Research Letter

DNA Extraction from *Nocardia* Species for Special Genes Analysis Using PCR

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Abstract

Background: *Nocardia* species have a complex cell wall structure similar to that of mycobacteria, and the extraction of DNA from this bacterium is extremely diffi cult. Currently, to identify *Nocardia* species particularly, it is essential to utilize molecular techniques. **Aims**: In the present study, we investigated STET (sodium chloride-TRIS-EDTA-triton) buffer for the extraction of high-quality genomic DNA from 20 clinical and environmental isolates. **Materials and Methods**: The extracted DNA was evaluated for portion of the 16S rRNA, 65-kDa heat-shock protein and 16S rRNA genes via polymerase chain reaction. **Results**: The extracted DNA had high molecular mass, and its concentration and purity was suitable when tested in 1% agarose gel, and using UV spectrophotometry. Amplifi cation of three different genes was successfully performed. **Conclusion**: This paper reveals an inexpensive, reproducible and effi cient method of DNA extraction from *Nocardia* species, which is appropriate for accurate identifi cation of this bacterium via polymerase chain reaction and polymerase chain reaction-restriction fragment length polymorphism.

Keywords: 16S rRNA, DNA extraction, hsp65, Nocardia, PCR

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Introduction

Nocardia species are gram-positive, partially acid-fast and non-motile bacteria that often form branched hyphae in both tissues and culture. *Nocardia* can be found around the world as saprophytic component of the normal soil microflora, marine water, dust, and air. Nocardial infections occur in both immunocompetent and immunosuppressive individuals, and respiratory tract is the primary site of nocardiosis.[1-4] In recent years, polymerase chain reaction (PCR) sequencing and PCR-restriction fragment length polymorphism (PCR-RFLP) are used for the accurate identification of *Nocardia* species.[3-6] Various methods, such as commercial kits and enzymatic methods (lysozyme, proteinase K), have been introduced for genomic DNA extraction from *Nocardia*. Enzymatic lysis is very expensive and time-consuming, thus molecular laboratories are searching for simple, inexpensive, rapid and acceptable methods for DNA extraction from bacteria.[7] Different methods have been introduced for DNA extraction from prokaryotic cells.[8,9] In this study, we investigated STET (sodium chloride-TRIS-EDTA-triton) buffer for DNA extraction from *Nocardia*.[10]

Access this article online	
Quick Response Code:	Website: www.najms.org
	DOI: 10.4103/1947-2714.132943

Materials and Methods

In this report, we describe a simple protocol for DNA extraction from the genus *Nocardia* using STET buffer. Pure colonies were picked from nutrient agar plate and inoculated in 5 mL of tryptic soy broth (TSB). The tube was incubated at 37°C and shaken until the turbidity of the bacterial suspension was adjusted to match 1.0 McFarland standard (approximately 3×10^8 bacterial cells). Bacterial suspension was pelleted via centrifugation at 13000 rpm for 5 min. The pellet was washed with sterile distilled water and re-suspended

in 200 µL of STET buffer (10 mM Tris-HCl, 0.1 M NaCl, 1 mM EDTA, 5% [v/v] Triton X100, pH 8.0), and the cell suspension was vortexed vigorously. The cell suspension was boiled at 100°C for 30 min and then centrifuged at 10000 rpm for 10 min. Supernatant fluid was transferred into a sterile Eppendorf tube. Subsequently, cold 95% ethanol was added to the supernatant and kept at −20°C for 60 min. After this stage, the solution was centrifuged at 13000 rpm for 10 min, the supernatant fluid was discarded, and DNA pellets were dried. DNA template was dissolved in 50 µL sterile distilled water and stored at −20°C until the PCR amplification. Purity and quality of the nucleic acid were determined by measuring A260/A280 ratio using UV spectrophotometry, and electrophoresis in 1% agarose gel (10 µL of DNA sample). PCR amplification of DNA was performed using partial sequence of 16S rRNA gene, 65-kDa heat-shock protein (*hsp*65) gene and 16S rRNA gene (universal bacterial 16S rRNA gene) [Table 1]. Following PCR amplification, 5 µL of PCR products were electrophoresed in 1.5% agarose gel at 70 V for 100 min in TBE buffer (Tris-HCl, Boric acid, EDTA), and stained with ethidium bromide (EtBr).

