

Research Article**A broad-spectrum antimicrobial activity of thermophilic *Nocardiopsis* sp. producing multiple extracellular enzymes of industrial and therapeutic use**Jyotsna Begani¹, Jyoti Lakhani², Dharmesh Harwani^{1*}¹Department of Microbiology, Maharaja Ganga Singh University, Bikaner, Rajasthan, India²Department of Computer Science, Maharaja Ganga Singh University, Bikaner, Rajasthan, India

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Abstract

Objective: An attempt has been made in the present study to evaluate the efficacy of six selective isolation media in the isolation of bioactive thermophilic actinomycetes native to the Bikaner region of the Thar desert in Rajasthan and to characterize them at polyphasic and chemical levels. **Material and methods:** Selective isolation media were used to recover thermophilic actinobacteria from the soil samples collected at the Bikaner region of the Thar desert. The isolates were characterized for their phenotype and physiology using Bergey's manual of determinative bacteriology. The molecular identification of actinobacteria was done using 16SrRNA sequence gene analysis and the nature of the bioactive chemical compound was determined by UV-visible absorption spectrum and thin layer chromatography. **Results:** Among different tested selective media, the highest plate counts (CFUs/gm of soil) of thermophilic actinomycetes were observed with Modified Actinomycete Selective (MAS)-DH-1 agar medium. The MAS-DH-1 agar medium permitted adequate growth of many different pigmented actinomycetes from the desert soil samples. Among various other bio-active actinomycetes strains, an actinobacterial strain D8 was identified on the basis of its broad-spectrum antibacterial and antifungal activities. The D8 strain was found to produce high levels of extracellular phosphatase, catalase, tyrosinase, lipase, cellulase, protease, amylase, and L-asparaginase enzymes. **Conclusion:** The polyphasic characterization of the isolate D8 assigned it to an anaerobic, Gram-positive, non-acid-fast, catalase-positive filamentous bacterium of the genus *Nocardiopsis* sp. (KM205637) in the order *Actinomycetales* that produces multiple enzymes of industrial and therapeutic use. The partial chemical characterization of the anti-microbial metabolite produced by the strain D8 revealed the presence of an aromatic ring compound.

Keywords: Thar desert, thermophilic actinomycetes, polyphasic characterization, phylogenetic tree, chromatography

Introduction

The rate of the discovery of novel metabolites from microorganisms has been diminished considerably. In addition, the most of antibiotic screening programs end up with already known chemical structures of secondary metabolites. For this reason, it is assumed that microorganisms have been exhausted for their secondary metabolites wealth. The rapid increase of multi-drug resistant pathogens also requires the world-wide attention for the development of novel antimicrobials, novel

treatment options, and alternative antimicrobial therapies. The nature remains the richest and versatile source of new antibiotics, therefore, to discover yet to be identified metabolite molecules, genuine efforts are needed to exploit the unexploited microbes from yet to be explored natural habitats. In the present work, in the course of isolation of thermophilic actinomycetes from uncommon and underexplored ecological niche of the Bikaner region of the Thar desert, one unusual and biotechnologically useful filamentous thermophilic bacterium D8 *Nocardiopsis* sp. (KM205637) has been identified that exhibit broad spectrum anti-bacterial and anti-fungal activities. The Bikaner region falling in the Thar desert extends from 27°11' to 29°3' North latitudes and 71°54' to 74°12' East longitudes. The major part of the region is comprised of desolate and dry regions which form part of the Great India Desert of Thar. At many places,

