate mycelium	Pigmentation
Tryptone Yeast-extract agar [ISP-1]	Good	White	Green	Green
YMD agar [ISP-2]	Excellent	White	Dark green	Dark green
Oat-meal agar [ISP-3]	Excellent	White	Green	Green
Inorganic salt starch agar [ISP-4]	Nil	No	No	No
Peptone yeast extract iron agar [ISP-6]	Poor	Pale white	Pale yellow	No
Tyrosine agar [ISP-7]	Moderate	Pale white	Pale Yellow	No
Nutrient agar medium	Good	White	Yellow	No
Actinomycetes isolation agar [AIA]	Moderate	White	Pale yellow	No
Starch peptone agar	Excellent	White	Yellow	No
Czapek Dox agar	Nil	No	No	No
Bennett's agar	Good	White	Green	Green

TABLE 3: MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL TRAITS OF VJRM- 7 AND VJRM-8

Morphology	**VJRM-7**	**VJRM-8**
Characteristic features	**Observation**	**Observation**
Sporophore morphology	Round to elongated shape	Coccoid to rod shape
Colour of Aerial mycelium	White	Creamy white
Colour of Substrate mycelium	Brown	Green
Physiological traits	**Response**	**Response**
Gram's reaction	Positive	Positive
Production of melanin pigment	Negative	Negative
Optimum temperature for growth	35 °C	32 °C
Range of temperature	35°-44 °C	29°-33 °C
Optimum pH for growth	7	8
Range of pH for growth	6-9	6-10
Sodium chloride tolerance	Up to 10%	Up to 7%
Biochemical characters	**Reaction**	**Reaction**
Indole production test	Negative	Positive
Methyl red test	Negative	Negative
Voges proskauer test	Negative	Negative
Citrate utilization test	Positive	Negative
Catalase production test	Positive	Positive
Starch hydrolysis test	Positive	Negative
Gelatin liquefaction test	Positive	Negative
Urease test	Positive	Positive
Hydrogen sulfide production test	Negative	Negative
Lipid hydrolysis test	Positive	Negative
Casein hydrolysis test	Positive	Positive

TABLE 4: UTILIZATION OF CARBON SOURCES AND STUDIES ON THE ENZYMATIC ACTIVITIES OF VJRM-7 AND VJRM-8

Carbon sources	**Results**	
	VJRM-7	**VJRM-8**
Dextrose	+++	+++
Arabinose	+	+
Mannose	++	++
Lactose	+	++
Mannitol	++	++
Sucrose	++	-
Rhamnose	++	-
Adonitol	+	-
Sorbitol	-	-
Raffinose	+	+
Maltose	++	-
(+++) = Good growth, (++) = Moderate growth, (+) = Poor growth, (-) = No growth		
Enzymes	**VJRM-7**	**VJRM-8**
Amylase	+	-
Glutaminase	+	-
Cellulase	-	-
Chitinase	-	-
Urease	+	+
Lipase	+	-
Arginase	-	+
Asparaginase	-	+
DNAase	+	-

*[(+) =Positive, (-) =Negative]

TABLE 5: ANTIBIOTIC SUSCEPTIBILITY/RESISTANCE OF VJRM-7 AND VJRM-8

Antibiotics (µg)	**Susceptibility/Resistance**	
	VJRM-7	**VJRM-8**
Chloramphenicol (50)	R	R
Metronidazole	R	I
Imipenem (10)	S	R
Clindamycin (2)	R	S
Cefepime (30)	S	S
Vancomycin (30)	R	S
Cephalexin (30)	R	R
Gentamicin (10)	S	R
Cefoxitin (10)	I	S

[R=Resistance, S=Susceptibility, Is=Intermediate]

TABLE 6: ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF STRAIN *NOCARDIOPSIS DASSONVILLEI* VJRM-7 AND *NOCARDIOPSIS* SP. VJRM-8

