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**SHORT COMMUNICATION**

# *IN VITRO* ANTIMICROBIAL POTENTIAL AND GROWTH CHARACTERISTICS OF *NOCARDIOPSIS* SP.JAJ16 ISOLATED FROM CRYSTALLIZER POND

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**ABSTRACT**

A valuable Actinomycete strain with antagonistic activity was isolated, identified and confirmed as Nocadiopsis sp. based on the 16S rRNA sequence analysis and biochemical examinations. Strain JAJ16 was subjected to cultural characterization with respect to aerial mycelium color, diffusible pigments production and the growth characteristics on different media. Extensive growth with bactericidal compound secretion was observed in ISP4 with 5 % (w/v) NaCl. Antimicrobial potential was screened against a list of bacteria and fungi. The strain JAJ16 showed good antimicrobial activity with significant minimum inhibitory concentration (MIC).



Actinomycetes are common soil inhabitants with an unprecedented ability to produce novel microbial products exhibiting antimicrobial, antiviral as well as antitumor properties. About 60% of the world's antibiotics were secreted by actinomyces (Liu Zh, 2002; Liu Zh and Jiang 2004). Halophilic actinomycetes are a type of extremophiles, classified as halophilic archaea, moderately halophilic bacteria and halotolerant bacteria according to their tolerance to NaCl concentration. Studies on alkaliphilic and alkali-tolerant actinomycetes found several antibiotics and enzymes (Mikami, 1982; Sato et al., 1983 and Mikami et al., 1985). Recent reports have revealed that salt requiring marine actinomycetes are a robust source of new natural products and serve as model systems in drug discovery (Marderosian, 1969; Molinski, 1993; Bernan et al., 1997; Fenical, 1997). This research communication deals with characteristics and antimicrobial activities of moderately halophilic *Nocardiopsis* species JAJ16 derived from saltpan soil.

The strain JAJ16 was isolated and cultured on a medium (modified from ISP4 medium) consisted of 1% soluble starch, 0.3% $CaCO_3$, 0.1% $K_2HPO_4$, 0.1% $MgSO_4$, 0.2% $(NH_4)_2SO_4$, 3% NaCl, 100% distilled water and 2% agar. The isolate was preliminarily identified according to traditional biochemical tests and morphological criteria, including utilization of various carbon sources, characteristics of colonies on the plate, morphology of substrate and aerial hyphae, morphology of spores and pigment produced (Good fellow and Cross, 1984). The isolate was identified and confirmed as *Nocadiopsis* sp. JAJ16 based on the 16S rRNA gene sequence analysis using BLAST sequence matching and bioinformatics tools such as RDP and PHYLIP.

Growth characteristics of isolate were recorded using different types of media, such as yeast malt extract agar (ISP2), oat meal agar (ISP3), inorganic salt - starch agar (ISP4), glycerol-asparagine agar (ISP5), tyrosine agar (ISP7), starch-asparagine agar, starch casein agar, glucose nitrate agar and glycerol yeast extract agar according to standard methods described for actinomycetes (Shirling and Gottlieb, 1966; Tresner et al., 1968 and Jensen et al., 1996). Each medium was supplemented with 3 to 8% (w/v) NaCl. The strain JAJ16 showed good growth on ISP4 and ISP5 containing 3 to 8% (w/v) NaCl, and hence the strain was considered as a moderately halophilic species. The aerial mycelium of JAJ16 was found to be white in colour on most of agar plates with the colony diameter of 5 to 7 mm. The substrate mycelium was gray to yellow in colour. The growth was found to take place in the temperature range of 15 to 45ºC and 29ºC was found to be optimum for growth. The strain was grown on ISP4 at different pH values such as 3.5, 5, 6, 7.5, 8 and 9 for 8 to 10 days, and pH 7.5 was found to be optimum for growth. The ability of the isolate to utilize various carbon sources were studied on carbon utilization agar (ISP 9) supplemented with 1% carbon sources (Nonomura, 1974). Strain JAJ16 utilized starch, sucrose, dextrose, mannitol

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**Table 1. Growth and biochemical characteristics of *Nocardiopsis sp. JAJ16***

| **Properties** | **Nocardiopsis sp. JAJ16** | | |
|---|---|---|---|
| **Growth Characteristics on*** | **Growth** | **Aerial mycelium** | **Pigment** |
| Yeast malt extract agar (ISP2) | Moderate | White | - |
| Oat meal agar (ISP3) | Moderate | White | - |
| Inorganic salt –starch agar (ISP4) | Abundant | Gray | Yellow |
| Glycerol-asparagine agar (ISP5) | Good | White | - |
| Tyrosine agar (ISP7) | Good | White | - |
| Starch-asparagine agar | Good | White | - |
| Starch casein Agar | Abundant | Gray | yellow |
| Glucose nitrate agar | Good | Gray | - |
| Glycerol yeast extract agar | Good | Gray | - |
| **Growth at** | **Positive /Negative** | | |
| 0% NaCl | – | | |
| 2% NaCl | + | | |
| 5% NaCl | ++ | | |
| 8% NaCl | ++ | | |
| 10% NaCl | – | | |
| 15% NaCl | – | | |
| **Growth at** | **Positive /Negative** | | |
| pH 3.5 | – | | |
| pH 5 | – | | |
| pH 6 | + | | |
| pH 7.5 | ++ | | |
| pH 8 | + | | |
| pH 9 | + | | |
| **Carbon source utilization**** | **Positive /Negative** | | |
| Starch | ++ | | |
| Sucrose | + | | |
| Dextrose | ++ | | |
| Mannitol | + | | |
| Raffinose | – | | |
| D-Xylose | – | | |
| D-Arabinose | – | | |
| L-Rhamnose | – | | |
| Glycerol | ++ | | |
| D-Mannose | – | | |
| **Nitrogen source utilization**** | **Positive /Negative** | | |
| Glutamic acid | ++ | | |
| Leucine | ++ | | |
| Methionine | ++ | | |
| Histidine | + | | |
| Serine | + | | |
| Tryptophan | + | | |