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one finds shifting sand-dunes of varying heights ranging from 6 to 30 meters. The D8 strain had phenotypic and chemotaxonomic characteristics similar to the genus *Nocardioopsis* sp. Earlier, based on the phenotypic appearance and biochemical features, *Actinomadura dassonvillei* (Brocq-Rousseu) Lechevalier and Lechevalier was transferred to a new genus *Nocardioopsis* (Meyer, 1976). The appearance of the colonies of the genus *Nocardioopsis* of the order *Actinomycetales* is similar to members of the genera *Nocardia* and *Actinomadura* (Cook and Meyers, 2003). Substrate and aerial mycelia of *Nocardioopsis* are well-developed and latter fragments into spores. Similar to the many actinobacterial species, *Nocardioopsis* also produces a huge variety of bioactive compounds such as Griseusins, Apoptolidins, Pendolmycin, lipopeptides, thiopeptides and naphthospiroones and other highly useful therapeutics (Sun et al., 1991; Kim et al., 1997; Li et al., 2007; Gandhimathi et al., 2009; Engelhardt et al., 2010; Begani et al., 2018) and diverse extracellular enzymes (Bennur et al., 2014). The strain D8 has been also identified for its production of higher amount of extracellular phosphatase, catalase, tyrosinase, lipase, cellulase, protease, amylase and L-asparaginase enzymes. In the following sections evaluation of the six different selective isolation media, polyphasic characterization, assessment of antimicrobial activities, production of different enzymes, and partial chemical characterization of antimicrobial substance from the *Nocardioopsis* strain D8 have been furnished. In addition, the importance of growth media with regard to occurrence, distribution, and ecology of actinomycetes in the desert environment has been discussed.

Materials and methods

Soil sampling and pre-treatment

Soil sample was collected from Bikaner region of Rajasthan. The region falls in the arid zone of the Thar in the Indian Territory. The soil sample was collected at the depth of 6-10 cm using a sterile scoop in sterile zipped bag. To avoid growth of undesired bacteria and for the selective isolation of thermophilic actinomycetes, the soil sample was air dried in a hot air oven at 50°C for 2h.

Evaluation of growth media for selective isolation of actinobacteria

Starch-casein medium (Kuester and Williams, 1964), ISP2 medium (International Streptomyces Project) (Shirling and Gottlieb, 1966), Benedict's modification of the Lindenbein medium (Osman and Abou-Zeid, 1968), R2YE medium (Kuznetsova et al., 1988), Gauze's medium (Williams and Cross, 1971), Modified Actinomycete Selective (MAS) DH agar (composition per litre; 1g starch, 0.1g sodium caseinate, 0.1g L-asparagine, 4g sodium propionate, 0.5g dipotassium phosphate,

0.1g potassium nitrate, 0.01g magnesium sulphate, 0.001g ferrous sulphate, 0.02g calcium carbonate, 22g Agar) were used in the present investigation for the selective isolation of thermophilic. Antibiotics kanamycin and cycloheximide were added to the media as selective agents to a final concentration of 25 and 50ug/ml respectively.

Selective isolation, enumeration and maintenance of putative thermophilic actinomycetes

Standard procedures for the selective isolation of actinomycetes were employed in the present investigation (Bredholdt et al., 2007; Okoro et al., 2009). Briefly, thermophilic actinobacteria were isolated by conventional serial dilution technique. After pre-treatment, exactly 1g of pre-treated, air dried, soil sample was taken to prepare dilutions. Aliquot (0.1 ml) from 10^{-4} and 10^{-5} dilutions were spread aseptically with sterile L-shaped glass rod using spread plating technique over the surface of selective isolation media. Inoculated plates were incubated at 45°C for three weeks. The numbers of presumptive culturable actinomycetes were counted and the results expressed as a mean colony forming units (CFUs) per gram dry weight of soil. The presumptive actinomycetes were purified by quadruplicate streak method and the stock cultures were prepared using 20% (w/v) sterile glycerol solution and stored at -20°C.

Primary Screening of antibacterial activity

For primary antibacterial screening, agar plugs (Gavin, 1957) of 6mm in diameter were cut from the 10 days old actinomycete agar culture and plugged into the wells bored using sterile cork borer (diameter of 6 mm) on MAS-DH-1 medium seeded with pathogenic strains in different experiments. The plates were incubated at 45°C for 24-48h and observed for zone of inhibition around the inserted agar plugs.

Secondary screening and analyses of antimicrobial product formation

The isolates exhibiting anti-bacterial activity in the primary screening was subjected to the secondary screening in the agar well diffusion method (Ghorbani-Nasrabadi et al., 2013). The antimicrobial activities were assayed at the interval of two days against *Staphylococcus aureus* (NCIM 2079), *Shigella flexneri* (NCIM 4924), *Escherichia coli* (UTI pathogen), *Klebsiella pneumoniae* (NCIM 2719) by the agar well diffusion method. For fermentation ISP2 medium was used. During the time course of fermentation, antibiotic production at different time points was also monitored by measuring optical densities at 560nm for 12 days at two days interval.

