Journal of Microbiology and Biotechnology
Vol. 6. No. 5, 364-365, 1996

NOTE

Nucleotide Sequence of 16S rRNA Gene from *Streptomyces melanosporofaciens* 7489

LEE, DONG-SUN, SUNG-OUI SUH, SEON-KAP HWANG, TAEG-KYU KWON, TAE-HO KIM, WOO-CHANG SHIN, AND SOON-DUCK HONG*

Department of Microbiology, College of Natural Science, Kyungpook National University, Taegu 702-701, Korea

A region encoding the 16S rRNA was cloned by PCR from *Streptomyces melanosporofaciens* 7489 and sequenced by the chain-termination dideoxy sequencing method. A phylogenetic tree constructed by sequence alignment of 24 *Streptomyces* species suggests that there is little evolutionary distance between this strain and *Streptomyces rimosus*.

A comparative sequence study of small subunit ribosomal RNAs (SSU rRNAs) is widely used to infer accurate phylogenetic relationship of various strains with high fidelity, because these molecules are highly conserved during evolution and also contain large amounts of information which can be practically used as an indicator of phylogeny (6).

In the prevoius papers (4, 5), we isolated a strain, that produces dibutyl phthalate, an inhibitor of DNA topoisomerase I, and identified as a *Streptomyces melanosporofaciens* 7489 via chemotaxonomical and numerical analyses. In order to infer the phylogenetic relationships of this strain to *Streptomyces* species, we have determined the nucleotide sequence of 16S rDNA by the following procedures.

For sequencing, the cells were cultured in LB liquid medium for 24 h at 28°C. Genomic DNA was extracted from 50 mg (wet weight) of cells and purified by repeated phenol treatment followed by RNase treatment (8). Polymerase chain reaction was performed to amplify the 16S rRNA coding region, using two oligonucleotide primers, 5'-AGTTTGATCCTGGCTC-3' (position 10 to 25 relative to *E. coli* 16S rRNA) and 5'-AGAAAGGAGGTGATC-3' (position 1541 to 1525), and then the PCR product with assumed size was cloned into pBluescript T vector. DNA sequencing reactions were performed with Sequenase™ V. 2.0 kit by the dideoxy chain termination method (9) using synthetic oligonucleotides as primers: 5'-CGCCCATTGTGCAATAT-3' (complementary to positions 365 to 349), 5'-AAAGCCTGATGCAGCGA-3' (same sequence as positions 366 to 382), 5'-GGGCTTTCACATACCGA-3' (complementary to position 578 to 562), 5'-GGGGCTTAACTCCGGGT-3' (same sequence as positions 579 to 595), 5'-GGGGGCACCACTCTCTG-3' (complementary to positions 995 to 979), 5'-TTGTGGTCGGTGTACAG-3' (same sequence as positions 996 to 1,012), 5'-AGCTCATTGTACCGGCC-3' (complementary to positions 1,230 to 1,214), 5'-GCGAAG-

```
AGTTTGATCC TGGCTCAGGA CGAACGCTGG AGGCGTGCTT AACACATGCA AGTCGAACGA      60
TGAACCGGTT TCGGCCGGGG ATTAGTGGCG AACGGGTGAG TAACACGTGG GCAATCTGCC     120
CTGCACTCTG GGACAAGCCC TGGAATCGGG GTCTAATACC GGATATGACA CGCTCCCGCA     180
TGGGATGTGT GTGGAAAGCT CCGGCGGTGC AGGATGAGCC CGCGGCCTAT CAGCTTGTTG     240
GTGGGGTGAT GGCCTACCAA GGCGACGACG GGTAGCCGGC CTGAGAGGGC GACCGGCCAC     300
ACTGGGACTG AGACACGGCC CAGACTCTAC GGGAGGCAGC AGTGGGGAAT ATTGCACAAT     360
GGGCGAAAGC CTGATGCAGC GACGCCGCGT GAGGGATGAC GGCTTCGGGT TGTAAACCTC     420
TTTCAGCAGG GAAGAAGCGA GAGTGACGGT ACCTGCAGAA GAAGCGCCGG CTAACTACGT     480
GCCAGCAGCC GCGGTAATAC GTAGGGCGCA AGCGTTGTCC GGAATTATTG GGCGTAAAGA     540
GCACGTAGGC GGCTTGTCGC GTCGGTATGT GAAAGCCCGG GGCTTAACTC CGGGTCTGCA     600
TTCGATACGG GCAGGCTAGA GTTCGGTAGG GGAGATCGGA ATTCCTGGTG TAGCGGTGAA     660
ATGCGCAGAT ATCAGGAGGA ACACCGGTGG CGAAGGCGGA TCTCTGGGCC GATACTGACG     720
CTGAGGAGCG AAAGCGTGGG GAGCGAACAG GATTAGATAC CCTGGTAGTC CACGCCGTAA     780
ACGTTGGGAA CTAGGTGTGG GCGACATTCC ACGTCGTCCG CGCCGCCAST AACGCATTAA     840
GTTCCCCGCC TGGGGRGTAC GGCCGCAAGG CTAAAACTCA AAGGAATTGA CGGGGGTCCG     900
CACAAGCGGC GGAGCATGTG GCTTAATTCG ACGCAACGCG AAGAACCTTA CCAAGGCTTG     960
ACATACACCG GAAACATCCA GAGAGTGGTG CCCCCTTGTG GTCGGTGTAC AGGTGGTGCA   1,020
TGGCTGTCGT CAGCTCGTGT CGTGTGATGT TGGGTTAAGT CCCGCAACGA GCGCAACCCT   1,080
TGTTCTGTGT TGCCAGCATG CCTTTCGGGG TGATGGGGAC TCACAGGAGA CTGCCGGGGT   1,140
CAACTCGGAG GAAGGTGGGG ACGACGTCAA GTCATCATGC CCCTTATGTC TTGGGCTGCA   1,200
CACGTGCTAC AATGGCCGGT ACAATGAGCT GCGAAGCCGT GAGGTGGAGC GAATCTCAAA   1,260
AGGCCGGTCT CAGTTCGGAT TGGGGTCTGC AACTCGACCC CATGAAGTCG GAGTCGCTAG   1,320
TAATCGCAGA TCAGCATTGC TGCGGTGAAT ACGTTCCCGG GCCTTGTACA CACCGCCCGT   1,380
CACGTCACGA AAGTCGGTAA CACCCGAAGC CGGTGGCCCA ACCCTTGTGG GGGGAGCCGT   1,440
CGAAGGTGGG ACTGGCGATT GGGACGAAGT CGTAACAAGG TAGCGTACCG GAAGGTGCGG   1,500
CTGGATCACC TCCTT                                                    1,515
```

