

Short Report

Identification of *Nocardia farcinica* by a PCR Primer Amplifying a Specific DNA Band for the Bacterium

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Abstract

A PCR primer specific to *Nocardia farcinica* was prepared based on sequence information of random amplified polymorphic DNA (RAPD) analysis. The PCR primer amplifies *N. farcinica* species only; no amplification was observed in 25 other *Nocardia* strains that we tested. Specificity of the primer for *N. farcinica* was also confirmed using other fungal and bacterial strains that are frequently isolated from clinical samples such as sputa and broncho alveolar lavage (VAL).

Key words: *Nocardia farcinica*, RAPD analysis, specific PCR primer, specific identification

Introduction

Members of the genus *Nocardia* are partially acid-fast and gram-positive bacteria with true branchings. *Nocardia* causes various human infections, including cutaneous, subcutaneous, pulmonary, cerebral, and lymphocutaneous infections¹⁾. More than 62 species of the genus *Nocardia* have now been reported²⁾. Of the greatest clinical importance within this genus is *Nocardia farcinica* because infectious cases attributable to this species are the most numerous among *Nocardia* species³⁾. Because it has been reported that *N. farcinica* species show a species-specific drug resistance pattern^{4, 5)}, early species identification of *Nocardia* strains are required to start proper chemotherapy^{5, 6)}. Traditional identification of *N. farcinica* using a battery of biochemical tests such as the growth temperature, utilization of acetamide as a nitrogen and carbon source, acid production from rhamnose, and resistance to tobramycin, is often laborious and time-consuming; species identification usually requires as long as 2 to 3 weeks⁵⁾. However, phenotypic identification to the species level within this genus using such

characteristics remains problematic because *N. farcinica* belongs to the *Nocardia asteroides* complex in such phenotypic characteristics^{7, 8)}. In fact, the RAPD method has been described in many bacterial and fungal identification studies as a useful method for species identification and intraspecies discrimination⁹⁾. Our recent RAPD pattern blot analysis showed that *N. farcinica* species show characteristic DNA bands common to all *N. farcinica* species tested. This paper describes the usefulness of the random amplified polymorphic DNA (RAPD) band information for differentiation of *N. farcinica* from other *Nocardia* species, and proposes a new PCR primer for specific identification of *N. farcinica*.

Preparation of genomic DNA samples for sequencing was performed using the guanidine thiocyanate method. As described previously, RAPD analysis was done using primer R-3=(5'-ATGGATCSSC)⁹⁾; PCR was performed with a DNA thermal cycler (TaKaRa, Japan) using 35 cycles and consisting of denaturation at 94°C for 60 s, primer annealing at 60°C for 60 s, and primer extension at 72°C for 120 s. The DNA sequences were determined using an automatic sequence analyzer (ABI PRISM™ 3100; PE Applied Biosystems, U.S.A.) with a dye

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terminator cycle sequencing kit (PE Applied Biosystems).

Figure 1 shows that RAPD band patterns are similar among the four strains of *N. farcinica* tested including the type strain of *N. farcinica* IFM 0284^T. However, the band patterns of *N. farcinica* differ from those of other *Nocardia* strains tested: *N. asteroides* IFM 0319^T, *Nocardia brasiliensis* IFM 0236^T, *Nocardia nova* IFM 0290^T, and *Nocardia otitidiscaviarum* IFM 0239^T, and *Nocardia transvalensis* IFM 0333^T. Therefore, one specific PCR band, which was common to all *N. farcinica* strains (Fig. 1), was extracted and sequenced. The size of the sequenced band was 412 bp; a BLAST search suggested that the

band belonged to *N. farcinica* species and it was found the band sequences are species-specific for strains of *N. farcinica*. Genome information related to the strain of *N. farcinica* IFM 10152 also showed that the band comprises a part of a putative gene (*nfa* 29510) and non ORF-gene of the bacterium. Based on the sequence information, the following PCR primer pair was designed and prepared: Nfar-T primer (forward: 5'-GGCGAGCCCAGTACCGATTAA and reverse: 5'-AAGCCACGCACCTGTTTC). Figure 2 shows that *N. farcinica* IFM 0284^T strain tested amplified the 283 bp PCR band, but no amplification was observed in other *Nocardia* type species such as *N. abscessus* IFM 10029^T, *N. nova* IFM 0290^T,