Antifungal activity

The antifungal activity of the isolate D8 (exhibiting broad

spectrum anti-bacterial activity in the secondary screening) was assayed against fungal pathogens *Alternaria* sp. and *Fusarium* sp. by the agar diffusion method utilizing Sabouraud agar medium.

Phenotypic characterization

The microscopic characterization of the presumptive actinomycete isolate D8 was carried out by slide culture method (Bredholdt et al., 2007). The mycelium structure, colour and arrangement of spores on the mycelium were observed through bright-field microscope (100X, Olympus). The cultural characteristic of the strain was studied on various ISP media (Shirling and Gottlieb, 1966).

Screening of D8 isolate for extracellular enzymes

Acid and alkaline phosphatase activities of the isolate D8 was determined as described by Ghorbani-Nasrabadi group (Mahon et al., 2011). The strain D8 was screened for its catalase activity using H₂O₂ (5%) (Sambasiva et al., 2012). Tyrosinase activity was determined in the agar medium containing L-tyrosine (Vishnupriya et al., 2010). Lipase activity of the D8 isolate was assessed in the modified growth medium (Rathnan and Ambili, 2011). Cellulase production was assessed in the agar medium supplemented with carboxymethyl cellulose (0.5%) as the only carbon substrate (Haritha et al., 2010). The protease production was monitored for the D8 isolate on the milk agar medium, containing starch casein provided with 20% of skimmed milk (Jani et al., 2012). The starch medium containing agar was used to detect the amylase activity (Haritha et al., 2010). The production of extracellular L-asparaginase was assessed for the strain D8 by a method described by Gulati group (Gulati et al., 1997).

Assimilation of carbon and nitrogen sources

The utilization of various carbon sources by the D8 isolate was monitored using different carbon sources (1%) i.e., D-xylose, sucrose, rhamnose, fructose, dextrose, lactose, salicin, meso-inositol, ribose, mannose, pectin, galactose, starch, D-glucose arabinose and mannitol prepared in minimal MAS-DH-1 agar medium. The plates were incubated at 45°C for 7 days. Similarly, the utilization of various nitrogen sources (1%) were studied by using alanine, glycine, histidine, methionine, glutamic acid, aspartic acid, proline, tyrosine, isoleucine, cysteine, tryptophane, arginine, asparagine prepared in MAS-DH-1 agar medium. The inoculated plates were incubated at 45°C for 7 days.

Stress tolerance assay

The different concentrations of NaCl (0, 1, 2.5, 5, 7.5, 10%), temperature range from 25-55°C and variable pH range (5, 7, 9 and 11) were selected to test the stress tolerance of the strain D8. The pH of the MAS-DH-1 broth medium was adjusted with 0.1N NaOH/0.1N HCl. The D8 strain was inoculated into the broth medium containing different salt and variable pH in different

assays and incubated at 45°C for 7 days. For temperature tolerance the broth medium inoculated for D8 strain was incubated at 25°C, 35°C, 45°C and 55°C separately. After incubation, the growth of the D8 isolate was observed by spectrophotometer at 560nm.

DNA preparation

The isolate D8 was grown for 7 days at 45°C in the MAS-DH-1 broth medium. Genomic DNA was isolated as described previously (Hopwood et al., 1985).

Phylogenetic assessment

The 16S rDNA of the isolate D8 was amplified using PCR with universal primers 27F 5'AGA GTT TGA TCC TGG CTC AG 3', 1492R GGT TAC CTT GTT ACG ACT T and 1525R 5' AAG GAG GTG ATC CAG CCG CA 3' that will amplify specifically 16SrDNA region (Stackebrandt et al., 1993; Frank et al., 2002). The reaction started with an initial denaturation at 95°C for 5min, followed by 30 cycles of DNA denaturation, at 95°C for 1min, primer-annealing at 50°C for 0.5 min and extension cycle at 72°C for 1.5 min, with a final extension at 72°C for 10 min. PCR-amplicons were visualized in 2% agarose gel electrophoresis and subsequently revealed with ethidium bromide staining. The amplified 16SrDNA gene product was sequenced using Sanger didoxy method. Manual sequence edition, alignment, and contig assembling were performed using Vector NTI v10 software package. Sequencing results were analyzed for chimeras using DECIPHER 1.4.0 program (Wright et al., 2012). The 16S rDNA sequence of D8 was deposited in NCBI and the sequences accession number was obtained. Reference sequences were downloaded from the Genbank Database (<http://www.ncbi.nlm.nih.gov/genbank>). The sequences were aligned by using the multiple sequence alignment program ClustalW. The Neighbor Joining algorithm (Saitou and Nei, 1987) using MEGA software version 6.0 (Tamura et al., 2013) with bootstrap values based on 1000 replications (Felsenstein, 1985) were used to construct a phylogenetic tree. Evolutionary distance matrices were generated as described by Jukes and Cantor (Jukes and Cantor, 1969).

