J. Microbiol. Biotechnol. (2003), 13(6), 846–852

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Differentiation of Actinomycete Genera Based on Partial *rpoB* Gene Sequences

KIM, BUM-JOON[1]*, YOUNG-HWAN KOH[2], JONGSIK CHUN[3], CHANG-JIN KIM[4], SEUNG-HYUN LEE[5], MOONJAE CHO[6], JIN-WON HYUN[6], KEUN-HWA LEE[1], CHANG-YONG CHA[1], AND YOON-HOH KOOK[1]

[1]*Department of Microbiology, College of Medicine, Seoul National University, Seoul 110-799, Korea*
[2]*Department of Food Science and Engineering, Cheju National University, Jeju 690-756, Korea*
[3]*School of Biological Sciences, Seoul National University, Seoul 151-742, Korea*
[4]*Department of Microbiology, College of Medicine, Konkuk University, Chugju 380-230, Korea*
[5]*Korea Research Institute of Bioscience and Biotechnology, Yusung, Taejon 305-600, Korea*
[6]*Department of Biochemistry, College of Medicine, Cheju National University, Jeju 690-756, Korea*

Received: March 20, 2003
Accepted: May 12, 2003

Abstract *rpoB* DNAs (279 bp) from 34 species of 5 actinomycete genera were sequenced and a phylogenetic tree was constructed based on the sequences obtained. The genera were clearly differentiated in the *rpoB* tree, forming clades specific to their respective genus. In addition, 2 signature amino acid residues specific to *Streptomyces* were found in a multiple alignment of the deduced amino acid sequences. To empirically confirm that this *rpoB* gene analysis system could be used to differentiate actinomycete isolates, the proposed system was used to identify 16 actinomycete isolates from Jeju Island. All isolates were successfully differentiated into the genera *Streptomyces* and *Micromonospora*. Accordingly, this is the first report that an *rpoB* sequence analysis has been effectively used to differentiate actinomycete strains at the genus level.

Key words: Phylogenetic relationship, actinomycete genera, *rpoB*, signature amino acids, differentiation, identification

The genus *Streptomyces* contains the largest number of species among actinomycete genera [3] and is most commonly isolated as the major source of antibiotics among the soil actinomycete population [1, 20]. More recently, so-called rare actinomycetes have also gained increasing importance as a new source of antibiotics [2, 13, 15]. As such, due to the high frequency of their isolation and their importance as a source of bioactive materials, the separation of actinomycete genera is important for their classification.

*Corresponding author
Phone: 82-2-740-8315; Fax: 82-2-743-0881;
E-mail: kbumjoon@snu.ac.kr

The identification of *Streptomyces* and other related genera at a genus level can be easily performed by the numerical classification method based on morphological and biochemical characteristics [23]. However, this method demands experts and laborious biochemical procedures for an exact distinction among actinomycete genera. Furthermore, in some cases, decisive results are impossible in contrast to a genetic analysis.

It has previously been reported that the *rpoB* gene encoding RNA polymerase can reveal relevant phylogenetic relationships among strains in the genus *Mycobacterium*, a member of the actinomycete genera, and even has an advantage over the 16S rDNA gene in species identification within this genus [8]. In addition, this target gene has also been successfully used for species or strain differentiation in other pathogenic genera, such as *Legionella* [9] and *Borrelia* [16]. Therefore, it is anticipated that the *rpoB* gene may also be effective for differentiation among actinomycete genera.

Accordingly, the present study was undertaken to determine the *rpoB* DNA from 34 species of 5 actinomycete genera for phylogenetic separation among the genera; subsequently, the system was applied to the genus differentiation of soil actinomycete isolates.

Materials and Methods

Bacterial Strains

Fourteen strains of *Streptomyces*, 11 strains of *Micromonospora*, 3 strains of *Amycolaptosis*, 4 strains of *Tsukamurella*, and 2 strains of *Nocardia* used in the current study were all provided by the Korean Collection for Type Cultures (KCTC) and Institute of Microbiology, Seoul National

Table 1. Reference strains used for *rpoB* gene phylogenetic analysis.

