

## Research article

**Antibacterial activity of aquatic actinomycete, *Actinoplanes digitatis* Dnj-2 (MN567713)**Ali Mohammed Bawazir<sup>1</sup>, M. Jayashankar<sup>2</sup> Manjula Shantaram<sup>3</sup><sup>1</sup>PhD Scholar, <sup>2</sup>Professor, Department of Studies and Research in Microbiology, Jnana Kaveri Post Graduate Centre, Mangalore University, Chikka Aluvara, Kodagu- 571 232, Karnataka, India<sup>3</sup>Professor and PhD Guide, Department of Studies and Research in Biochemistry, Jnana Kaveri Post Graduate Centre, Mangalore University, Chikka Aluvara, Kodagu- 571232, Karnataka, India

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Corresponding author: **Manjula Shantaram**. Email: manjula59@gmail.com**ABSTRACT**

**Introduction and Aim:** There are centers for antibiotics in the nature to treat disease by looking for different sources, for example, actinomycetes, are approximately two-thirds of naturally occurring antibiotics, having much of medical importance, have been isolated from actinomycetes. The aim of the present study was to isolate and to identify the actinomycetes having antagonistic activity.

**Materials and Methods:** An actinomycetes strain isolated from dam soil sample collected from Alnajl dam of Hadhramout Yemen, showed antibacterial activity against selected microbial pathogens. The nutritional requirements and cultural conditions for maximal growth to produce secondary metabolites have been optimized under shake flask conditions. The growth and production of secondary metabolites were maximal with the use of SCA medium supplemented at pH 7.0, and incubation temperature and time of 37°C and 20 days respectively.

**Results:** Based on morphological, biochemical, physiological and phylogenetic characterization, the strain was identified as *Actinoplanes digitatis*Dnj-2 (MN567713). The ethyl acetate extract (1g/mL) obtained from the isolate showed significant antibacterial activity against Gram-negative bacteria- *Escherichia coli* (21 cm) and *Pseudomonas aeruginosa* (29 cm) and Gram-positive bacteria- *Bacillus subtilis* (31 cm) and *Staphylococcus aureus* (22 cm) when compared with penicillin G 10 units.

**Conclusion:** In conclusion, the isolated strain has broad spectrum of antagonistic activity against Gram-positive and Gram-negative bacteria.

**Keywords:** Actinomycetes; *Actinoplanes digitatis*Dnj-2; antibacterial activities.

**INTRODUCTION**

Natural products stay to be the most hopeful source of antibiotics. There are approximately 32,500 natural products reported from microbial sources (1). In addition, at present, two-thirds of natural antibiotics are obtained from actinomycetes and they serve as substitutional sources of biologically active substances (2). Actinomycetes from the genera *Actinoplane*, *Streptomyces*, and *Actinopolyspora* have been reported to produce over 300 broad-spectrum antibiotic substances (3, 4) and representatives of these genera are widely abundant in aquatic ecosystems.

The genus *Actinoplanes* was classified under the group *Actinoplanetes* in Bergey's Manual of Determinative Bacteriology (5). Genera in this group were distinguished from each other based on the shape of the sporangium, number, shape, and arrangement of spores in the sporangium, motility, and flagellation of the spores and other such characters. The isolate was identified as belonging to *Actinoplanes*. Identification of isolate to the species level was done based on the morphology of sporangium, presence or absence of aerial mycelium and assimilation of carbon sources as well as a molecular genome. Species belonging to this genus were identified as *Actinoplanes digitatis*. At the

time of writing report, the genus involves 45 species with validly published names, including as those of late described *Actinoplanes lichenis* (6), *Actinoplanes subglobosus* (7), *Actinoplanes subglobosus* (8), *Actinoplanes ramoplaninifer* (9) and *Actinoplanes sediminis* (10). A study of the morphology, biochemical characters in agreement with 16S rRNA gene sequence-based phylogenetic analysis, showed later that a member of a second species of the genus *Actinoplanes* related to this strain had been found. This strain has been described previously as *Actinoplanes digitatis* (11).

The Hadhramout-Yemen has not been studied extensively with respect to the antagonistic properties of actinomycetes. In the course of our screening program for new antagonistic actinomycetes, the strain, *Actinoplanes digitatis*Dnj-2 (MN567713) was isolated from the soil sample collected from Rahbat bin Junaid Hadhramout Yemen capable of producing antibiotics that strongly inhibit the growth of Gram-positive and Gram-negative bacteria. In the present study, we have reported the antimicrobial activity of *Actinoplanes digitatis*Dnj-2 (MN567713).

**MATERIALS AND METHODS**

This study was carried out during December 2018 to June 2019 in the Jnana Kaveri Post Graduate Centre,

Mangalore University, Chikka Aluvara, Kodagu, Karnataka, India.