Fermentation, extraction, purification and partial characterization of antibiotic compound

For small scale fermentation the D8 strain was cultured in a 1L flask containing 500mL of ISP2 medium. The fermentation was carried out at 45°C for 8 days at an agitation rate of 120 rpm. A range of extraction solvents was used including n-hexane, benzene, dichloromethane, n-butanol and ethyl acetate. The 500mL of culture broth was centrifuged (3000g, 15 min) and the supernatant was extracted twice with the ethyl acetate. The extract was concentrated to dryness. Chemical screening of partially

purified compound was performed to determine the functional group present in the bioactive compound (Fiedler, 1993; Marston, 2011). To find out the best solvent system to separate the crude compound in TLC, solvents such as methanol, chloroform, acetic acid, n-butanol, n-hexane and water were used in different proportions. In the present work, commercially available readymade TLC sheet (Merck; silica gel 60-F254nm) was spotted with methanolic crude compound. TLC was run using chloroform: methanol (18:2). After running, the sheet was kept at room temperature for complete drying and the separated spot was visualized in UV (254nm) and in iodine vapour. The resolving factor (Rf) value was determined by the following equation: $R_f = \frac{\text{distance traveled by the solute}}{\text{distance traveled by the solvent}}$ (Marston, 2011). For partial characterization and identification of chromophore in the compound, UV-visible absorption spectrum (200-600nm) was recorded on spectrophotometer (Shimadzu).

Results

Evaluation of growth media and isolation of thermophilic actinomycetes

Since selective media has a major influence on the number of isolates obtained, different types of selective media were used in the present study to screen the most preferred medium for the selective isolation of thermophilic actinobacteria from the Thar desert soil. The six growth media were plated with a similar soil sample and compared for the emergence of discrete bacterial colonies after incubation at 45°C. The highest number of presumptive actinomycete colonies was scored with MAS-DH-1 isolation agar medium (Figure 1). The population density of presumptive actinobacterial colonies was recorded to be 40×10^4

cfu gm^{-1} soil for MAS-DH-1 medium followed by 31×10^4 cfu gm^{-1} soil for ISP2 and 30×10^4 cfu gm^{-1} soil for R2YE media. The relative density of presumptive actinomycete population was observed to be lower for Starch-casein medium, Benedict's modification of the Lindenbein medium and Gauze's medium (Figure 1). The MAS-DH-1 medium yielded superior results as shown mainly by its high selectivity for actinobacteria. It should be emphasized here that the selective medium does not have to be one on which the growth of actinobacterial isolate is luxuriant. But it is enough that they grow, be it meagerly, while other culturable eubacteria and fungi do not. It is known that actinomycetes can survive and grow to some extent on very small amounts of nutrients that may be found as impurities in some of the non-nutrient substances such as agar. This is why such a medium as water and agar may also serve sometimes as a medium for the isolation of actinomycetes. The growth of individual colonies of actinomycetes was observed to be better when MAS-DH-1 medium was used, but also utilizing this medium the lowest relative numbers of other eubacteria and fungi were observed. The results suggest that the MAS-DH-1 medium is more advantageously selective for actinomycetes, native to the Thar desert than the five other media used. In addition, the MAS-DH-1 medium permitted the adequate growth of many different types of pigmented actinomycetes from the desert soil sample. An earthy, soil smell, opaque, sometimes chalky appearance in mature colonies, darker in the center and lighter farther from the center, irregular, fuzzy edge, of hyphal appearance, strong adherence to medium, and