Species or subspecies	Strain
Streptomyces	
S. anthocyanicus	KCTC 9755 (=ATCC 19821)
S. antimycoticus	KCTC 9694 (=ATCC 23880)
S. carpinensis	KCTC 9128 (=ATCC 27116)
S. citreofluorescens	KCTC 9710 (=ATCC 15858)
S. diastaticus	KCTC 9142
S. djakartensis	KCTC 9722 (=ATCC 13441)
S. flavofuscus	KCTC 9737 (=ATCC 19908)
S. limosus	KCTC 9033 (=ATCC 19778)
S. noursei	KCTC 1083 (=ATCC 11455)
S. paradoxus	KCTC 9118 (=ATCC 15813)
S. phaeochromogenes	KCTC 9763 (=ATCC 23945)
S. plicatus	KCTC 9040 (=ATCC 12957)
S. spectabilis	KCTC 9218 (=ATCC 27465)
S. viridosporus	KCTC 9145 (=ATCC 27479)
Micromonospora	
M. carbonacea	KCTC 1053(=ATCC 27114)
M. chalcea	KCTC 9070 (=ATCC 12452)
M. citreasp	KCTC 9367 (=ATCC 35571)
M. coerulea	KCTC 9368 (=ATCC 27008)
M. echinorunnea	KCTC 9548 (=DSM 43913)
M. echinospora echinospora	IMSNU 21292 (=ATCC 15837)
M. echinospora ferrunginea	KCTC 9549 (=ATCC 15836)
M. olivasterospora	KCTC 1052 (=ATCC 21819)
M. pallida	KCTC 1051 (=ATCC 15838)
M. purpureochromogenes	KCTC 9369 (=ATCC 27007)
M. yulongensis	IMSNU 21304(=ATCC 43540)
Tsukamurella	
T. inchonensis	KCTC 9866 (=DSM 44067)
T. paurometabola	KCTC 9821 (=ATCC 8368)
Tsukamurella sp.	KCTC 9827
Tsukamurella sp.	KCTC 9832
Amycolatopsis	
A. albidoflavus	KCTC 9471 (=ATCC 53205)
A. mediterranei	KCTC 1739 (=ATCC 13685)
A. rubida	KCTC 19909 (=DSM 44637)
Nocardia	
N. brevicatena	IMSNU 21207
N. farcinia	IMSNU 21224

KCTC, Korean Collection for Type Cultures; ATCC, American Type Culture Collection; DSM, Deutsche Sammlung von Microorganismen; IMSNU, Institute of Microbiology, Seoul National University.

University (IMSNU) (Table 1). All but five, including *S. diastaticus* (KCTC 9142), *Tsukamurella* sp. (KCTC 9827), *Tsukamurella* sp. (KCTC9832), *N. brevicatena* (IMSNU 21207), and *N. farcina* (IMSNU 21224) were type strains.

Soil Actinomycete Isolation

Soil samples collected during 2002 from several sites near Halla Mountain on Jeju Island, Korea, were used to isolate actinomycete strains [11]. A starch-casein agar was used as the isolation medium, and cycloheximide was added to a concentration of 50 μg/ml to inhibit fungal growth [6, 7, 14]. Fourteen colonies exhibiting typical actinomycete characteristics were used in the *rpoB* gene analysis for differentiation at the genus level.

Preparation of DNA

The DNAs were prepared using the bead beater-phenol extraction method [8]. A loopful of the culture of each isolate was suspended in 200 μl of a TEN buffer (10 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl; pH 8.0) and placed in a 2.0-ml screw-cap microcentrifuge tube filled with 100 μl (packed volume) of glass beads (diameter, 0.1 mm; Biospec Products, Bartlesville, Okla., U.S.A.) and 100 μl of phenol-chloroform-isopropyl alcohol (50:49:1). To disrupt the bacteria, the tube was oscillated on a Mini-Bead Beater (Biospec Products) for 1 min, and the phases were then separated by centrifugation (12,000 ×g, 5 min). After the aqueous phase was transferred into another tube, 10 μl of 3 M sodium acetate and 250 μl of ice-cold ethanol were added, then the mixture was kept at 20°C for 10 min to precipitate the DNA. Thereafter, the DNA pellet was washed with 70% ethanol, dissolved in 60 μl of TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0), and used as the template for PCR.

