The Journal of Microbiology, June 2002, p.178-181 Vol. 40, No. 2
Copyright © 2002, The Microbiological Society of Korea

NOTE

Characterization of a Streptomycete Isolate Producing the Potent Cytotoxic Substance, Nonadecanoic Acid

Jin-cheol Yoo[1], Ji-Man Han[1], Seung-Kwan Nam[1], Keun-shik Baik, Jung-sun Jo, and Chi-nam Seong*

Department of Biology, Sunchon National University, Sunchon, 540-742,
[1]Department of Pharmacy, Chosun University, Gwangju, 501-759, Korea

(Received April 19, 2002 / Accepted May 30, 2002)

Streptomycete isolate, strain M0137 showed cytotoxic effect on THP-1 cells. One of the purified substances produced from the strain was identified as nonadecanoic acid. Morphological and physiological properties, phylogenetic analysis, and genomic fingerprinting of strain M0137 were determined. Strain M0137 showed a high similarity with *Streptomyces scabiei*, phenotypically and phylogenetically. In contrast, genomic fingerprinting and G+C content analysis revealed that strain M0137 could be distinguished from *S. scabiei* ATCC49173^T. We propose to name strain M0137 as *Streptomyces scabiei* subsp. *chosunensis*.

***Key words*:** cytotoxic substance, nonadecanoic acid, genomic fingerprinting, *Streptomyces scabiei* subsp. *chosunensis*

Actinomycetes are widely distributed in natural and man-made environments, and play an important role in the degradation of organic matter. They are also well known as a rich source of antibiotics and bioactive molecules, and are of considerable importance in industry. Tissue culture microtiter-plate based screens were developed for the screening of novel microorganisms producing antitumor or cytotoxic agents (Mirabelli *et al.*, 1985; Iwai and Takahashi, 1992; Kanzaki *et al.*, 2000).

Until now, cytotoxic substances such as actinomycin D, mitomycin C, bleomycin and doxorubicin originating from streptomycetes were used for cancer therapy. To overcome the secondary effects of these compounds, intensive approaches aimed at searching for more selective and novel structural agents have started. In recent years, vicenistatin M, albonoursin, migrastatin, valinomycin, and fattiviracin A1 produced by *Streptomyces* spp. have been under screening (Nakae *et al.*, 2000; Paananen *et al.*, 2000; Matsushima *et al.*, 2001).

It is one of the major concerns of the researchers whether the screened agents are novel or not. Thus, the screening programs for the isolation of novel microorganisms, such as the use of diverse sources and pretreatments have been improved. Recognition as a novel taxa of the isolate has been very important for both practical and taxonomical purposes. Recently, molecular systematic methods, notably 16S rDNA sequencing, are having an ever increasing impact on streptomycete systematics (Stackebrandt *et al.*, 1991; van Wezel *et al.*, 1991; Kim *et al.*, 1996). DNA or chemical fingerprinting was recognized as one of the most useful tools for the definition of a novel strain (O'Donnell, 1985).

Six hundred streptomycete isolates were obtained from soil samples from various locations in the province of Chonnam, Korea. Among them, one streptomycete isolate, M0137, was found to produce a potent cytotoxic substance. The conditions for culturing microorganisms and for extracting substances were described in Nam (2002). The purified compound 0116p, which was recovered from Sephadex LH-20 column chromatography and silica-gel column chromatography, showed *in vitro* anticancer and immunosuppressive activities. Compound 0116p was identified to be a nonadecanoic acid ($C_{19}H_{38}O_2$, M.W. 301.10) by NMR spectral analysis (Nam, 2002). Nonadecanoic acid has been known to be isolated from several sources such as fungi, marine sponges, plants, and larvae, which have inhibitory effects on fibrinolysis and plasmin activity (Kawashiri *et al.*, 1986). Thus, M0137 is the first strain producing nonadecanoic acid among the genus *Streptomyces*. Also, there have been no reports on the anti-tumor

* To whom correspondence should be addressed.
(Tel) 82-61-750-3613; (Fax) 82-61-750-3608
(E-mail) scnu@sunchon.ac.kr

and immunosuppressive activities of nonadecanoic acid.

