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Isolation and Molecular Characterization of Hydrocarbon Degrading *Nocardia* Isolated from Hospital Environments in Isfahan Province

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ABSTRACT

Background: Biodegradation is the metabolic ability of some microorganisms in degrading or transforming the organic and inorganic contaminants into less harmful and non-hazardous substances, which are then integrated into the natural biogeochemical cycles. Some microorganisms, mainly the members of family *Actinomycetes*, were found with the capability of transforming and degrading the polluting agents. In this study, three different *Nocardia* species with the ability to biodegrade organic and inorganic compounds were isolated from soil in Isfahan province.

Materials & Methods: The soil samples were collected from the hospital environments. Isolation process was done according to the standard methods. The identification and characterization of the isolates were based on the conventional and molecular methods, including direct sequence analysis of almost full length of 16S rRNA gene.

Results: Almost, the complete 16S rRNA gene sequences of the strains under study revealed that the isolates coded as NR6, NR17, NR18, NR25, NR26, and NR28 were the strains of *N. cyriacigeorgica*; NR7, NR34, and NR50 were the strains of *N. coubleae*; and NR4 was the strain of *N. otitidiscaviarum*. The relationship between the isolates under study and standard strain of *Nocardia* was supported by a phylogenetic tree of 16S rRNA gene.

Conclusion: In this study, 10 *Nocardia* strains with the capability of biotransforming polluting agents were isolated from the hospital environments. It was the first study conducted on the isolation and characterization of *Nocardia* strains, with the capability of degrading polluting agents, from Iranian hospitals. This study can be considered as a pioneer to develop a new insight about the study of microbial diversity in Iran using an applied approach to deal with environmental challenges.

Keywords: Nocardia, 16SrRNA, Biodegradation, Hospitals, Environmental samples

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Introduction

With the technological advancements and the increase in the global population, plastic, rubber, petrochemical products, crude oil derivations, and other organic and inorganic materials have been widely used in all aspects of life and industries [1]. However, most of these materials are non-biodegradable, and their increased accumulation in the environment is a serious threat to the life of the planet [1]. Most components of the organic and inorganic materials are non-degradable and toxic to humans, wildlife, and environment; therefor, these materials with long-term survival in the environment could be entered into the soil and aquatic ecosystems through different ways and then into the normal life of human, which cause irreparable damages to natural resources and human health [1, 2].

To overcome the high risk of non-degradable pollutants for the environment and human life, there is a need for practical and affordable techniques to degrade or transform and remove hazardous waste from the environment. Biodegradation is one of the best methods for the removal of these pollutants from the environment, in which the metabolic ability of some microorganisms is used to degrade or transform organic and inorganic contaminants into less harmful, non-hazardous substances, which are then integrated into the natural biogeochemical cycles [3, 4].

The ability of some microorganisms to degrade a wide range of pollutants, previously considered as non-degradable, has led to the excessive studies to be done on microorganisms in order to identify efficient strains for the biodegradation process [5]. One of these biodegrading microorganisms are the members of family *Nocardiaceae* known for their ability to degrade crude oil hydrocarbons [6, 7]. *Nocardia* species are abundance in the soil around the world and affect the ecosystem and the environment inhabitants [8]. Therefore, the isolation and identification of these agents in soil is an appropriate and easy way to use these microorganisms in the biodegradation process.

However, non-degradable materials has been shown to be biodegradable, there is insufficient data about the microorganisms which are capable of degradation and little information about the mechanisms or biochemistry of this process.

Objective: The present study was conducted with the aims of isolation and characterization of *Nocardia* strains, with the ability to biodegrade organic and inorganic compounds, from the soil of hospital environments in Isfahan province in Iran.

Materials and Methods

Sampling: A total of 90 soil samples were collected during October 2013 to November 2014 from Isfahan province. Sampling and decontamination were carried out according to the standard methods [9]. Briefly, up to 1 g of soil samples stirred for 30 min in 100 ml of sterile Ringer's solution (5% v/v). Tenfold dilutions of the homogenized suspensions were prepared, and 200 µl of each of the pretreated 10^{-2}, 10^{-3}, and 10^{-4} dilutions were inoculated into the sauton's media containing antibacterial and antifungal antibiotics; including nystatin, kanamycin, and nalidixic acid (each at 50µg/ml); and incubated at 25°C, 32°C, and 37°C for 3 weeks.