PCR Amplification

A set of primers (MF, 5'-CGACCACTTCGGCAACCG-3'; MR, 5'-TCGATCGGGCACATCCGG-3') previously used to differentiate strains within the genus *Mycobacterium* [8] was also used to amplify the *rpoB* DNA (351-bp) in the actinomycete strains (Fig. 1). The template DNA (50 ng) and 20 pmol of each primer were added to a PCR mixture tube (AccuPower PCR PreMix; Bioneer, Chungbuk, Korea) containing 1 U of Taq DNA polymerase and 250 μM each of the following: deoxynucleoside triphosphate, 50 mM Tris-HCl (pH 8.3), 40 mM KCl, 1.5 mM $HgCl_2$, and a gel

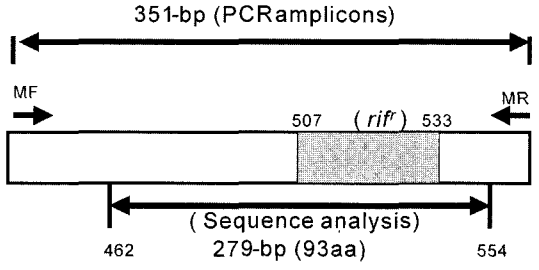

Fig. 1. Strategy for PCR amplification and DNA sequence analysis of *rpoB* gene.

The PCR was performed using the MF-MR primer set, as previously described, producing 351-bp *rpoB* amplicons. Automatic DNA sequencing was then performed with the forward primer, MF, and 279-bp from each strain were sequenced for the phylogenetic analysis. Numbers indicate *rpoB* codons of *E. coli*.

loading dye, and the volume was then adjusted with distilled water to 20 µl. The reaction mixture was subjected to 30 cycles of amplification (30 s at 95°C, 30 s at 60°C, and 45 s at 72°C) followed by a 5-min extension at 72°C (model 9600 Thermocycler; Perkin-Elmer Cetus). The PCR products were electrophoresed on a 3% agarose gel and purified using a QIAEX II gel extraction kit (Qiagen, Hilden, Germany).

To differentiate the 17 soil isolates at a genus level, the partial 16S rDNA sequences (500-bps) containing the hypervariable region of each strain were analyzed. As previously described [22], a set of primers (16Sf, 5'-TCA-CGGAGAGTTTGATCCTG-3'; 16Sr, 5'-GCGGCTGCT-GGCACGTAGTT-3') was used for the 16S rDNA analysis. The PCR conditions for the 16S rDNA analysis were the same as those for the *rpoB* analysis, as described above.

The template DNA (50 ng) and 20 pmol of each primer were added to a PCR mixture tube (AccuPower PCR PreMix; Bioneer, Chungbuk, Korea) containing 1 U of Taq DNA polymerase and 250 µM each of the following: deoxynucleoside triphosphate, 50 mM Tris-HCl (pH 8.3), 40 mM KCl, 1.5 mM $HgCl_2$, and a gel loading dye, then the volume was adjusted with distilled water to 20 µl. The reaction mixture was subjected to 30 cycles of amplification (30 s at 95°C, 30 s at 60°C, and 45 s at 72°C) followed by a 5-min extension at 72°C (model 9600 Thermocycler; Perkin-Elmer Cetus). The PCR products were separated by electrophoresis on a 3% agarose gel and purified using a QIAEX II gel extraction kit (Qiagen, Hilden, Germany).

Nucleotide Sequencing

For the *rpoB* gene analysis, the *rpoB* DNA sequences (279-bp) of the purified PCR products (351-bp) were directly determined with the forward primer (MF) using an Applied Biosystems Automatic Sequencer (model 373A) and BigDye Terminator Cycle Sequencing kit (Perkin-Elmer Applied Biosystems, Warrington, United Kingdom) (Fig. 1). For the 16S rDNA analysis, the nucleotide sequences (500-bp) of the amplified products were directly determined with the forward (16Sf) and reverse (16Sr) primers. For this sequencing reaction, 60 ng of the PCR-amplified DNA, 3.2 pmol of the primer, and 8 µl of the BigDye Terminator RR mix (Perkin-Elmer Applied Biosystems; part no. 4303153) were mixed, and the mixture was adjusted to a final volume of 20 µl by the addition of distilled water. The reaction was run with 5% (vol/vol) dimethyl sulfoxide for 30 cycles of amplification, 15 s at 95°C, 10 s at 50°C, and 4 min at 60°C.

Sequence Analysis

The *rpoB* sequences of the 34 reference strains used in the current study were aligned using the multiple alignment algorithm in the MegAlign package (Windows version 3.12e; DNASTAR, Madison, Wis., U.S.A.). A phylogenetic tree of the actinomycetes was constructed using the MEGA program [8, 14]. A bootstrap analysis (100 repeats) was also performed to evaluate the topology of the phylogenetic tree.

