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Preliminary screening of Ni(II) metal tolerance and dye-decolorizing by *Nocardiopsis* sp. SD8

Ramasamy Thangaraj[1], Saha Subhasish[2], Dharumadurai Dhanasekaran[1], Nooruddin Thajuddin[1*]

[1]*School of Life Science, Department of Microbiology, Bharathidasan University, Tiruchirappalli-620 024, Tamil Nadu, India*

[2]*Key Laboratory of Marine Bio-resources Sustainable Utilization, Center for Marine Microbiology, Research Network for Applied Microbiology, Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Rd., Guangzhou 510301, China*

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ABSTRACT

Objective: To reveal the screening of metal tolerance and dye-decolorizing of *Nocardiopsis* sp.
Methods: $NiSO_4$ and Congo red dye were used for evaluating the metal tolerance and dye-decolorizing of the randomly selected actinobacterial isolates.
Results: *Nocardiopsis* sp. SD8 showed a better efficiency in Ni(II) tolerance, though a longer lag phase was observed for this microorganism grown for 7 days in integrated mismatch negativity. Interestingly, we also found that *Nocardiopsis* sp. SD8 had dye-decolorizing, hemolytic, lipase and protease activity.
Conclusions: The present results revealed the bioremediation of metal resistant and diverse properties of *Nocardiopsis* sp. SD8 and further investigations are needed to extract and identify the potent molecule.

1. Introduction

Several industrial activities including electrolytic treatment, ceramic production, fertilizer production and pigments production can create severe heavy metal pollution. They have high mobility in aquatic systems and in general may produce high toxicity[1]. The use of conventional technologies, such as ion exchange, chemical precipitation, reverse osmosis and evaporative recovery, for this purpose is often inefficient and/or very expensive.

For the last decades, research in the area of biodegradation of metals and azo dyes has expanded in recent years due to its potential use in different areas. The development of this line of research is of vital importance, mainly in view of the present concern regarding the protection of the environment. Actinobacteria are exciting structures inhabiting almost all possible niches[2]. They are filamentous in nature and are considered as an intermediate group between bacteria and fungi[3]. Screening and isolation of promising actinobacteria with potential antibiotics are still a thrust area of research and it is suggested that the exploration of materials from new areas and habitats has a vital role to play in the search for new microbes and novel metabolites and is urgent to counter the threats posed by the fast emerging phenomenon of antibiotic resistance. Many microorganisms show adaptation to the toxic materials constantly released into their environment. They have developed strategies to resist, tolerate, metabolize and to detoxify these toxic substances. They also play important role in mineralization process in nature. Due to their ability of producing bioactive compounds, their role in bioremediation of harmful dye and heavy metals and their role in soil fertility, actinobacteria group is the subject of interest for scientists[4,5].

Ni(II) is more toxic and carcinogenic metal when compared with Ni(IV). Due to their toxic effects on living systems stringent limits have been stipulated for the discharge of nickel into the environment. According to Indian Standards Institution: Bureau of Indian Standard, the industrial effluent permissible discharge level of Ni(II) into inland water is 0.1 and 3.0 mg/L. Among various metal ions, Pb, Hg, Cd, Ni(II) and Cr(VI) are at the top of the toxicity list. International Agency for Research on Cancer has identified Ni as one of the three metals established to be a human carcinogen[6].

Azo dyes are the predominant class of dyes that is extensively used in textile, food, paper, leather and cosmetics industries. These sulphonated azo dyes are not only toxic in excessive quantities but also be carcinogenic. Releases of these dyes are of major concern since they cause a serious health hazards to humans and animals. Amoroso *et al*. have reported that metal resistance and biosorption capability may be widespread among actinobacteria growing in contaminated environments[7]. Metal-resistant actinobacteria, and their potential use for bioremediation strategies, have been

*Corresponding author: Nooruddin Thajuddin, Department of Microbiology, Bharathidasan University, Tiruchirappalli-620024, Tamil Nadu, India.
Tel: 0431 2407082, 98423 79719
Fax: 0431 2407045
E-mail: thajuddin@gmail.com
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described[8-10]. Richards *et al*. have studied the heavy-metal resistance patterns of *Frankia* isolates[11]. Ni-resistant actinobacteria were isolated from U mining areas where a high level of Ni co-contamination exists[12]. The rate of discovery of new compounds from terrestrial actinobacteria has decreased, whereas the rate of reisolation of known compounds has increased. Our search for a potential was led to the *Nocardiopsis* sp. SD8 with diverse properties.