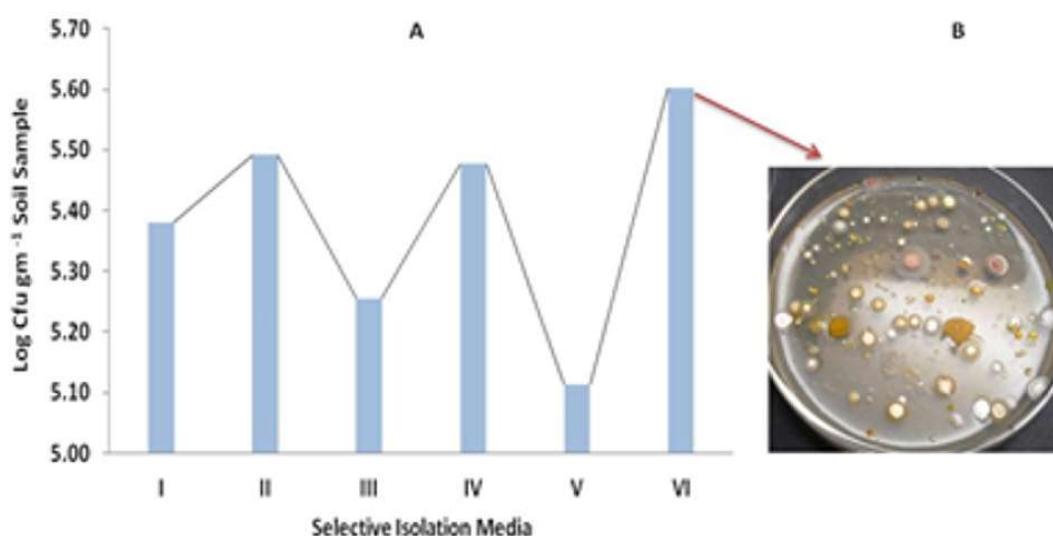


Figure 1. A. Evaluation of six growth media (I) Starch-casein medium, (II) ISP2 medium, (III) Benedict's modification of the Lindenbein medium, (IV) R2YE medium, (V) Gauze's medium, and (VI) Modified Actinomycete Selective-DH-1 medium for the selective isolation of thermophilic actinomycetes. B. Pigmented and non-pigmented presumptive actinomycete isolates on MAS-DH-1 agar medium.

leathery textured colonies were selected for the total count. These isolates were checked for their typical mycelial structure by Gram's staining at 100x oil immersion. The results from the present investigation are also supported by the other reports describing the prevalence of actinomycete in desert eco-system (Badji et al., 2006; Song et al., 2007; Santhanam et al., 2012; Harwani, 2013; Jiao et al., 2018).

Screening for antibacterial activity and antibiotic product formation

Actinomycete isolates were analyzed for their antibacterial activity in primary and secondary screening. Based on the results observed in the primary screening, actinomycete isolates showed inhibition zones for one or more test pathogens were shortlisted for their subsequent secondary screening. Out of

these actinomycete isolates, the strain D8 showed broad spectrum antimicrobial activity with inhibition zone diameter of 24mm against *Shigella flexneri*, 17mm against *E. coli*, 14mm against *Staphylococcus aureus* and 13mm against *Klebsiella pneumoniae* (Figures 2A and 2B). Biological extracts (supernatant) collected from D8 strain exhibited the maximum growth inhibition of the tested pathogens after eight days of incubation and then declined (Figure 2B). Interestingly, the D8 strain also exhibited the growth inhibitory effect against fungal pathogens *Alternaria* sp. and *Fusarium* sp. by producing inhibition zone diameter of 8 and 13mm respectively (Figure 3). The broad spectrum antibacterial and antifungal potential in terms of growth suppression (inhibition zone) of pathogens was observed to be associated only with the D8 strain.