Identification of Soil Actinomycete Isolates

The differentiation of 16 soil actinomycete isolates at a genus level was performed based on a comparative analysis of 279-bp sequences in the *rpoB* gene and by the BLAST program from GenBank using a 16S rDNA analysis [6, 7, 14].

RESULTS

rpoB PCR and Sequencing

rpoB DNA (351-bp) was successfully amplified from all 34 reference strains and 17 soil isolates by a PCR using the primer set of MF and MR (Fig. 2). When all these amplicons were sequenced unidirectionally by the PCR direct sequencing method using the forward primer MF, the 279-bp DNAs, excluding the primer sequences and those exhibiting ambiguous results within 351-bp (from the first nucleotide at codon 462 to the third nucleotide at codon 554), were successfully determined (Fig. 1).

rpoB DNA Sequence Comparison Among Actinomycete Strains

When the 279-bp *rpoB* sequences of the 34 reference strains were aligned, no insertion or deletion was observed. That is, all the actinomycetes strains had identical 279-bp *rpoB* sequences. When the sequences of 14 *Streptomyces*, 11 *Micromonospora*, 4 *Tsukamurella*, 3 *Amycolaptosis*, and 2 *Nocardia* strains were compared for pairwise similarity, the results showed that the sequences of the strains within the same genus were closely related and distinct from those in another genus. In general, 92.1-98.6%, 89.6-97.5%, 94.3-97.1%, 90.0-98.6%, and 93.5% similarities were observed among the *Streptomyces*, *Micromonospora*, *Tsukamurella*, *Amycolaptosis*, and *Nocardia* strains,

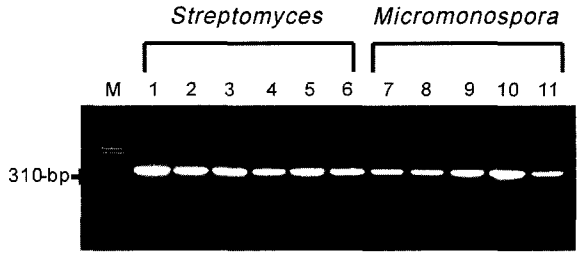

Fig. 2. Partial amplification of *rpoB* DNA by PCR using DNA from *Streptomyces* (lanes 1 to 6) and *Micromonospora* (lanes 7 to 11) strains as templates.

The PCR amplicons were separated by electrophoresis on a 3% agarose gel. Lanes: M, φX174/RF DNA/*Hae*III digest; 1, *S. anthocyanicus*; 2, *S. antimycoticus*; 3, *S. djakartensis*; 4, *S. limosus*; 5, *S. phaeochromogenes*; 6, *S. spectabilis*; 7, *M. chalcea*; 8, *M. citreasp*; 9, *M. coerulea*; 10, *M. pallida*; 11, *M. yulongensis*.

Table 2. Signature amino acid residues specific to genus *Streptomyces*.

Site[a]	*Streptomyces*	Other *Actinomycetes*
466	Threonine	Valine
469	Alanine	Serine

[a]Numbers indicate *rpoB* codons of *E. coli*.

respectively. All strains in the same genus showed a sequence divergence of no more than 10%, when their sequences were compared to those of strains in another genus.

Comparison of Amino Acid Sequences Between *Streptomyces* and *Micromonospora* Strains

The deduced amino acid sequences, comprising 93 amino acid residues of 15 *Streptomyces*, 12 *Micromonospora*, 4 *Tsukamurella*, 3 *Amycolaptosis*, and 2 *Nocardia* strains, were multiple aligned. The results showed that the amino acid sequences among strains within one genus were more conserved than the nucleotide sequences. In the multiple alignment of the amino acid sequences, 93.5-100%, 94.6-100%, 97.8-100%, 94.6-100%, and 100% similarities were observed among the *Streptomyces*, *Micromonospora*, *Tsukamurella*, *Amycolaptosis*, and *Nocardia* strains, respectively. In addition, signature amino acid residues specific to *Streptomyces* strains were observed at codons 466 and 469 (Table 2).