To our best knowledge, this is the first report for metal tolerance and dye-decolorizing activity of *Nocardiopsis* sp. The present study was undertaken with the view of exploring actinobacteria to address these environmental problems. Hence, in the present study, the actinobacteria were maintained aseptically under laboratory conditions, and were evaluated for their efficiency as potential dye-decolorizing and for their ability to bio-remediate Ni(II).

2. Materials and methods

2.1. Cleaning of glasswares

Clean Borosil glasswares were used. They were soaked in tap water for few minutes and thoroughly washed in tap water. They were soaked in dichromate solution for few hours to remove tough residues. Then, they were again washed in tap water.

2.2. Sterilization of glasswares and chemicals

All types of glassware such as conical flask, Petri plates, test tubes, pipettes and starch casein agar were sterilized at 121 ˚C for 15 min for 15 pounds pressure in autoclave.

2.3. Collection of actinobacterial isolates

Among 25 actinobacterial isolates, only five isolates were morphologically identified and molecularly characterized by Saha *et al*. and Saha and Dhanasekaran. and other 20 unidentified isolates were collected from Germplasm, Department of Microbiology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India[13,14]. These isolates were randomly selected and used for this study.

2.4. Screening of Ni(II) metal tolerance in actinobacterial isolates

$NiSO_4$ was used for evaluating the heavy metal tolerance of the isolates. Primary qualitative screening assay was carried out in plates containing starch casein agar supplemented with $NiSO_4$. Resistant isolates that were capable of growing up to the highest concentrations were amended with higher $NiSO_4$ concentrations. In this case, 5, 10 and 15 mg/1 000 L were made in Petri dishes with various concentrations of $NiSO_4$, then isolates were inoculated by streaking. Actinobacterial growth was used as the qualitative parameter of metal tolerance. After the incubation at 40 ˚C for 7 days, plates were observed for Ni tolerance.

2.5. Screening of dye-decolorizing ability of actinobacterial isolates

Congo red was used for evaluating the dye-decolorizing activity of the isolates. Primary qualitative screening assay was carried out in the plates containing filtered and sterilized congored dye. Decolorizing isolates that were capable of decolorizing the Congo red dye were supplemented with starch casein agar medium. Zone of clearance were indicated around the isolates decolorized Congo red dye. After the incubation at 40 ˚C for 7 days, plates were observed for zone of clearance for dye decolorization.

2.6. Screening of diverse properties of Nocardiospsis sp. SD8

2.6.1. Hemolytic activity

Hemolysis was carried out using blood agar plate and it was prepared by adding sheep blood (5%) to blood agar base. The purified cultures were inoculated and the blood agar plates were incubated at 40 ˚C for 7 days. The plates were then examined for zone of clearance around the colonies.

2.6.2. Lipase activity

The screening of actinobacterial isolates for lipase activity was studied by following the method of Gandhimathi *et al*. with slight modification[15]. Lipase activity by actinobacterial isolates was screened using tributyrin agar plates by adding 1% tributyrin to starch casein agar. The plates were incubated at 28 ˚C for 3–7 days. After incubation, the plates were examined for the formation of clear zone around the colonies.

2.6.3. Protease activity

Protease activity was carried out using skim milk agar. The purified cultures were inoculated and the agar plates were incubated at 40 ˚C for 7 days. The plates were then examined for zone of clearance around the colonies.

3. Results

Our research was focused to identify a novel *Nocardiopsis* sp. SD8, which had diverse properties.

3.1. Screening of potent Ni(II) metal tolerance isolate

Selected isolates were screened for Ni(II) metal tolerance ability and primary screening method of Ni(II) metal tolerance study was carried out using Ni(II) concentration 5 mg/L supplemented with starch casein agar. Out of them, only five isolates including *Nocardiopsis* sp. SD5, *Nocardiopsis* sp. SD6, *Nocardiopsis* sp. SD7, *Nocardiopsis* sp. SD8 and *Streptosporangium* sp. SD9 exhibited heavy metal tolerance and their growth was observed at that concentration (Figure 1). *Nocardiopsis* sp. SD5, *Nocardiopsis* sp. SD6, *Nocardiopsis* sp. SD7 and *Streptosporangium* sp. SD9 showed less metal tolerance of Ni(II). In secondary screening of Ni(II) metal tolerance ability for tolerance level of potent isolate at the highest concentration, we prepared different concentrations of Ni(II) 5, 10 and 15 mg/L supplemented with starch casein agar. Isolates of *Nocardiopsis* sp. SD8 which had high tolerance level (up to 15 mg/L) was occurred (Figure 2). *Nocardiopsis* sp. SD8 showed potent candidate for Ni(II) metal tolerance ability.