Fig. 1. Nucleotide sequence of 16S rDNA from *S. melanosporofaciens* 7489.
The underlined sequences represents the primers used in PCR.

*Corresponding author
Key words: phylogenetic analysis, 16S rRNA gene, *Streptomyces melanosporofaciens* 7489

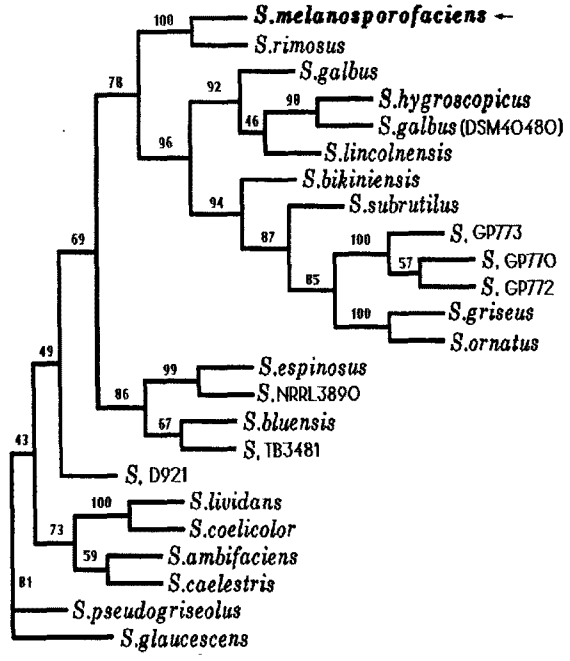

Fig. 2. Phylogenetic tree of 24 *Streptomyces* species based on the 16S rDNA sequence.

Each number indicates the percentage of bootstrap samplings, derived from 100 samples, supporting the internal branches. The position of *S. melanosporofaciens* 7489 is indicated by arrow.

CCGTGAGGTGG-3' (same sequence as positions 1,231 to 1,247). As a result, 1,515 nucleotides of 16S rDNA sequence were determined (Fig. 1).

To construct a phylogenetic tree, the rDNA sequence determined in this work was aligned using the CLUSTALW program (10) with those of 24 other strains collected from data bases. Phylogeny was estimated using Kimura's two parameter method (3) and Neighbor-joining analysis (6), in which 100 bootstrap resamples were made and used to produce the most probable phylogenetic tree by the SEQBOOT, DNADIST, NEIGHBOR, and CONSENSE programs with the PHYLIP 3.5c package (1, 2). All sites with gaps in any sequences were excluded for determination of evolutionary distances. *S. glaucescens* was used as an outgroup for this analysis. As shown in Fig. 2, *S. melanosporofaciens* 7489 is closely related with *S. rimosus*, and both strains form a group, with a supporting probability of 78%, separate from other strains.

REFERENCES

1. Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783-791.
2. Felsenstein, J. 1993. Distributed by the author, Department of Genetics, University of Washington, Seattle.
3. Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.*, **16**: 111-120.
4. Lee, D. S., S. C. Ha, S. Y. Lee, J. G. Kim, and S. D. Hong. 1996. DNA topoisomerase I inhibitor by *Streptomyces* sp. 7489. *J. Microbiol. Biotechnol.* **24**: 101-104.
5. Lee, D. S., S. C. Ha, W. C. Shin, T. H. Kim, H. J. Kim, Y. H. Park, J. G. Kim, and S. D. Hong. 1995. Numerical identification of an actinomycetes strain producing an antitumor antibiotic with inhibitory activity against DNA topoisomerase I. *J. Microbiol. Biotechnol.* **23**: 123-130.
6. Olsen, G. J., R. Overbeek, N. Larsen, T. L. Marsh, M. J. McCaughey, M. A. Maciukenas, W. M. Kuan, T. J. Macke, Y. Xing, and C. R. Woese. 1992. The ribosomal database project. *Nucl. Acids Res.* **20**(supplement): 2199-2200.
7. Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, **4**: 406-425.
8. Sambrook, J., R. Maniatis, and E. F. Fritsch. 1989. *Molecular cloning: a laboratory manual.* Cold Spring Harbor, New York.
9. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**: 5463-5467.
10. Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTALW: improving the sensitivity of progressive multiple alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**: 4673-4680.

(Received April 16, 1996)