Phylogenetic Tree

A phylogenetic tree to provide the basis for differentiation among the actinomycete genera was constructed using the neighbor-joining method (Fig. 3). All the tested actinomycete

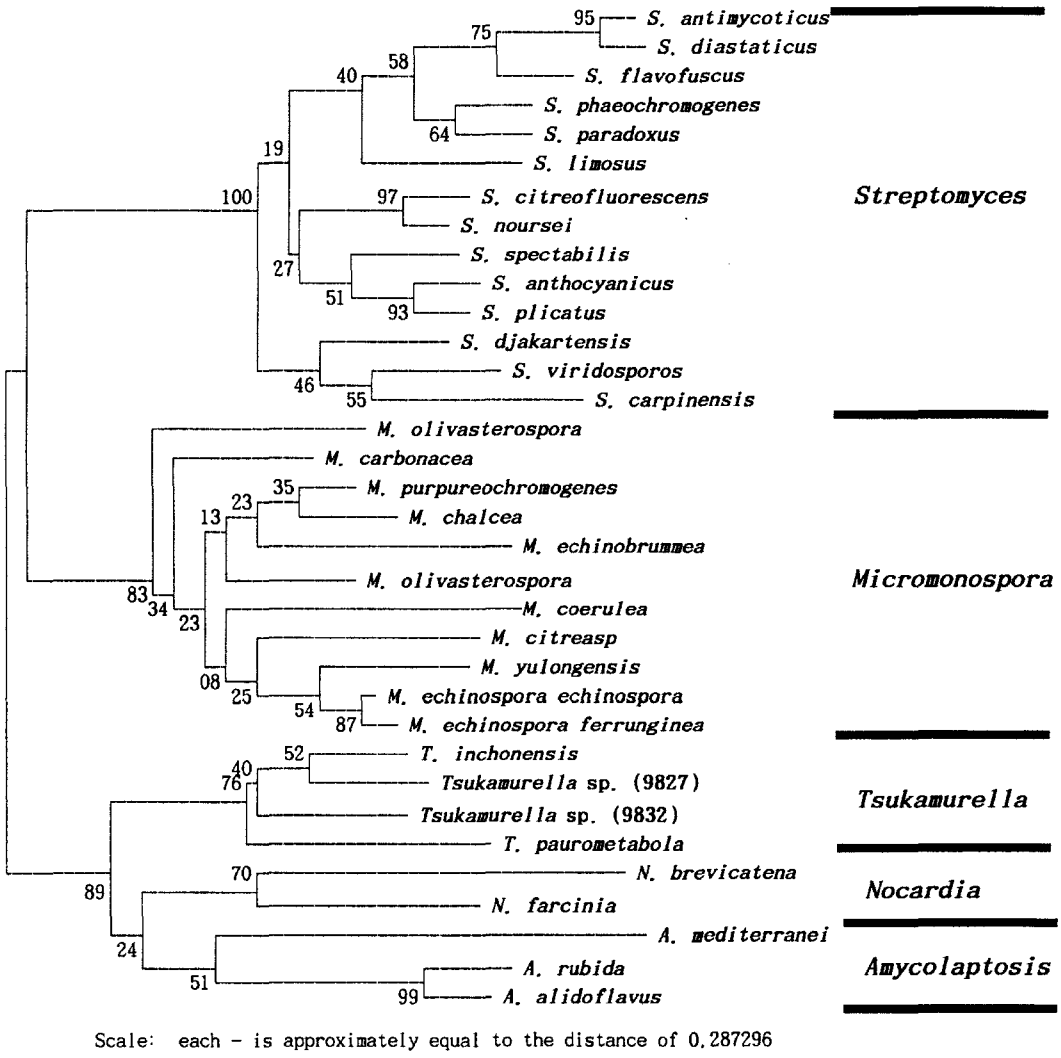

Fig. 3. Phylogenetic tree based on 279-bp *rpoB* sequences of 34 actinomycete strains.
This tree was constructed by the neighbor-joining method. The topology was also evaluated by a bootstrap analysis (MEGA program, 100 repeats). The numerical values in the tree represent the bootstrap results. The distance between two strains is the sum of the branch lengths between them.

strains exhibited a good separation at the genus level. In other words, the 15 *Streptomyces*, 12 *Micromonospora*, 4 *Tsukamurella*, 3 *Amycolaptosis*, and 2 *Nocardia* strains could be clearly differentiated in the *rpoB* tree, forming clusters specific to each genus and distinct from the other actinomycete genera. The reliability of the phylogenetic tree was verified by the bootstrap method.