Test organism	Zone of inhibition (mm)		
Bacteria	**VJRM-7**	**VJRM-8**	**Positive control #**
Xanthomonas campestris	21	20	22
Bacillus megaterium	25	22	32
Escherichia coli	18	11	24
Pseudomonas aeruginosa	23	18	22
Staphylococcus aureus	19	12	18
Bacillus subtilis	20	19	28
Klebsiella pneumoniae	16	15	20
Bacillus cereus	22	20	25
Fungi			
Aspergillus flavus	17	16	26
Penicillium citrinum	15	18	20
Candida albicans	19	18	27

#Positive control: Streptomycin against bacteria, Cycloheximide against yeast and Flucanazole against fungi

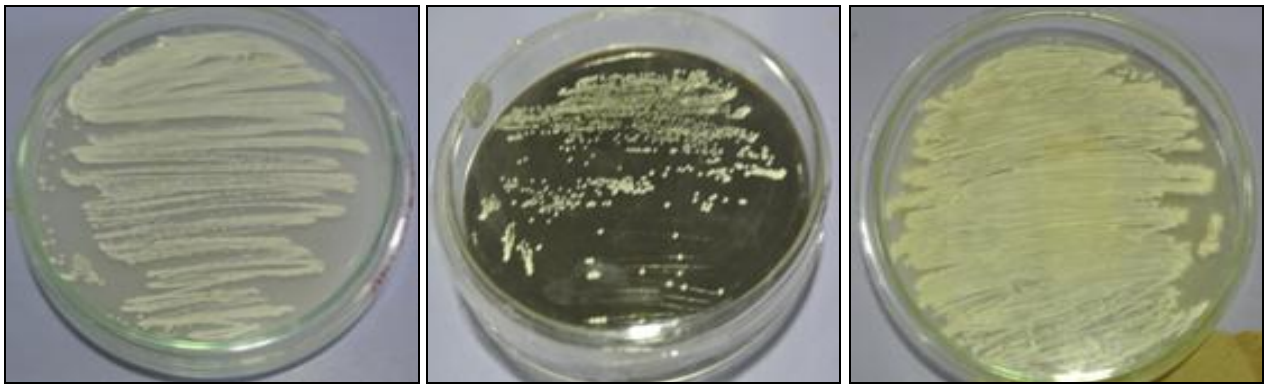

FIG. 1: CULTURAL CHARACTERISTICS OF STRAINS VJRM-7ANDVJRM-8

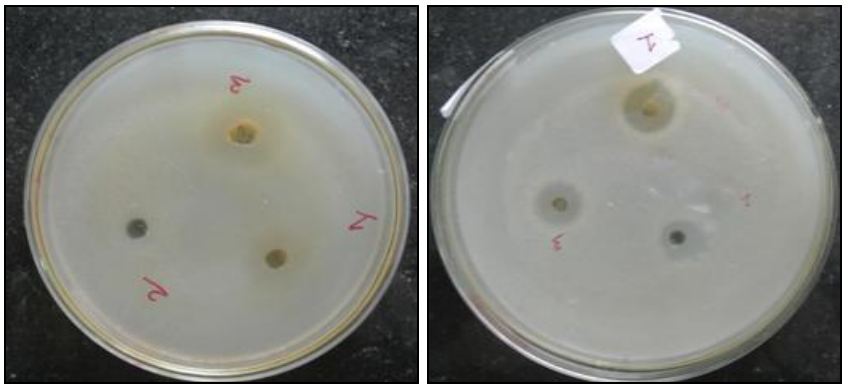

FIG. 2: ANTIMICROBIAL ACTIVITY OF VJRM-7 AND VJRM-8 STRAINS

Zone of inhibition recorded in millimeter (*1 indicates VJRM-7 and 3* indicates VJRM-8 and 2* is control)