*++, Strongly positive utilization; +, Positive utilization; –, Utilization negative*
**Each medium was prepared in distilled water containing 3% to 8% (w/v) NaCl.*
***Carbon utilization and nitrogen utilization were recorded on carbon utilization agar (ISP 9) with 1% (w/v) carbon or nitrogen source at 29ºC temperature and observed after 12 days*

**Table 2. Antimicrobial activity of *Nocardiopsis* sp.JAJ16**

| **Test Organisms** | Zone of inhibition (mm)* | MIC ($\mu g\ Ml^{-1}$)* |
|---|---|---|
| **Bacteria** | | |
| *Staphylococcus aureus* | 31 | 0.13 |
| *Bacillus subtilis* | 32 | 0.72 |
| *Salmonela typhi* | 30 | 0.70 |
| MRSA | 32 | *0.72* |
| *Klebsiella pneumoniae* | 19 | 1.25 |
| *Pseudomonas aeruginosa* | 18 | 1.19 |
| *Enterobacter* sp. | 17 | 1.15 |
| **Fungi** | | |
| *Candida albicans* | 15 | 1.35 |
| *Fusarium oxysporum* | 17 | 1.15 |
| *Aspergillus flavus* | 13 | 1.62 |

*The values shown are mean value of three replicates

and glycerol as a carbon source for growth; however, raffinose, D-xylose, D-arabinose, L-rhamnose and D-mannose were not utilized. Glutamic acid, Leucine, Methionine, Histidine, Serine, and Tryptophan were used as a nitrogen source for growth. Production of enzymes such as amylase, protease and lipase was also recorded. The growth and biochemical characteristics of *Nocardiopsis* sp. JAJ16 were summarized in Table 1.

JAJ16 was screened for antimicrobial activity by agar plug method (Shomura et al., 1979) The strain was grown on modified ISP4 agar media at 28ºC for 10 days and then agar plugs were cut out and transferred onto plates containing test organisms. The plates were incubated at 37ºC for 24 h for bacteria and at 28ºC for 4 days for fungi, and the zone of inhibition around the isolates were observed. JAJ16 showed good antibacterial activity against bacteria such as *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi*, Methicillin-resistant *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterobacter* sp. and *Pseudomonas aeruginosa.* The antifungal activity was observed against fungi such as *Candida albicans*, *Aspergillus flavus* and *Fusarium oxysporum*. Diameter of zone of inhibition varied from 15 to 32 mm (Table 2), highest activity was observed against *Bacillus subtilis* and Methicillin-resistant *Staphylococcus aureus* (MRSA) and lowest activity was observed against a fungi *Aspergillus flavus.* Antimicrobial activity of the strain JAJ16 against MRSA was exhibited in Figure 1.

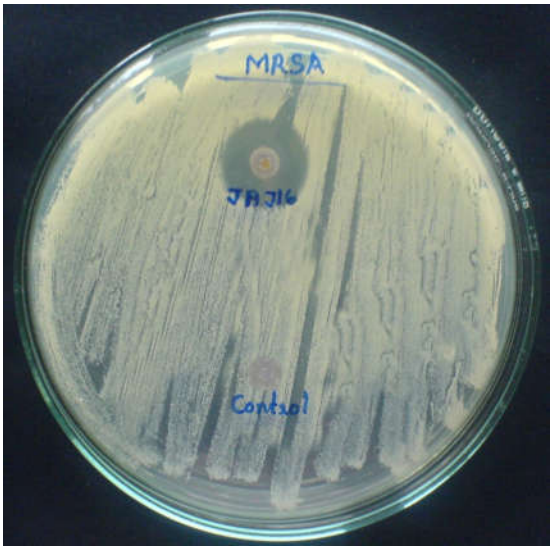


**Figure 1. Bioassay plate showing antibacterial activity of Strain JAJ16 against MRSA by agar plug method**

Seed culture of strain JAJ16 was prepared by growing the antagonistic strain in 50 ml of production medium containing 10g soluble starch, 3g $CaCO_3$, 1g $K_2HPO_4$, 1g $MgSO_4$, 2g $(NH_4)_2SO_4$, 30g NaCl and 1000ml distilled water at 29ºC for 10 days. The seed culture was then transferred to a flask containing 1000ml of production medium. After the incubation, the culture broth was collected and the bioactive compounds were extracted using equal volume of ethyl acetate. Then the extract was screened for antimicrobial activity. Thus, the isolated actinomycete was a moderately halophilic *Nocardiopsis* species exhibited significant antibacterial activity.

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