Identification of Soil Isolates at Genus Level

The *rpoB* sequences of the 16 soil actinomycete isolates were determined and used for differentiation at the genus level. The differentiation was performed based on a comparative sequence analysis using a *rpoB* gene database of the 34 reference strains (Fig. 4). All 16 isolates were clearly differentiated into the genus *Streptomyces* (13 strains) and *Micromonospora* (3 strains) according to the *rpoB* gene analysis. When the results were compared to those obtained from the 16S rDNA analysis, the two methods produced 100% identical results (Table 3). When the signature amino acids of the soil isolates were analyzed, 13

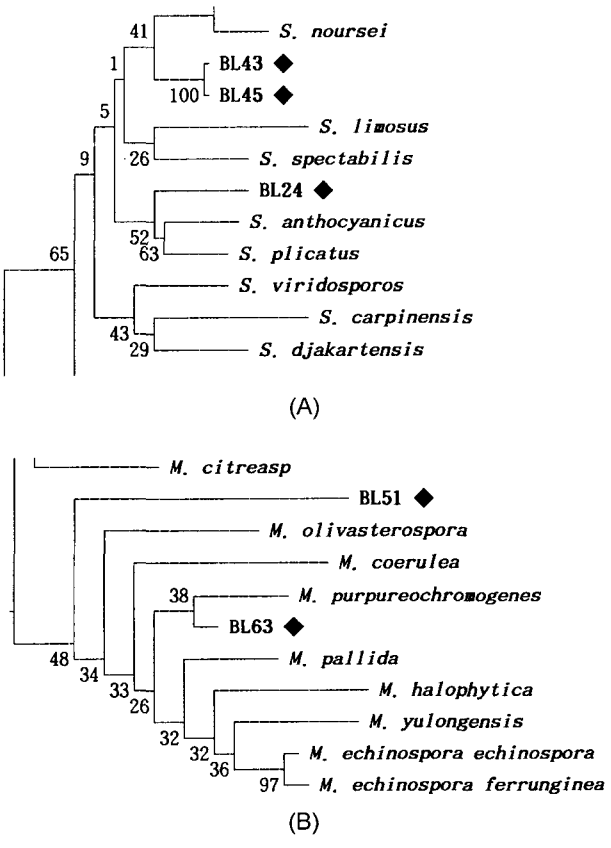

Fig. 4. Differential identification of actinomycete isolates from Jeju Island at the genus level by comparative sequence analysis of the *rpoB* gene (279-bp).

(A) Isolates BL24, BL43, and BL45 were identified as strains of genus *Streptomyces* based on *rpoB* gene analysis. (B) Isolates BL51 and BL63 were identified as strains of genus *Micromonospora* based on *rpoB* gene analysis.

Table 3. Differentiation of actinomycete isolates from Jeju island at the genus level by *rpoB* and 16S rDNA analyses.

No.	Isolates	*rpoB* gene analysis	16S rDNA analysis
1	BL11	Stm.	Stm.
2	BL22	Stm.	Stm.
3	BL24	Stm.	Stm.
4	BL29	Stm.	Stm.
5	BL33	Mim.	Mim.
6	BL40	Stm.	Stm.
7	BL43	Stm.	Stm.
8	BL45	Stm.	Stm.
9	BL48	Stm.	Stm.
10	BL51	Mim.	Mim.
11	BL53	Stm.	Stm.
12	BL63	Mim.	Mim.
13	BL67	Stm.	Stm.
14	BL71	Stm.	Stm.
15	BL75	Mim.	Mim.
16	BL79	Stm.	Stm.

Stm.; *Streptomyces*, *Mim.*; *Micromonospora*.

Streptomyces strains had amino acid residues specific to the corresponding genus.

DISCUSSION

Despite the significance of actinomycete classification for industrial microbiology [2, 10, 14], only a few phylogenetic analyses on differentiation of actinomycete genera have been published. Furthermore, almost all these previous studies have been limited to the genus *Streptomyces* [3, 5, 12, 17, 18].