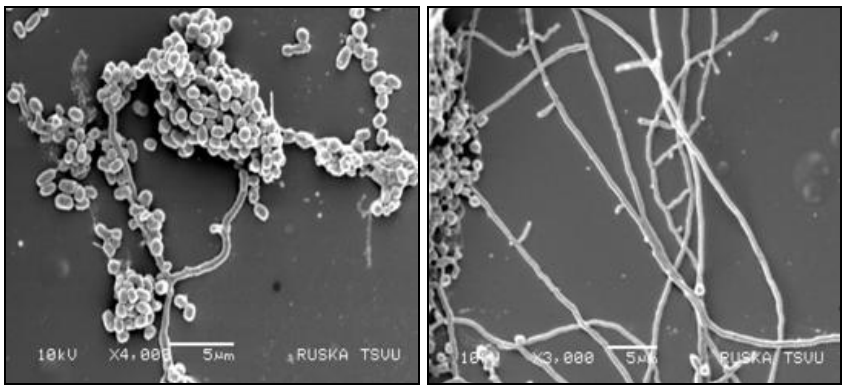

FIG. 3: SCANNING ELECTRON MICROSCOPIC PHOTOGRAPH OF *NOCARDIOPSIS DASSONVILLEI* VJRM-7

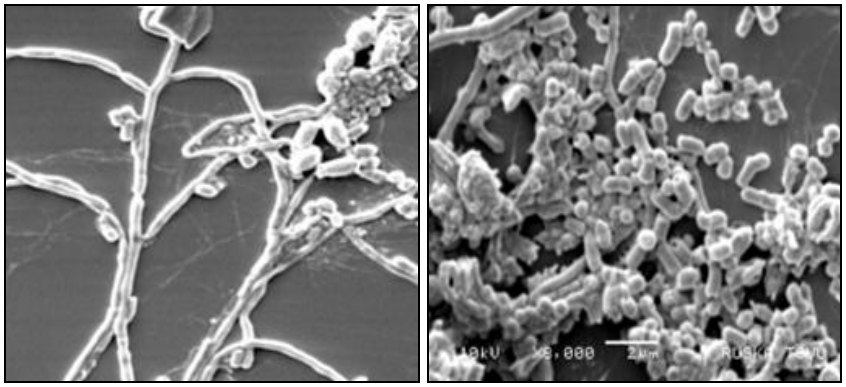

FIG. 4: SCANNING ELECTRON MICROSCOPIC PHOTOGRAPH OF *NOCARDIOPSIS* SP. VJRM-8

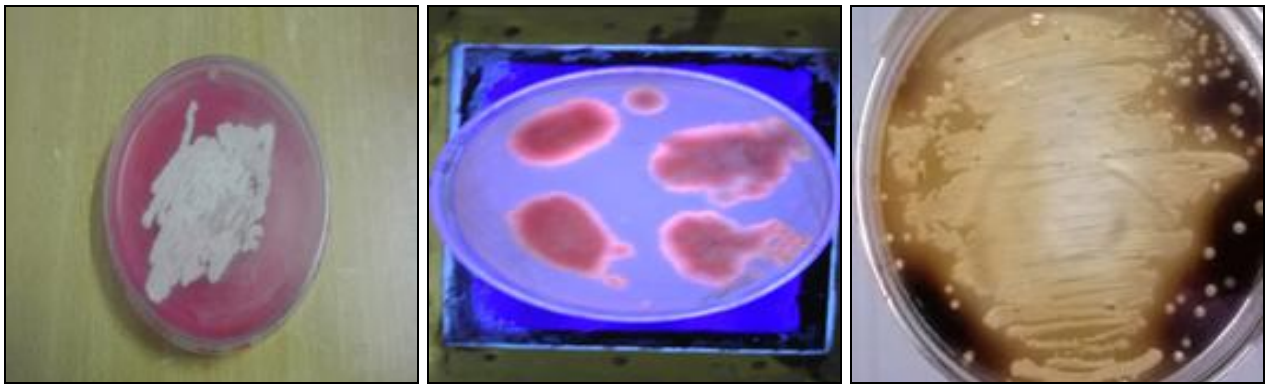

FIG. 5: ENZYMATIC SCREENING RESULTS OF (A) GLUTAMINASE, (B) LIPASE AND (C) AMYLASE FOR STRAIN VJRM-7

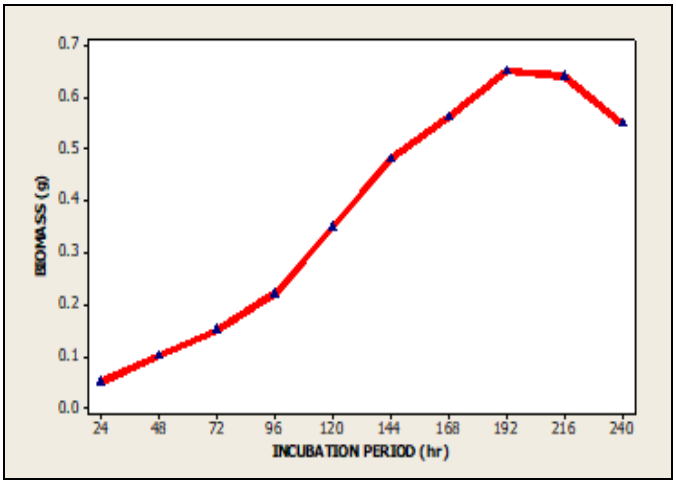

FIG. 6: GROWTH PATTERN OF *NOCARDIOPSIS DASSONVILLEI* VJRM-7

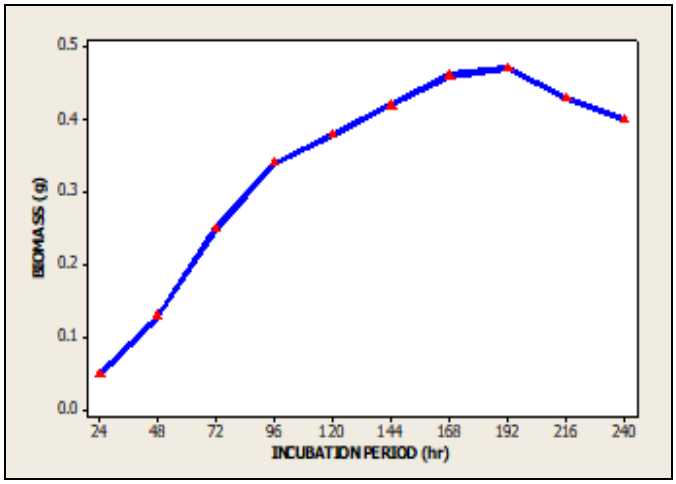

FIG. 7: GROWTH PATTERN OF *NOCARDIOPSIS* SP VJRM-8

Nucleotide Sequence Accession Numbers: DNA sequences of *Nocardiopsis dassonvillei* and *Nocardiopsis* sp obtained in this study were deposited in GenBank with the accession numbers KP299158 and KP299159.

Statistical Analysis: The antimicrobial assay was accomplished in the triplicate process. Readings were taken as the mean ± standard deviation of the mean of three replicates calculated using Microsoft Excel XP 2007.