### Microorganisms and culture conditions

The strain Dnj-2 was isolated from a soil sample collected from Alnajl dam, Hadhramout –Yemen. It was subjected to serial dilution (up to  $10^6$  dilution) by adding 1 g of the soil sample in 10 mL of distilled water. About 1.0ml of diluted sample plated on starch casein agar containing 10 g starch, 1.0 g casein, 0.2 g calcium carbonate, 0.01 ferrous sulfate (anhydrous), 2.0g potassium nitrate, and 20g agar in distilled water at pH 7.2, incubated at 37°C for two weeks (12).

### Cultural and morphological characterization

Cultural characteristics of strain Dnj-2 were compared on the base of observations made after 14 days incubation starch casein agar, nutrient agar sabouraud dextrose agar, yeast malt extract agar, glycerol asparagines agar, tyrosine agar, Czapeck's dox thom agar, glycerol yeast extract agar and starch yeast extract agar (13). Morphology was examined by light microscope.

### Physiological characterization

The utilization of carbohydrate was investigated with a basal carbon nutrient medium (13, 14). Methods and media used for physiological tests were as described by Williams *et al.*, (15). All cultures were incubated at 37°C for 10 days. The assay for enzymatic activity was performed (16-18).

### Genotypic characterization

DNA was isolated from Actinoplane strains using the method of Healy and Lambert (19). DNA extraction, PCR amplification, Gel verification, purification, sequencing, assembling, phylogenetic tree.

### Fermentation and preparation of crude extract

The actinomycetes are inoculated on starch casein agar plates and incubated at 37°C 7 days. The spores were harvested in sterile 0.01% (v/v) Tween-20 in distilled water. 300 ml SCB medium was inoculated with 10%

(v/v) spore's suspension of actinomycetes, the flasks incubated at 37°C on rotary incubator shaker, at 200 rpm for 14 days, and OD of the growth was recorded every day at  $A_{540}$ . Fermentation broth was centrifuged at 10,000 rpm for 20 minutes to separate the mycelial biomass. Ethyl acetate was added to the supernatant for extraction of antibiotics, in 1:1 proportion. Solvent-supernatant mixture was agitated for 45 minutes with homogenizer. Solvent was separated from broth by separating funnel. Solvent was centrifuged at 5,000 rpm for 10 minutes to remove traces of fermentation broth and crude antibiotic stored in Eppendorf tubes at 4°C (20).

### Microbial pathogens

Bacterial pathogens, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* were used.

### Method of antimicrobial activity

The antibacterial activity of secondary metabolites (1g/ml) extracted by the ethyl acetate solvent was tested by the agar diffusion method. The plates were incubated at 37°C for 24 h during which activity was evidenced by the presence of a zone of inhibition surrounding the well. Each test was repeated three times and the antibacterial activity was expressed as the mean of a diameter of the inhibition zones (mm) produced by the secondary metabolite when compared to controls.

## RESULTS AND DISCUSSION

### Characterization of *Actinoplanes digitatis* Dnj2 isolates

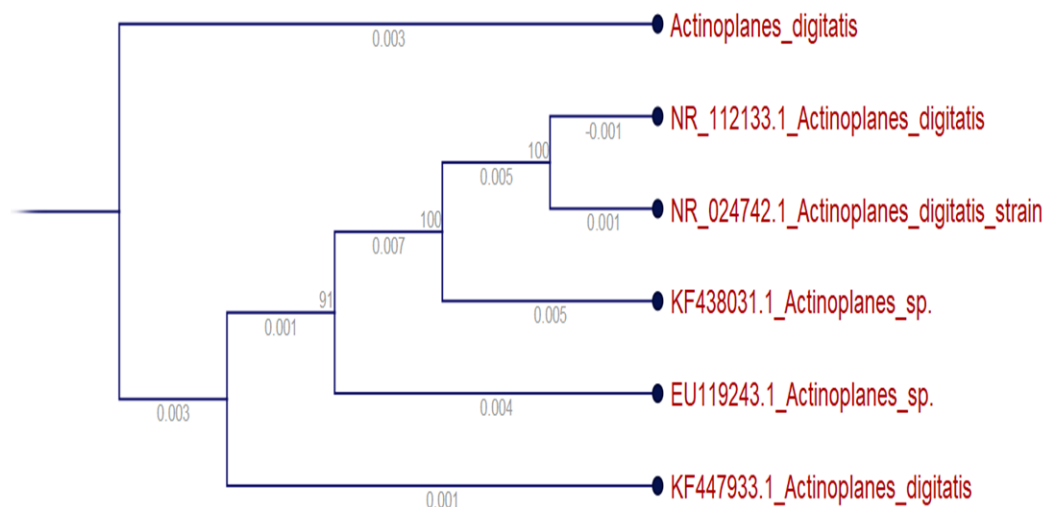
The strain Dnj-2 was isolated from Alnajl dam on SCA. The early work had shown that the morphological and physiological characteristics of the strain Dnj-2 was observed using agar cultures incubated for 21 days at 37°C on the various media described by Shirling and Gottlieb (13): YMA, GAA and TA, but we added various media as SCA, PDA, NA, SYA, and GYA (Table 1).