Results and Discussion

Chromosomal DNA was extracted from 20 clinical and environmental isolates within 110 minutes. Using this method, the extracted DNA gave an A260:A280 ratio of 1.9:2.0, and the concentration of DNA was 142.35 µg/mL. The extracted DNA had a high molecular weight in 1% agarose gel electrophoresis [Figure 1]. PCR amplification of *hsp*65, partial sequence of 16S rRNA and 16S rRNA gene (universal primer) regions yielded 439-bp, 999-bp,and 1500-bp fragments, respectively (data not shown). Details are shown in Figure 2. The rapid method described here could be used as an effective method for DNA extraction from *Nocardia* species. Other procedures for DNA extraction from the genus *Nocardia* are expensive and time-consuming.[12-14] Loeffelholz and Scholl in 1989 established a difficult method for DNA extraction from *Nocardia* species. This procedure was time-consuming and monotonous.[5] Recently, STET buffer solution has been used for other bacteria, including *Lactobacillus* species, *Clostridium perfringens,* and *Listeria monocytogenes*.[15-17] In the present paper, we report a method for DNA extraction from *Nocardia* species that has not been used before for this specific bacteria. This DNA extraction method using STET buffer showed acceptable and satisfactory results for molecular epidemiology techniques such as PCR and PCR-RFLP. The described method is simple, fast, cost-effective, sensitive, and highly reproducible for DNA extraction from *Nocardia*, and there is no need for a skillful specialist to perform this method.

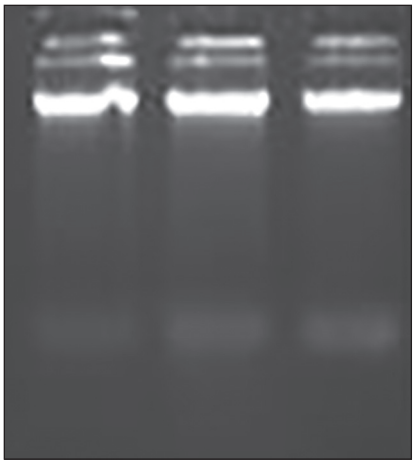

Figure 1: 10 µl of DNA template electrophoresed in 1% agarose gel

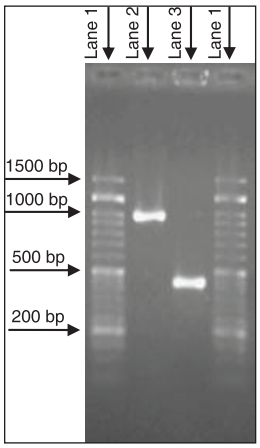

Figure 2: Lane 1, 50-bp DNA ladder; PCR amplification of the 999-bp (lane 2) and 439-bp (lane 3) fragments

Table 1: Primers used for PCR amplification in our study

Primer	Target gene	Sequence (5′-3′)	Reference
TB11: f	HSP	ACCAACGATGGTGTGTCCAT	[11]
Tb12: r	HSP	CTTGTCGAACCGCATACCCT	[11]
f	Part of 16S rRNA	CGAACGCTGGCGGCGTGCTTAAC	[7]
r1	Part of 16S rRNA	CCTGTACACCGACCACAAGGGGG	[7]
r2	Part of 16S rRNA	ACCTGTACACCAACCACAAGGGGG	[7]
p27: f*	16S rRNA	AGAGTTTGATCMTGGCTCAG	[14]
p1525: r*	16S rRNA	AAGGAGGTGWTCCARCC	[14]

f = Forward, r = Reverse*, universal primer

Acknowledgments

This study was supported by Tehran University of Medical Sciences, Deputy of Research.

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How to cite this article: Bafghi MF, Eshraghi SS, Heidarieh P, Habibnia S, Nasab MR. DNA extraction from *nocardia* species for special genes analysis using PCR. North Am J Med Sci 2014;6:231-3.

Source of Support: Nil. **Conflict of Interest:** None declared.