Metabolite Extraction and Antimicrobial Assay: Yeast-extract glucose broth (Yeast extract 0.4 g/100 mL; Glucose 0.4 g/100 mL) was used to obtain a sufficient quantity of the active secondary metabolites from potent strains VJRM-7 and VJRM-8. The bioactive metabolites of both strains were recovered from the harvested medium by the solvent extraction method. The filtrate was assorted with ethyl acetate (1:1 v/ v) and shaken vigorously in a solvent extraction funnel.

The solvent extracts were concentrated and were tested for their bioactivity spectrum. The stationary phase of VJRM-7 continued from 192 h to 216 hours of the incubation period. Bioactive metabolites resulted from the VJRM-7 culture stationary phase exhibited antimicrobial activity against test organisms (listed in table-6). Maximum antagonistic activity against *Pseudomonas aeruginosa, Bacillus megaterium, Xanthomonas campestris, Bacillus subtilis, Candida albicans,* and *Aspergillus flavus,* for the strain VJRM-8 stationary phase extended from 168h to 192 h of the incubation period. It exhibited good antagonistic activity against *Xanthomonas campestris, Bacillus subtilis, Bacillus cereus, Penicillium citrinum, and Candida albicans.* Antimicrobial activities of the two strains were compared with that of known commercially available antibiotic streptomycin.