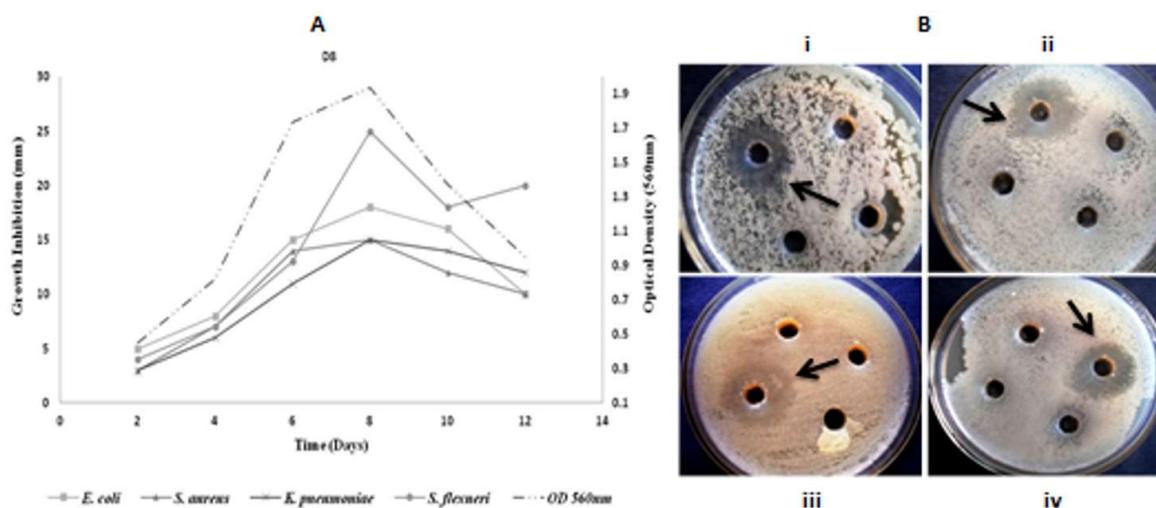


Figure 2. A. Analyses of antimicrobial product formation, optical growth density and growth inhibition of tested pathogens by the D8 strain. B. Secondary screening for the assessment of antibacterial activity of the biological extract from D8 strain by agar well diffusion method against (i) *S. flexneri*, (ii) *E. coli*, (iii) *S. aureus*, (iv) *K. pneumoniae*



Figure 3. Screening of antifungal activity of the biological extract from D8 strain by agar well diffusion method against (i) *Alternaria* sp. and (ii) *Fusarium* sp.

Morphological and physiological characteristics of the strain D8

The strain D8 showed typical mycelial structure in Gram's staining and well developed grey-white aerial and pale-yellow substrate mycelium (Figure 4). The details of the physiological characteristics of the D8 strain have been furnished in Table 1. The strain D8 efficiently utilized galactose, D-xylose, sucrose, rhamnose, fructose, dextrose, lactose, salicin, meso-inositol, ribose, mannose, pectin, starch, D-glucose but was not observed to utilize arabinose and mannitol (Table 1). The strain D8 efficiently utilized various nitrogen sources such as alanine, glycine, histidine, methionine, glutamic acid, aspartic acid, proline, tyrosine, isoleucine, cysteine, tryptophane, arginine, asparagine. The strain D8 exhibited good growth on yeast extract-malt extract-dextrose (ISP-2) agar, starch-inorganic salts (ISP-4) agar and glycerol-asparagine (ISP-5) agar. The growth was observed to be moderate on oat-meal agar (ISP-3) and tyrosine agar (ISP-7) whereas D8 grew poorly on tryptone-yeast extract (ISP-1) agar and starch casein agar (ISP6) (Table 1). The growth of the strain D8 observed in the pH range of 5-11 with growth optima at pH 7. The strain D8 grew well in temperature range of 25-55°C with the optimum growth temperature was observed to be 45°C (Table 1). The strain exhibited salt tolerance up to 7.5% with

Table 1. Physiological characteristics of the D8 strain

Parameters	Variables	Growth
Media	ISP1	+
	ISP2	+++
	ISP3	++
	ISP4	+++
	ISP5	+++
	ISP6	+
	ISP7	++
Carbon Sources	Arabinose	-
	D-Mannitol	-
	Galactose	++
	D-Xylose	++
	Sucrose	++
	Rhamnose	++
	Fructose	++
	Dextrose	++
	Lactose	++
	Salicin	++
	Meso-inositol	++
	Ribose	++
	Mannose	++
	Pectin	++
Starch	++	
D-Glucose	++	

+++ Good, ++ Moderate, + Poor, - Negligible

Table 1. Continue

Nitrogen Sources	Alanine	++
	Glycine	++
	Histidine	++
	Methionine	++
	Glutamic acid	++
	Aspartic acid	++
	Proline	++
	Tyrosine	++
	Isoleucine	++
	Cysteine	++
	Tryptophane	++
	Arginine	++
	Asparagine	++
pH	5	-
	7	+++
	9	++
	11	+
Temperature (°C)	25	+
	35	+++
	45	+++
	55	++
NaCl (%)	0	+
	1	++
	2.5	+++
	5	+
	7.5	+
	10	-
Staining	Gram	+++
	Acid fast	-