**Table 1:** Growth and cultural characteristics of the strain Dnj-2 on different media

Culture medium	growth	Arial mycelium	Substrate mycelium	Soluble pigments
Starch casein agar	Good	White	Melon yellow	None
Nutrient agar	Moderate	None	Cream	None
Sabouraud dextrose agar	Good	White	Yellow	None
Yeast malt extract agar	Good	White	Brown	brownish
Glycerol asparagine agar	Moderate	Yellow	Beige	None
Tyrosine agar	Moderate	None	Yellow orange	None
Czapeck's dox thom agar	Moderate	Golden yellow	Yellow	None
Starch yeast extract agar	Good	White	Golden yellow	None
Glycerol yeast extract agar	Good	White	Cream	None

The nucleotide sequence of the 16S rRNA gene of strain Dnj-2 showed 99.59% similarity to *Actinoplanes digitatis*. The phylogenetic tree (Fig.1) from representative strains of the related species indicated that strain Dnj-2 should be placed in the *Actinoplanes digitatis*. During comparison of 16S rRNA gene

sequences, Dnj-2 was mostly related to *Actinoplanes digitatis* strain OKG1 (KF447933.1) (99.59%), *Actinoplanes digitatis* strain JCM 3060(NR\_112133.1) (97.96%), and *Actinoplanes digitatis* strain IFO 12512 (NR\_024742.1) (98.04%).



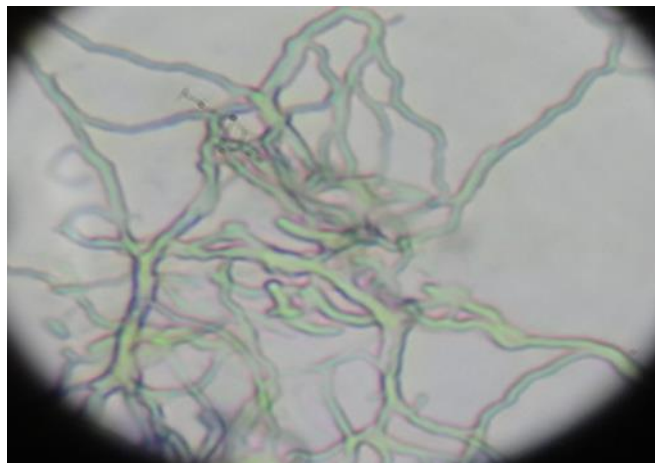
**Fig. 1:** Phylogenetic tree based on the 16s rRNA sequence Dnj-2.

By comparing the results between strain Dnj-2 and closely related species of the genus, *Actinoplanes* revealed that it is similar to *Actinoplanes digitatis* strain OKG1 (KF447933.1) in morphological, cultural, and physiological characteristics (table 2). The aerial mycelium of strain Dnj-2 was initially white and become orange brown on CDTA, produce diffusible pigment as brownish in YMA. A similar pattern of results was obtained in the aerial mycelium of *Actinoplanes digitatis* as white and soluble pigments were produced. Overall, these findings are in accordance with findings reported by Stackebrandt and Kroppenstedt (6). Notably, all prior carbon source utilization was determined by the methods described by Shirling and Gottlieb (13) and Locci (21). Nitrogen source utilization was determined according to Williams *et al.*, (22). In line with previous studies, our results were casting a new light on carbon sources. Therefore, the Dnj-2 strain of *Actinoplanes* has the same physiological characters such as the ability to utilize

sole carbon and nitrogen sources: D- glucose, D- mannose, L- arabinose, glycerol, D- maltose, sucrose, and lactose but not sodium acetate and sucrose (sole carbon sources) and glycerol, alanine and L-asparagine (sole nitrogen sources). Growth of strain Dnj-2 was observed at a wide range of temperatures (20-45°C), although the optimal temperature range was 37°C. The initial pH range for which growth of strain Dnj-2 was observed between pH 7-10; however, the optimal pH value for growth was determined to be 7.0. Strain Dnj-2 was also capable of growth in the presence of 4% NaCl. In addition, the strain Dnj-2 reduced nitrate to nitrite and hydrogen sulfide was not produced. Starch, casein, gelatin, starch, chitin, lecithin, pectin and urea were degraded by Dnj-2 but not, keratin and lipid. Based on the genotypic and phenotypic evidence, it is suggested that strain Dnj-2 is a species of the genus *Actinoplanes*, for which the name is *Actinoplanes digitatis* Dnj2 under accession number MN567713.