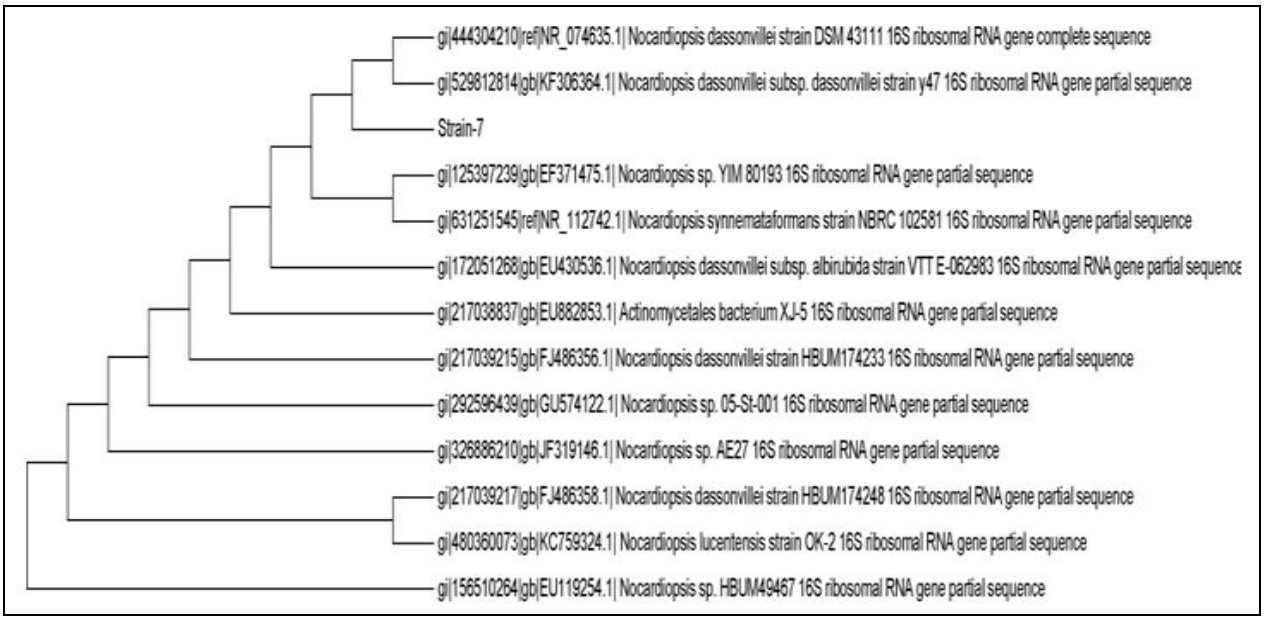

FIG. 8: PARSIMONY METHOD BASED ON 16S rRNA GENE SEQUENCES SHOWING RELATIONSHIPS BETWEEN STRAIN VJRM-7 AND CLOSELY RELATED MEMBERS OF THE GENUS NOCARDIOPSIS