+++ Good, ++ Moderate, + Poor, - Negligible

optimum growth at 2.5% NaCl. The results of the screening of the strain D8 for its production of extracellular enzymatic activities revealed that the strain is a good producer of lipase, cellulase, amylase and L-asparaginase enzymes (Table 2). For tyrosinase enzyme the strain D8 was found to be the least producer. The enzymes phosphatase, catalase and protease were noticed to be produced in higher amounts extracellularly in the growth medium by the strain D8 (Table 2).

Table 2. Production of extracellular enzymes by the D8 strain

Enzymes	Activity
Phosphatase	+++
Catalase	+++
Tyrosinase	-
Lipase	++
Cellulase	+
Protease	+++
Amylase	++
L-asparaginase	++

+++ Good, ++ Moderate, + Poor, - Negligible

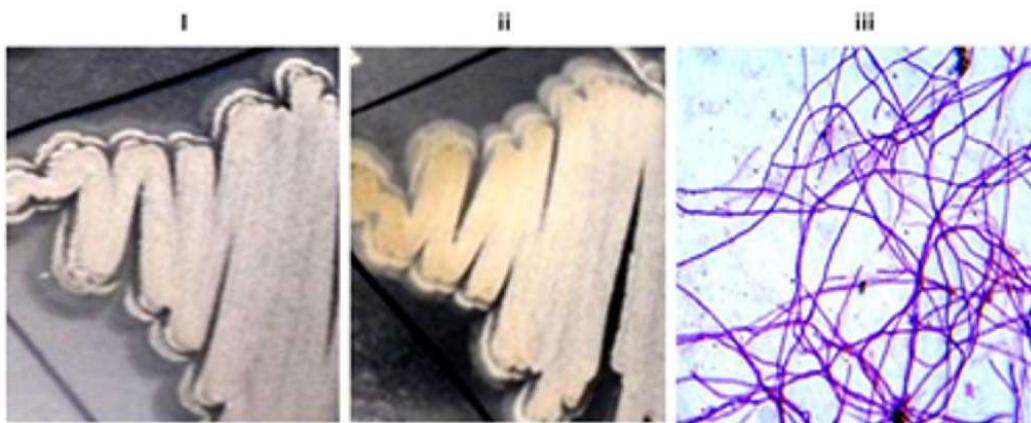


Figure 4. (i) A well developed grey-white aerial (ii) pale-yellow substrate mycelium and (iii) micro-morphological details of mycelial structure of the D8 strain under bright field microscope (1000x)

Molecular characterization of the strain D8

After Sanger sequencing, the 1456bp 16SrDNA sequence of D8 strain was submitted to the GenBank database under an accession number KM205637. The sequence was aligned and compared with the available 16SrDNA gene sequences in the GenBank database by using the multi-sequence advanced BLAST comparison tool. The highest 16SrDNA sequence similarity of 97% of the strain D8 was found to be with *Nocardioopsis* sp. The phylogenetic analysis of the 16SrDNA gene sequence of D8 and 16SDNA gene sequences of its closely related member species (reference sequences) were aligned using ClustalW programme with Molecular Evolutionary

Genetics Analysis (MEGA) 6 to construct the phylogenetic tree using neighbor joining method. Sequence comparison of the strain D8 with its corresponding reference sequences of *Nocardioopsis* strains from the GenBank database showed that the strain formed a close distinct phyletic line with the clade *Nocardioopsis dassonvillei* (Figure 5).