Microbiological analysis: The Iranian isolates coded as NR4, NR6, NR7, NR17,NR18, NR28, NR34, and NR50"

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were identified by conventional phenotypic tests; including partial acid fast staining; and standard biochemical assays; including resistance to lysozyme, hydrolysis of tyrosine, xanthine, and hypoxanthine tests [8].

Molecular identification: Chromosomal DNA was extracted using modified boiling method in which the conditions was change into an optimum conditions for *Nocardia* [10]. Briefly, a few colonies of bacteria was added into 200 ml of TE buffer (Tris EDTA), boiled for 30 min, and centrifuged at 10000 for 10 min. The supernatant was transferred to another sterile microtube and centrifuged at 13000 for 10 min. Supernatant was discarded, and 50 ml of distilled water was added to microtube then stored at -20 ° C.

The phenotypically identified isolates were analyzed at genus and species level using *Nocardia*-genus specific PCR reaction targeting 596 bp region of the 16S rRNA gene, recommended by Laurent et al. (2000) [11]. The amplification and direct sequence analysis of 16S rRNA gene were carried out as described previously [12]. The sequence data received from Bioneer Company (South Korea with an ABI 3100 genetic analyzer) were aligned manually with the existing sequences of *Nocardia*, retrieved from GenBankTM database, and analyzed using the Blast program in GenBank and the jPhydit program [13].

Nucleotide sequence accession numbers: The GenBank accession numbers for *Nocardia* isolates 16S rRNA gene sequencing determined in this study were as follows: *N. cyriacigeorgica*, KX685347; *N. coubleae*, KX685350; and *N. otitidiscaviarum*, KX685341.

Findings

The strains coded as NR4, NR6, NR7, NR17, NR18, NR25, NR26, NR28, NR34, and NR50 were recovered from the soil samples gathered from different environments. The strains coded as NR4, NR6, and NR18 were isolated from the soil and dust collected from pediatrics department of Seyedalshohada hospital in Isfahan. The strains coded as NR25 and NR26 were isolated from water and soil of Shahid Montazeri hospital in Najaf Abad. The strains coded as NR7 and NR17 were isolated from soil samples collected from Lenjan area in Zarinshahr, and the strains coded as NR28, NR34, and NR50 were isolated from the soil samples collected from teaching hospital of Isfahan University of Medical Sciences.

The recorded temperature and pH of the soil samples were in the ranges of 11 to 21 and 7.1 to 8.5, respectively. Phenotypic and biochemical characteristics of *Nocardia* strains isolated in this study are shown in Table 1.

The genus-specific PCR amplification was performed for the *Nocardia* isolates, producing a 596-bp amplicon of the 16S rRNA gene [11]. The results confirmed that the isolates belonged to the genus *Nocardia*.

The 16S rRNA gene sequences of the strains coded as NR6, NR17, NR18, NR 25, NR 26, and NR28 showed 100% similarities with the type strain of *N. cyriacigeorgica* DSM44484T. The strains coded as NR7, NR34, and NR50 showed 99.75 % similarities with the type *N. coubleae* DQ235688T strain, and the isolate coded as NR4 showed 100 % similarities with the type *N. otitidiscaviarum* DSM43242^T strain. The relationship between the standard species of *Nocardia* and this study isolates was supported by a phylogenetic tree of 16S rRNA gene (Fig. 1).

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Table 1) Samples profile and phenotypic and molecular features of biodegrading *Nocardia* isolates.