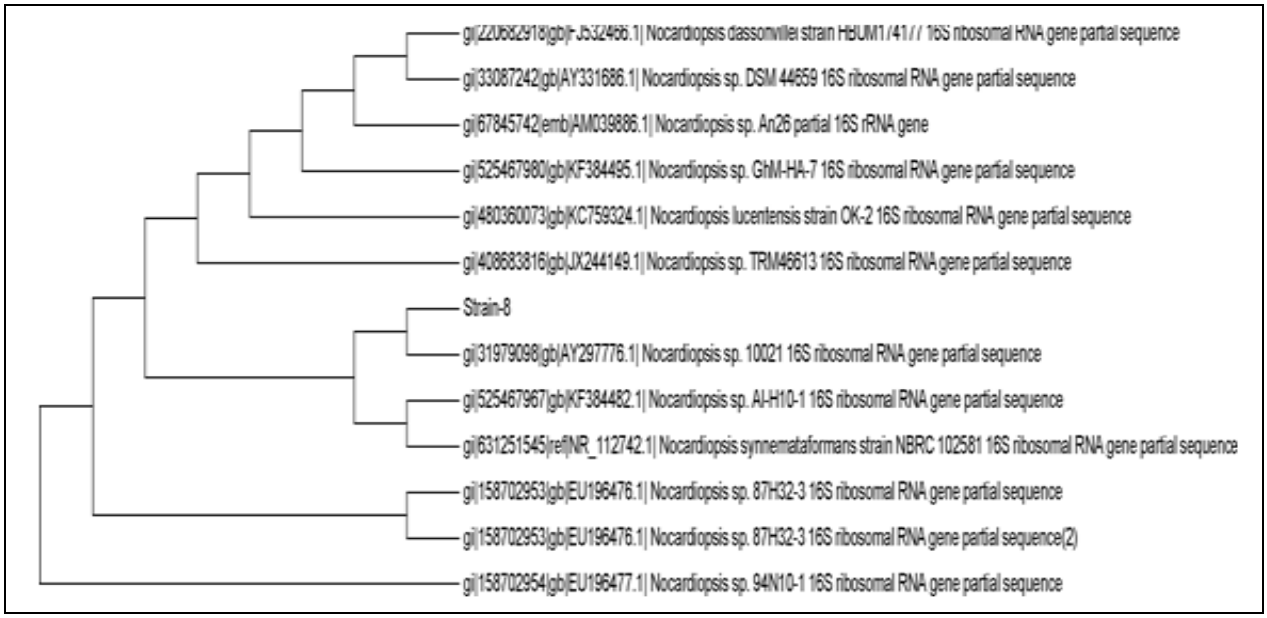

FIG. 9: PARSIMONY METHOD BASED ON 16S rRNA GENE SEQUENCES SHOWING RELATIONSHIPS BETWEEN STRAIN VJRM-8 AND CLOSELY RELATED MEMBERS OF THE GENUS *NOCARDIOPSIS*

DISCUSSION: Immoderate, misapply of antibiotics, economic burden, and change in the genotype of pathogens led to the evolution of resistant lethal strains and infections globally. This multidrug resistance crisis challenged mores to focus on the discovery of new drugs or metabolites

from underexplored marine recess habitats compared to terrestrial ones. The isolation of rare marine actinomycetes accorded the discovery of distinctive, non-toxic benign, efficacious, broad-spectrum antimicrobial compounds. Our current study mainly emphasized on the exploration of some part of marine ecosystems of Andhra Pradesh to isolate and screen the puissant actinobacteria that render new classes of therapeutics against drug-resistant pathogens.

In the avenue of our screening for pharmacologically active agents from marine actinomycetes, we have isolated fifteen actinobacterial strains. All the isolates were assessed for their antimicrobial activity. Among them, ethyl acetate culture broth of two halophilic actinobacterial strains *Nocardiopsis dassonvillei* and *Nocardiopsis* sp. were found to be active against selected microbial pathogens.

Actinomycetes derived enzymes have an assemblage of biological, industrial, and environmental applications, like polymer hydrolysis, synthesis of chemicals, soil neutralization, biological dominance of diseases, and putrefaction of organic matter. Both strains exhibited positive outcomes in the production of enzymes (amylase, asparaginase, glutaminase, streptokinase, DNase, and lipase). These two actinomycetes explicited to generate an extensive range of enzymes in screening, which might be the byproduct of natural selection of microorganisms in order to outlive in contending habitats. Moreover, the present research proved that Nizampatnam and Kakinada seashores are eminently suitable ecosystems for screening programs designed to isolate strains producing new bioactive compounds.

CONCLUSION: In our study, two actinobacteria, Nocardiopsis dassonvillei and Nocardiopsis species were isolated from Kakinada and Nizampatnam areas of Andhra Pradesh and characterized cultural, morpho-physiological and biochemical traits. These isolated actinobacteria displayed potent antimicrobial, Glutaminase, Lipase, and Amylase activities warranting detailed mechanistic Investigation. This study also contributed in exploring some of the underexploited areas of Kakinada and Nizampatnam as a source of microbial multifariousness. Currently, studies on purification and chemical elucidation of the bioactive compounds are in progress.

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CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest.

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