Partial characterization of antibiotic compound from D8

The cell-free fermentation broth (500ml) of D8 strain prepared in the ISP2 medium was extracted by ethyl acetate. The organic phase was concentrated to dryness, recuperated in methanol and chromatographed by TLC. Among the different solvent systems tested in various proportions,

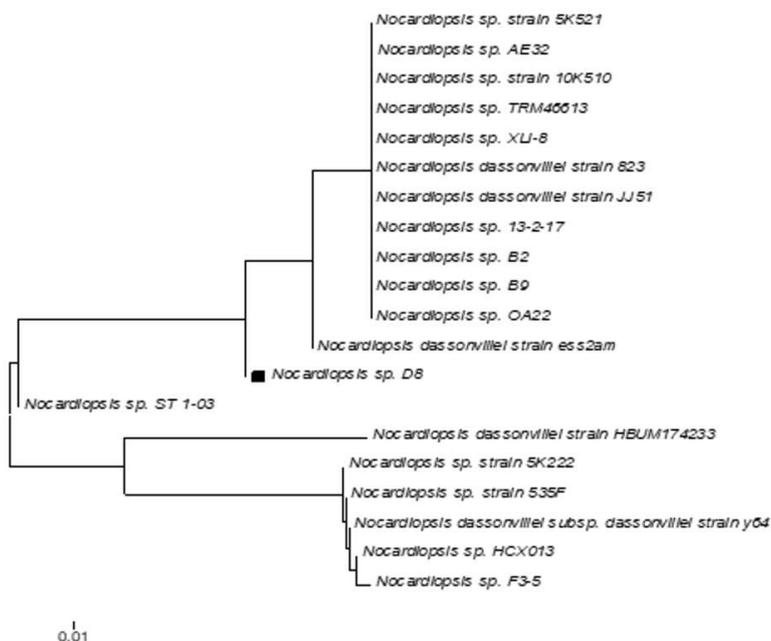


Figure 5. Evolutionary relationship of the D8 strain. The evolutionary history was inferred using the Neighbor-Joining method using MEGA6. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter =1). The analysis involved a total of 20 16SrDNA nucleotide sequences.

chloroform: methanol in 18:2 proportion showed good separation in TLC and allowed to identify one active spot when exposed to UV light (254nm) and iodine spray. The Rf value of the separated spot was calculated to be 0.76. The compound extracted from D8 strain shown UV-VIS spectrum absorbance maxima in ethyl acetate at 272 nm. The antibiotic localized at 0.76 Rf value showed strong antibacterial and antifungal activities.

Conclusion

Considerable progress has been made within the fields of chemical and engineered biosynthesis of antibacterial compounds; nature remains the richest and the versatile source for new antibiotics. The present investigation was focused on the bioprospecting of thermophilic actinomycetes for their bio-active antibacterial substances of therapeutic use from yet unexplored ecosystem. Therefore, in the present study, the soil sample was collected from the Bikaner region of Thar desert in Rajasthan which is known as the tropical desert of Asia that extends to India through Rajasthan and Gujarat and called as the Thar. We have found that the thermophilic actinomycetes have wide distribution in this area and show variation in their population dynamics. Among six tested growth media, MAS-DH-1 agar medium yielded the highest number of presumptive actinobacterial colonies from the Thar desert soil sample. In short, the new recipe described in the present study, facilitates cost and labour efficient, massive isolation of soil actinomycetes. The less complex isolation procedures will contribute in improving our knowledge concerning the occurrence, distribution, ecology, taxonomy and evolution of actinomycetes native to the Thar. The present work clearly establishes the wide diversity and huge occurrence of actinomycetes in the Bikaner desert eco-system. The 16s rRNA gene sequence analysis revealed that the D8 strain belongs to a species of the genus *Nocardopsis*. Further, the strain D8 was observed to efficiently utilize various carbon and nitrogen sources. The D8 strain was also observed to produce various industrially useful extracellular enzymes like lipase, cellulase, tyrosinase, amylase and L-asparaginase enzymes. Interestingly, the strain D8 was found to produce higher amount of extracellular phosphatase, protease and catalase in the growth medium. Chemical characterization of the metabolites produced by the D8 suggested the presence of an aromatic ring compound. The overall results of the present study suggest that the unexplored Bikaner ecosystem of the Thar desert is a valuable source of bio-active actinomycetes. The strain D8 may be considered as a vital source for the isolation of bioactive compound(s) that may be tested further for its screening to procure antibiotics against drug resistant bacterial and fungal pathogens. Additionally, the production of an excess amount of extracellular enzymes is an indicative that the bioprospecting of thermophilic actinomycetes from the Thar desert ecosystem may

lead to the future discovery of novel, stable and useful compounds and enzymes of therapeutic use.

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Conflict of interest

The authors declare no conflict of interest.

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