16S rRNA analysis	**Identification**	*N. cyriacigeorgica*	*N. coubleae*	*N. otitidiscavarum*	
	Similarity (%)[1]	**100**	**99.5**	**100**	
Phenotypic features	Opt. Tm^2	35	30	35	
	Decomposition of Hypoxanthine	-	-	+	
	Decomposition of Xanthene	-	-	+	
	Decomposition of Tyrosine	-	-	-	
	Pigment	White	White	White	
	Lysozyme Resistance	+	+	+	
Sample profile	TDS	365	-	-	
	Tm^2	11-16	18	18	
	pH	7.5-8.5	9	8.2	
	Source	Soil/ water	Soil	Soil	
	Hospital Department	Infectious disease	Pediatrics	Neurosurgery	
Isolates	Designation	NR 25/NR 17/ NR 18/ NR 6/ NR 26/ NR 28	NR 34/NR 7/NR50	NR 4	
	No	6	3	1	

1; Similarity; % similarity to the nearest validated species

2; Temperature (°C)

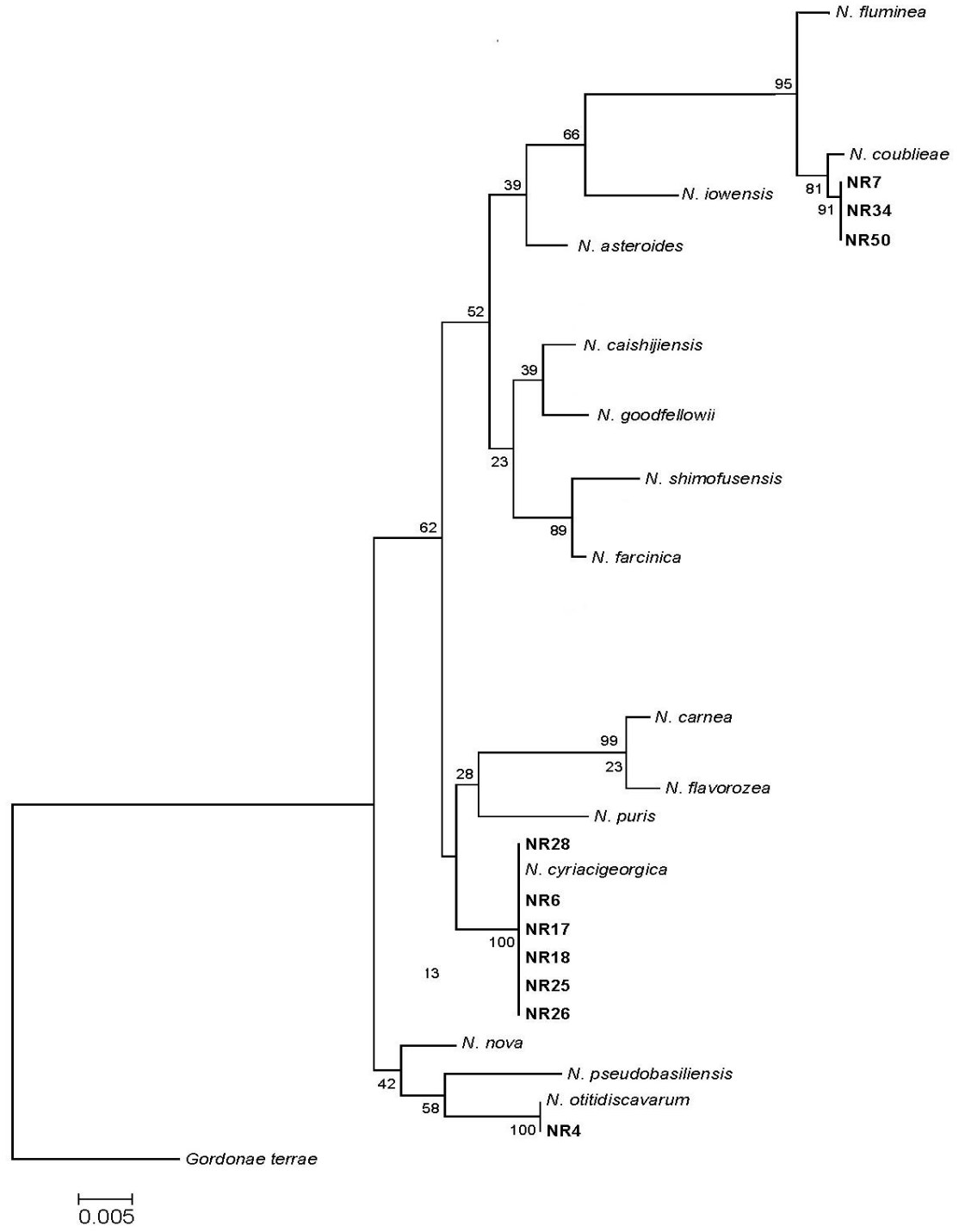

Fig 1) 16S rRNA gene sequence based phylogenetic tree for biodegrading *Nocardia* isolates and nearest validated species of *Nocardia* by using the neighbor-joining method. The figures at each node represent bootstrapping values. The tree was rooted with *Gordonae terrae*.