Although the differentiation of actinomycetes at the genus level can be easily performed based on a combination of chemical and morphological properties, this classical taxonomy has reproducibility problems, and demands laborious procedures and a large-scale numerical phenetic survey to clarify the infrastructure of the genus [3, 12, 23]. Therefore, several genetic methods targeting the 16S rDNA and *gyrB* gene have been developed to differentiate strains within the genus *Streptomyces* [5, 17, 18] and other actinomycete genera [4, 10]. As a result, the 16S rDNA method is the most commonly used and produces the most reliable phylogenetic relationships among acinomycete genera [10] or strains within a genus [17]. However, this method has certain disadvantages regarding the taxonomy of actinomycete genera, since some strains within the genus *Mycobacterium* and *Streptomyces* have multi-copy genes of 16S rDNA within one organism that exhibit different sequences [19, 22], and also two distinctly different species within the genus *Mycobacterium* have completely identical sequences [21]. Therefore, an alternative genetic method that targets a different gene is required for the classification of actinomycetes. Therefore, to solve these problems, the

current study was undertaken to develop a novel method for differentiating among actinomycete genera that uses the *rpoB* gene as the target molecule, which has already been successfully used to differentiate strains within the genus *Mycobacterium*, one of the actinomycete genera [8].

There are two advantages to exploit the *rpoB* gene over 16S rDNA for differentiating actinomycete genera. First, *rpoB* is a one-copy gene. Therefore, when the direct sequencing method is used to target this gene, there are no ambiguous results arising from different sequences of a multi-copy gene. Second, *rpoB* is a functional gene, therefore, in addition to DNA sequence information, the deduced amino acid sequence can also be used for genus separation.

The phylogenetic tree presented in Fig. 3 revealed a good phylogenetic relationship among the actinomycete genera; 5 actinomycete genera formed distinct clusters specific to their own genus, indicating that *rpoB* sequences can be effectively used to differentiate actinomycete genera.

Two signature amino acid residues specific to the genus *Streptomyces* were observed in the deduced amino acid sequence alignment. As such, the nucleotides encoding these signature amino acid residues could be used to develop a *Streptomyces*-specific PCR or hybridization method that could selectively identify *Streptomyces* strains from actinomycetes. The biological role of the two signature amino acids specific to the genus *Streptomyces* in the function of the RNA polymerase β subunit remains to be studied. No other region in the DNA sequences and deduced amino acid sequence alignment was identified that could be used for inter-general identification.

To evaluate the *rpoB* analysis system for the differentiation of strains at the genus level, the proposed analysis was applied to 16 soil actinomycete isolates. The results were in complete agreement with those obtained with a 16S rDNA analysis. Therefore, the *rpoB* gene analysis appears to be a useful genetic method for the differentiation of actinomycetes at the genus level.

Acknowledgment

This work was supported by a Korea Research Foundation Grant (KRF--2001-015-DP0462).