**Table 2:** Biochemical characterization of actinomycetes isolates

Physiological test	Result	Enzyme test	Result	Carbon source	Result	Nitrogen source	Result
Gram stain	+	Amylase	+	D Glucose	+	Alanine	+
Motility	+	Caseinase	+	Citric acid	-	Glycine	+
Colony shape	Irregular			D Fructose	+	L Tyrosine	+
Spores	Single	Chitinase	+	Lactic acid	+	L Asparagine	+
Starch hydrolysis	+	Gelatinase	+	D Mannitol	+	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	+
No <sub>3</sub> reduction	+	Keratinase	—	L Arabinose	+	Sodium nitrate	+
H <sub>2</sub> S production	+	Lecithinase	+	Sucrose	+		
NaCl tolerance	2%	Lipase	—	D Mannose	+		
Growth temperature	20-45°C	Pectinase	+	Dextrose	+		
pH range	7-10	Urease	+	Lactose	+		

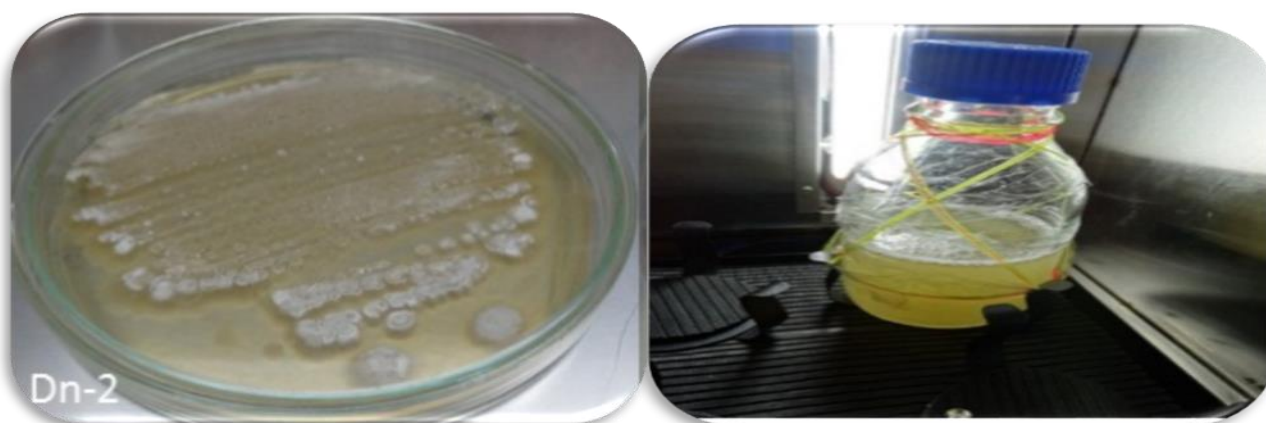


**Plate 1 :** Microscopic view of spore morphology of *Actinoplanes digitatis*Dnj-2 (MN567713)

### The antibacterial activity

Growth of Dnj-2 isolate on SCB media to produce antibacterial antibiotics was carried out in a 300ml flask. The color of growth media was observed in plate no.2. The isolates Dnj-2 produced antibacterial antibiotic and green yellow pigment. This color was due to antibacterial compound. This antibacterial gave maximum zone of inhibition for *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*. The ethyl acetate (1g/ml) obtained from the isolate showed significant

antimicrobial activity against selected Gram -negative bacterial pathogens, *E. coli* (21mm), *P. aeruginosa* (29mm), and Gram -positive bacteria *B. subtilis* (31mm) and *S. aureus* (22mm) when compared with the standard, penicillin. The antagonistic secondary metabolites produced by this *Actinoplanes digitatis*Dnj-2 (MN567713) needs to be studied further to identify its chemical nature and characterization of its biological activity.



**Plate 2:** The growth and fermentation culture of DNJ-2

**Table 3:** Extraction of antibacterial antibiotics of actinomycetes isolates grow in SCB at pH 7.0 and 37°C for 21 days at 200 rpm

Isolate name	SCB (ml)	Biomass dry weight (mg)	Extract solvent	Supernatant Color	Crude antibiotic (mg)	Color of antibiotic residue	Antibiotic zone (mm)			
							<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>E. coli</i>
<i>Actinoplanes digitatis</i> Dnj-2	300	450	Extract solvent	Light green	50mg	Light yellow	31	29	22	21

### CONCLUSION

Our studies will establish the rich bioactive actinomycete of the Hadhramout region. Therefore, further intensive studies are required on the actinobacterial diversity of bioactive compounds in Hadhramout- Yemen, which could form an important input into pharmaceutical industries.

### ACKNOWLEDGMENT

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### CONFLICT OF INTEREST

Authors declare no conflict of interest.

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