Discussion

In the new era, the development of new technologies in the field of organic and inorganic materials chemistry has led to the production and manufacturing of a huge collection of materials and equipment associated with these compounds, such as explosives, a variety of agricultural fertilizers,

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cosmetics, petroleum compounds, plastic, and rubber. These hard degradable or non-degradable materials are imported into the environment during the production or transportation processes or after the application and cause the pollution of the area they are disposed in [14, 15]. In addition, these compounds are non-degradable, which have high toxicity for human, animals, and the environment in which they are excreted [16, 17]. Therefore, to eliminate these non-degradable materials from the environment, appropriate practical methods are required. Bioremediation is a cost-effective way that can be used for the decomposition of toxic compounds into the safe or low-risk compounds.

In recent years, many bacterial and fungal species, especially the members of family *Actinomycetes*, have been reported to be able to transform or break down polluting agents [18]. These group of organisms have an ability to decompose a wide range of environmental pollutants, such as polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls, chlorophenols, sulfonated azo dyes, and alkanes [3, 19, 20]. Among the members of family *Actinomycetes*, *Nocardia* genus due to its ability to overcome the adverse conditions in polluted environments could be used as a great source of bacteria for bioremediation process [21].

In the present study, the isolation and characterization of 8 biodegrading *Nocardia* strains was reported, isolated from soil samples collected from Isfahan province. They had potentially an ability to degrade and transform many hazardous compounds present in water, soil, and environment by applying them into the bioremediation process and source of pollution.

Based on the phenotypic and biochemical profiles of the isolates under study and molecular test used in this study, the isolates coded as NR6, NR17, NR18, and NR28 were identified as *N. cyriacigeorgica.* Various studies results showed that *N. cyriacigeorgica* strains are able to biotransform aliphatic branched chains, aromatic hydrocarbons, and thymol [22, 23].

The isolates coded as NR7, NR34, and NR50 were identified as *N. coubleae,* which was first isolated and characterized in 2007 from oil-contaminated soil in Kuwait [24]. Previous studies showed that this species has the ability to degrade and decompose hydrocarbons derived from petroleum [25].

The isolate coded as NR4 was identified as *N. otitidiscaviarum.* In recent studies, it has been reported that this organism is able to degrade different polycyclic aromatic hydrocarbons (PAHs) such as n-alkanes and phenol [26].

Conclusion

In conclusion, the present study dealt with the isolation and identification of 8 rare *Nocardia* strains, which were hard to isolate and characterize. *Nocardia* strains were isolated from unsuitable environments of Isfahan province, such as those that may be encountered in water and soil located in special ecosystems near to the factories, industries, forests, deserts, and hospitals. This study indicated that *Nocardia* isolates under study possessed biochemical and ecological capacity to biodegrade and break down organic and inorganic pollutants and to decrease the risks associated with these contaminants. This findings emphasis the idea that unlike being abundant in environment, *Nocardia* strains are not considered for a remarkable usage. In fact, there is an untapped capability for biodegrading *Actinomycetes* and in particular family *Nocardia* that has not been yet discovered and exploited in bioremediation of hazardous materials.

In this study, the isolation and biodegradation capacity of the following strains were

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reported, including the strains coded as NR6, NR17, NR18, and NR28 belonging to the *N. cyriacigeorgica* species; the strains coded as NR7, NR34, and NR50 belonging to the *N. coubleae* species; and the strain coded as NR4 belonging to the *N. otitidiscaviarum* species; all of which were isolated from soil samples collected from Isfahan hospitals environment. Because of their broad biodegradation capacity, these strains could be useful for the application in bioremediation technologies.

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