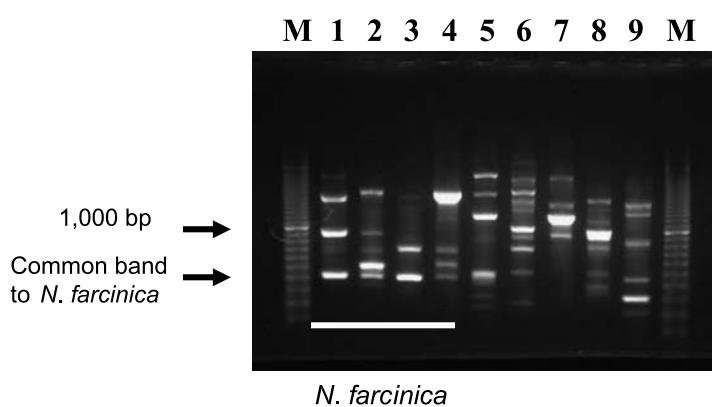


Fig. 1. RAPD band patterns of four strains of *N. farcinica* in comparison with those of other *Nocardia* species which are frequently isolated from clinical specimens in Japan.

(M; 100 bp ladder marker, 1; *N. farcinica* IFM 0284^T, 2; *N. farcinica* IFM 10823, 3; *N. farcinica* IFM 10826, 4; *N. farcinica* IFM 10152, 5; *N. asteroides* IFM 0319^T, 6; *N. transvalensis* IFM 0333^T, 7; *N. nova* IFM 0290^T, 8; *N. brasiliensis* IFM 0236^T, 9; *N. otitidiscaviarum* IFM 0239^T)

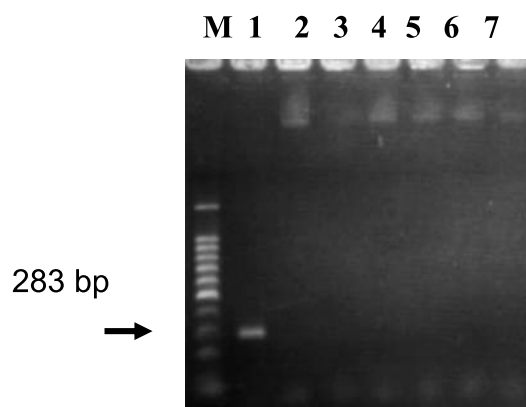


Fig. 2. Specific identification of *N. farcinica* species by a newly developed specific PCR primer for the bacterial species.

(M; 100 bp ladder marker, 1; *N. farcinica* IFM 0284^T, 2; *N. abscessus* IFM 10029^T, 3; *N. nova* IFM 0290^T, 4; *N. higoensis* IFM 10084^T, 5; *N. asteroides* IFM 0319^T, 6; *N. shimofusensis* IFM 0333^T, 7; *N. puris* IFM 10064^T)

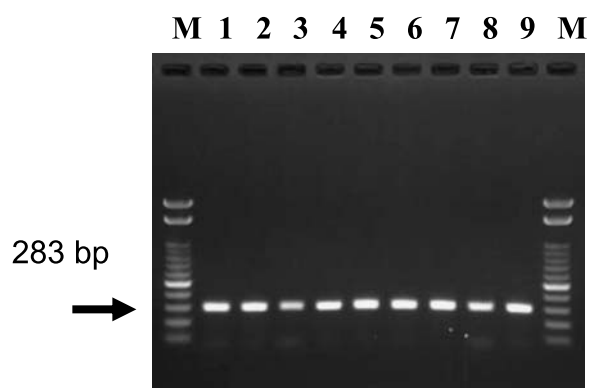


Fig. 3. Specific amplification of *N. farcinica* by a newly developed specific PCR primer for the detection of the bacterial strains.

(M; ladder marker, 1; *N. farcinica* IFM 10152, 2; IFM 10580, 3; IFM 10821, 4; IFM 10823, 5; IFM 10826, 6; IFM 10835, 7; IFM 10836, 8; IFM 10840, 9; IFM 10853)

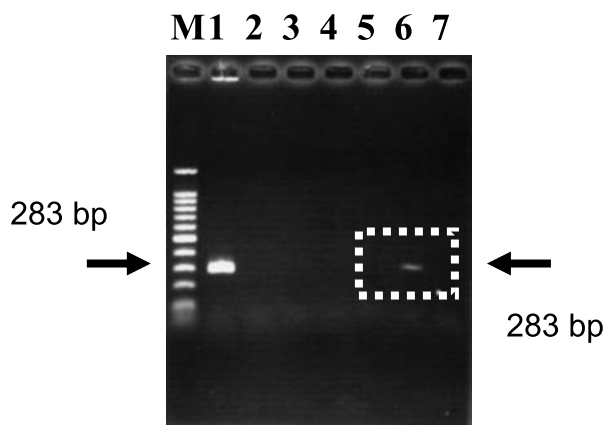


Fig. 4. Detection of the bacterial DNA from blood sample of experimental *N. farcinica* infection in mice by newly developed specific PCR primer. Dotted square (in lane 6) indicates amplification of PCR band from one blood sample from one of the infected mice.