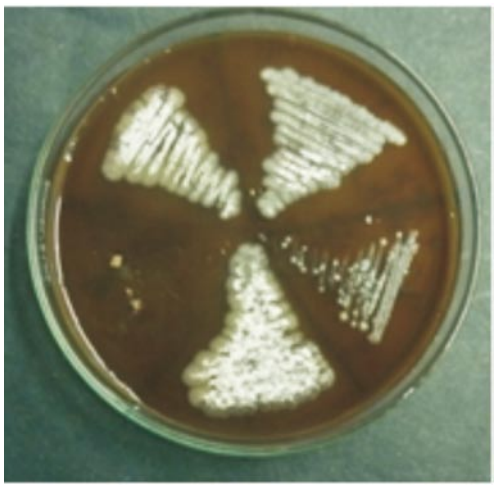

Figure 1. Preliminary screening of Ni(II) metal tolerance of actinobacterial isolates.

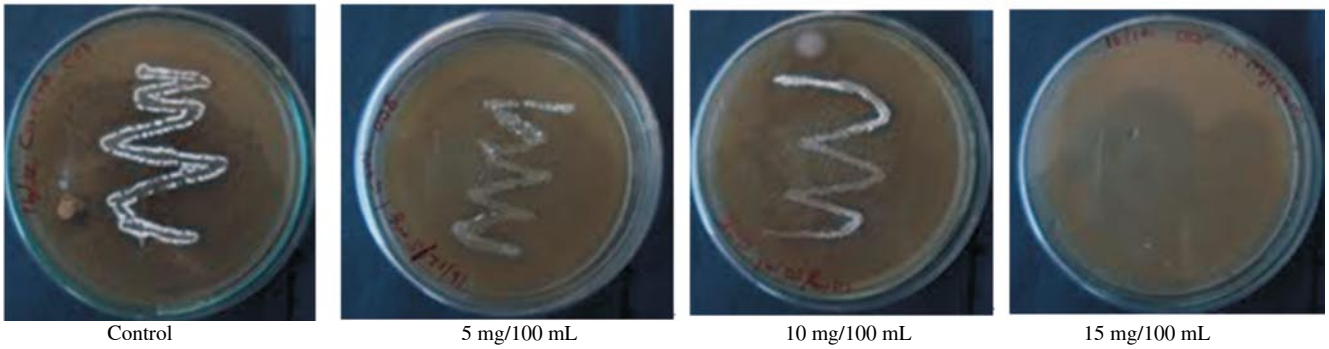

Figure 2. Secondary screening of *Nocardiopsis* sp. SD8 tolerance at different concetraitons of Ni(II) metal.

3.2. Dye-decolorizing activity

Out of 25 isolates, four isolates (*Nocardiopsis* sp. SD5, *Nocardiopsis* sp. SD6, *Nocardiopsis* sp. SD7, *Nocardiopsis* sp. SD8) had dye-decolorizing ability. *Nocardiopsis* sp. SD8 showed significantly decolorizing-dye indicated by zone of clearance around the colonies. *Nocardiopsis* sp. SD8 showed the zone of clearance up to 50 mg/L at the highest concentration of Congo red (Figure 3).

Figure 3. Dye-decolorizing activity of *Nocordiopsis* sp. SD8.

3.3. Screening of diverse properties of Nocardiospsis sp. SD8

3.3.1. Haemolytic activity

We also found that *Nocardiopsis* sp. SD8 had haemolytic activity tested by sheep blood agar medium prepared 5% blood with blood base agar medium. *Nocardiopsis* sp. SD8 of β-haemolysis showed maximum haemolytic activity (23 mm) (Figure 4).

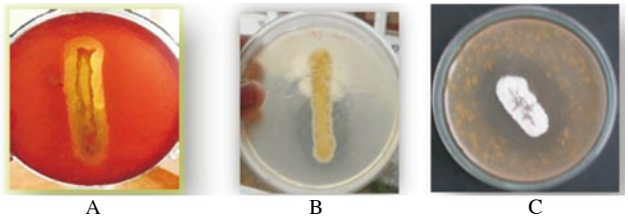

Figure 4. Hemolysis (A), lipase (B) and protease (C) activity showed by clear region around the growth of the *Nocardiopsis* sp. SD8.