REFERENCES

1. Choi, W. C., S. Y. Hwang, T. K. Park, and S. K. Kim. 2002. Identification of *Streptomyces* sp. producing new polyene antibiotics and *in vivo* antimicrobial activity of tetrin C against phytopathogenic fungi. **12:** 204-208.
2. Colquhoun, J. A., S. C. Heald, L. Li, J. Tamaoka, C. Kato, K. Horikoshi, and A. T. Bull. 1998. Taxonomy and biotransformation activities of some deep-sea actinomycetes. *Extremophiles* **2:** 269-277.
3. Goodfellow, M., E. V. Ferguson, and J. J. Sanglier. 1992. Numerical classification and identification of *Streptomyces* species - a review. *Gene* **115:** 225-233.
4. Kasai, H., T. Tamura, and S. Harayama. 2000. Intrageneric relationships among *Micromonospora* species deduced from *gyrB*-based phylogeny and DNA relatedness. *Int. J. Syst. Evol. Microbiol.* **50:** 127-134.
5. Kataoka, M., K. Ueda, T. Kudo, T. Seki, and T. Yoshida. 1997. Application of the variable region in 16S rDNA to create an index for rapid species identification in the genus *Streptomyces*. *FEMS. Microbiol. Lett.* **151:** 249-255.
6. Kim, B. J., G. J. Choi, K. Y. Cho, H. Yang, C. Shin, C. H. Lee, and Y. Lim. 2002. Antifungal activities against *Plasmodiophora brassicae* causing club root. *J. Microbiol. Biotechnol.* **12:** 1122-1125.
7. Kim, B. J., M. Cho, J. C. Kim, K. Y. Cho, G. J. Choi, C. H. Lee, and Y. Lim. 2001. *Streptomyces* showing antifungal activities against six plant pathogenic fungi. *J. Microbiol. Biotechnol.* **11:** 1120-1123.
8. Kim, B. J., S. H. Lee, M. A. Lyu, S. J. Kim, G. H. Bai, G. T. Chae, E. C. Kim, C. Y. Cha, and Y. H. Kook. 1999. Identification of mycobacterial species by comparative sequence analysis of the RNA polymerase gene (*rpoB*). *J. Clin. Microbiol.* **37:** 1714-1720.
9. Ko, K. S., H. K. Lee, M. Y. Park, K. H. Lee, Y. J. Yun, S. Y. Woo, H. Miyamoto, and Y. H. Kook. 2002. Application of RNA polymerase beta-subunit gene (*rpoB*) sequences for the molecular differentiation of *Legionella* species. *J. Clin. Microbiol.* **40:** 2653-2658.
10. Koch, C., R. M. Kroppenstedt, F. A. Rainey, and E. Stackebrandt. 1996. 16S ribosomal DNA analysis of the genera *Micromonospora*, *Actinoplanes*, *Catellatospora*, *Catenuloplanes*, *Couchioplanes*, *Dactylosporangium*, and *Pilimelia* and emendation of the family *Micromonosporaceae*. *Int. J. Syst. Bacteriol.* **46:** 765-768.
11. Kwon, O. S., D. J. Park, C. Y. Lee, and C. J. Kim. 1996. Distribution pattern of soil actinomycetes at Cheju islands. *Kor. J. Appl. Microbiol. Biotechnol.* **24:** 399-403.
12. Langham, C. D., S. T. Williams, P. H. Sneath, and A. M. Mortimer. 1989. New probability matrices for identification of *Streptomyces*. *J. Gen. Microbiol.* **135:** 121-133.
13. Lazzarini, A., L. Cavaletti, G. Toppo, and F. Marinelli. 2001. Rare genera of actinomycetes as potential producers of new antibiotics. *Antonie Van Leeuwenhoek* **79:** 399-405.
14. Lee, C. H., B. J. Kim, G. J. Choi, K. Y. Cho, H. Yang, C. Shin, S. Min, and Y. Lim. 2002. *Streptomyces* with antifungal activitiy against rice blast causing fungus, *Magnaporthe grisea*. *J. Microbiol. Biotechnol.* **12:** 1026-1028.
15. Lee, J. Y. and B. K. Hwang. 2002. Diversity of antifungal actinomycetes in various vegetative soils of Korea. *Can. J. Microbiol.* **48:** 407-417.
16. Lee, S. H., B. J. Kim, J. H. Kim, K. H. Park, S. J. Yeo, S. J. Kim, and Y. H. Kook. 2000. Differentiation of *Borrelia burgdorferi* sensu lato on the basis of RNA polymerase gene (*rpoB*) sequences. *J. Clin. Microbiol.* **38:** 2557-2562.

17. Mehling, A., U. F. Wehmeier, and W. Piepersberg. 1995. Nucleotide sequences of streptomycete 16S ribosomal DNA: Towards a specific identification system for Streptomycetes using PCR. *Microbiology* **141:** 2139-2147.
18. Ochi, K. 1995. A taxonomic study of the genus *Streptomyces* by analysis of ribosomal protein AT-L30. *Int. J. Syst. Bacteriol.* **45:** 507-514.
19. Reischl, U., K. Feldmann, L. Naumann, B. J. Gaugler, B. Ninet, B. Hirschel, and S. Emler. 1998. 16S rRNA sequence diversity in *Mycobacterium celatum* strains caused by presence of two different copies of 16S rRNA gene. *J. Clin. Microbiol.* **36:** 1761-1764.
20. Rhee, K. H., K. H. Choi, C. J. Kim, and C. H. Kim. 2001. Identification of *Streptomyces* sp. AMLK-335 producing antibiotic substance inhibitory to vancomycin-resistant enterococci. *J. Microbiol. Biotechnol.* **11:** 469-474.
21. Rogall, T., J. Wolters, T. Flohr, and E. C. Bottger. 1990. Towards a phylogeny and definition of species at the molecular level within the genus *Mycobacterium*. *Int. J. Syst. Bacteriol.* **40:** 323-330.
22. Ueda, K., T. Seki, T. Kudo, T. Yoshida, and M. Kataoka. 1999. Two distinct mechanisms cause heterogeneity of 16S rRNA. *J. Bacteriol.* **181:** 78-82.
23. Williams, S. T., M. Goodfellow, G. Alderson, E. M. Wellington, P. H. Sneath, and M. J. Sackin. 1983. Numerical classification of *Streptomyces* and related genera. *J. Gen. Microbiol.* **129:** 1743-1813.