(M; ladder marker, 1; *N. farcinica* IFM 0284^T, 2-6; blood samples obtained from different mice infected with *N. farcinica* IFM 0284^T, 7; blood sample from control mice)

Nocardia higoensis IFM 10084^T, *N. asteroides* IFM 0319^T, *N. shimofusensis* IFM 10031^T, or *Nocardia puris* IFM 10064^T. Figure 3 shows that 9 clinical isolates of *N. farcinica* amplified the 283 bp band, and confirmed the usefulness of this PCR primer for the specific identification of *N. farcinica* strains.

No amplification was observed in these strains when the specificity of this PCR primer was tested using specimens frequently isolated in clinical situations such as *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus*. Mice and human gene samples also showed no amplification of the PCR band.

Figure 4 shows that the PCR primer amplified the DNA band from one blood sample (lane 6) obtained from experimental nocardiosis in mice, suggesting the usefulness of the PCR primer for the specific detection of the gene of *N. farcinica* in clinical specimens. Since the detection rate of PCR band from blood samples was low (DNA band amplification was only observed in one blood sample from one infected mouse out of 5 mice), further improvements in the detection methods are now in progress in our laboratory using various techniques such as the Real-Time PCR method. In summary, this method is simple and is useful for rapid identification of species of *N. farcinica* to enable an earlier diagnosis of infected patients, and engender optimization of their antimicrobial therapy to improve patient outcome.

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References

- 1) McNeil MM, Brown JM: The medically important aerobic actinomycetes: epidemiology and microbiology. *Clin Microbiol Rev* **7**: 357-417, 1994.
- 2) Hoshino Y, Watanabe K, Iida S, Suzuki S, Kudo T, Kogure T, Yazawa K, Ishikawa J, Kroppenstedt RM, Mikami Y: *Nocardia terpenica* sp. nov., isolated from Japanese patients with nocardiosis. *Int J Syst Evol Microbiol* **57**: 1456-1460, 2007.
- 3) Ishiwaka J, Yamashita A, Mikami Y, Hoshino Y, Kurita H, Hotta K, Shiba T, Hattori M: The complete genome sequence of *Nocardia farcinica* IFM 10152. *Proc Natl Acad Sci USA* **101**: 14925-14930, 2004.
- 4) Poonwan N, Kusumu M, Mikami Y, Yazawa K, Tanaka Y, Gono T, Hasegawa T, Konyama K: Pathogenic *Nocardia* isolated from clinical specimens including those of AIDS patients in Thailand. *Eur J Epidemiol* **11**: 507-512, 1995.
- 5) Kageyama A, Yazawa K, Ishikawa J, Hotta K, Nishimura K, Mikami Y: Nocardial infections in Japan from 1992 to 2001, including the first report of infection by *Nocardia transvalensis*. *Eur J Epidemiol* **19**: 383-389, 2004.
- 6) Brown-Elliott B, Brown JM, Conville PS, Wallace RJ: Clinical and laboratory features of *Nocardia* spp. based on current molecular taxonomy. *Clin Microbiol Rev* **19**: 259-282, 2006.
- 7) Alfaresi M, Eikosh A: Rapid identification of clinically relevant *Nocardia* species using real-time PCR with SYBR Green and melting-curve analysis. *J Med Microbiol* **55**: 1711-1715, 2006.
- 8) Brown JM, Pham NK, McNeil MM, Lasker BA: Rapid identification of *Nocardia farcinica* clinical isolates by a PCR assay targeting a 314-base-pair species-specific DNA fragment. *J Clin Microbiol* **42**: 3655-3660, 2004.
- 9) Mekha N, Poonwan N, Mikami Y, Yazawa K, Gono T, Hasegawa S, Nishimura K: Random amplified polymorphic DNA (RAPD) analysis of *Penicillium marneffeii* strains isolated from AIDS patients in Thailand. *Mycoscience* **38**: 97-100, 1997.