3.3.2. Lipase activity

Nocardiopsis sp. SD8 was screened for lipase activity on tribuytrin agar plate. *Nocardiopsis* sp. SD8 was significantly produced lipase activity. The zone of clearance around the colonies indicated the organisms which were responsible for hydrolysis of the lipid molecule (Figure 4).

3.3.3. Protease activity

Nocardiopsis sp. SD8 was screened for protease activity on skim milk agar plate. *Nocardiopsis* sp. SD8 was significantly produced protease activity. The zone of clearance around the colonies indicated the organisms which were responsible for hydrolysis of the milk protein molecule (Figure 4).

4. Discussion

Contributions to Ni in the ambient air are made by combustion of fossil fuels, Ni plating and other metallurgical processes. The most common oxidation state of Ni is the divalent (Ni^{2+}) form. Elemental Ni is a malleable and silvery-white metal that is highly resistant to strong alkali. Because of its corrosion resistance, Ni is used in the production of stainless steel, permanent magnets and other alloys that require resistance to extremes of temperature or stress. Ni is also used in electroplating baths, batteries, textile dyes and catalysts[16].The processes through which microorganisms interact with toxic metals are biosorption, bioaccumulation and enzymatic reduction[17].

Actinobacteria were accounted for more than 45% of all bioactive metabolites discovered in nature[18]. The genus *Nocardiopsis* was described by Meyer[19]. *Nocardiopsis* genus is an aerobic actinobacteria that includes several species[20]. According to the key of McCarthy, *Nocardiopsis* is easily differentiated from other isolates[21]. A protease-producing, crude oil degrading marine *Nocardiopsis* sp. NCIM 5124 has been reported[22]. Biosurfactant producing marine actinobacteria, *Nocardiopsis alba* MSA 10 have been reported[23]. The sponge associated actinobacteria, *Nocardiopsis dassonvillei* MAD08 having 100% activity against multidrug resistant pathogens have been reported[24]. *Nocardiopsis* sp. has been reported in shore marine environment and mangrove ecosystem at 8 different locations of Kerala, West Coast of India[25]. *Nocardipsis* sp. could be used as potential probiotic bacteria for shrimp aquaculture[26]. Above literature evidenced that actinobacterium *Nocardipsis* sp. has enormous novel application. In our earlier investigation, *Nocardiopsis* sp. SD8 isolated from keratin waste and the organisms having keratinolytic property were reported by Saha and Dhanasekaran.[14].

In this study, we screened Ni(II) metal tolerance of selected isolates. Out of them, *Nocardiopsis* sp. SD8 actinobacteria showed high Ni(II) metal resistance property observed high growth in the agar media supplemented with $NiSO_4$. High growth of Ni, Cr, Hg, Cu and Pb resistance levels were found in several *Streptomyces* isolates by performing an agar media test[12,16,26]. Interestingly, we

found the best result in the *Nocardiopsis* sp. SD8 for Ni(II) metal tolerance. Literature evidenced that nocardioform actinomycetes in general play a crucial role in the degradation of multiring hydrocarbon in soils[27].

In this study, we also found that, 25 isolates, only four of the isolate have dye-decolorizing ability. Among the four isolates, *Nocardiopsis* sp. SD8 was efficiently decolorizing the dye. Release of dyes in general into the nearby water bodies pollutes the land and water micro-flora and fauna, and macro-flora and fauna but also directly and indirectly affects human beings and the dependent animals. Degradation of sulphonated azo dyes by actinobacteria has been reported by Pasti-Grigsby *et al*.[28]. In this study, we also found the potential isolate of *Nocardiosis* sp. SD8 having with diverse properties of haemolytic, protease and lipase activity. Literature evidenced that haemolytic and lipase activity is best screening method for biosurfactant producing isolates. Carrillo *et al*., found an association between hemolytic activity and surfactant production and they recommended the use of blood agar lysis as a primary method to screen biosurfactant production[9]. None of the studies reported the possibility of biosurfactant production without a hemolytic activity[22,23,25,29]. So, *Nocardiopsis* sp. SD8 could be having a role of biosurfactant producer. Further studies are in progress with respect to the extraction and identification of the potent molecule with Ni(II) metal resistance and dye-decolorizing activity from the diverse properties of *Nocardiopsis* sp. SD8.

Conflict of interest statement

We declare that we have no conflict of interest.

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