Morphological and physiological properties of strain M0137 were determined as described in Williams *et al.* (1983). Aerial spore mass color and spore chain morphology of the strain was observed on inorganic salts-starch agar (ISP medium 4: Difco) (Shirling and Gottlieb, 1966). Physiological properties were examined using procedures described in a previous study (Williams *et al.*, 1983).

In order to characterize strain M0137 by molecular methods, G+C content of the DNA, 16S rDNA sequencing and genomic fingerprinting were carried out. Chromosomal DNA was isolated from the test strain using a procedure (Chun and Goodfellow, 1995) slightly modified from that of Pitcher *et al.* (1989). The G+C contents were determined using the thermal denaturation (*Tm*) method (Mandel and Marmur, 1968).

PCR amplification of 16S rDNA was performed as described by Kim *et al.* (1996). The amplified fragments were directly sequenced by using a *Taq* DyeDeoxy terminator Cycle Sequencing Kit (Applied Biosystems) and previously described oligonucleotide primers (Chun and Goodfellow, 1995). Sequencing gel electrophoresis was carried out and nucleotide sequences were automatically obtained using an Applied Biosystems DNA sequencer (model 373A) and software provided by the manufacturer. The 16S rDNA sequence of strain M0137 has been deposited in the GenBank database under accession number AY057090. The nucleotide sequence was aligned manually against corresponding streptomycete nucleotide sequences retrieved from the GenBank database. An unrooted evolutionary tree was constructed by the neighbor-joining method (Saitou and Nei, 1987).

Repetitive sequence based PCR was performed using a thermal cycler (Perkin-Elmer). Each 25 μl PCR reaction contains 50 pmol each of two opposing oligonucleotide primers (Versalovic *et al.*, 1994), 100 ng of chromosomal DNA, 1.25 mM of each of 4 dNTPs, 2 units of *Taq* DNA ploymerase (Bioneer) in a reaction buffer with 10% DMSO (v/v). The following conditions were used for amplification: denaturation at 95°C for 30 sec, annealing at 40°C (for BOXA1R, 52°C) for 1 min, and elongation at 65°C for 8 min for each cycle. A total of 30 cycles were performed followed by a final elongation step at 65°C for 16 min. For the comparative analysis of DNA fingerprint patterns, 5 μl of each PCR reaction was electrophoresed at 5 V/cm directly on 1% agarose gels containing 1×Tris acetate-EDTA(TAE) and 0.5 μg/ml ethidium bromide.

Strain M0137 was closely related to *S. scabiei* strains including the type strain *S. scabiei* ATCC49173^T (Lambert and Loria, 1989). Strain M0137 formed extensively branched substrate mycelia and aerial hyphae which carried rectiflexible/open spiral and grey spore chains, produced melanin and contained LL-diaminopimellic acid. The strain was also sensitive to most of the antibiotics, and did not inhibit the growth of target microorganisms. Strain M0137 did not produce an earthy odor. Other characteristics are described in Table 1.

Table 1. Phenotypic properties of strain M0137

Unit Character	M0137	*Streptomyces scabies* ATCC 49173^T*
Aerial spore mass color	Grey	Grey
Spore chains	Open spiral	Spiral
Substrate mycelium color	-	
Melanin production	+	+
Diffusible pigment	-	-
Lipolysis	-	
Pectin hydrolysis	+	
Earthy odor	-	+
Degradation of:		
Elastin	-	
Xanthine	-	-
Growth in the presence of (%):		
NaCl (7)	-	-
Sodium azide (0.01)	-	-
Thallous acetate (0.01)	-	-
Phenol (0.1)	-	-
Growth at 45°C	-	-
Resistance to antibiotics (μg/ml)		
Rifampicin (50)	-	
Oleandomycin (100)	-	-
Penicillin G (10 i.u.)	-	-
Growth on carbon sources (1.0%):		
meso-Inositol	+	+
L-Rhamnose	+	+
Raffinose	+	+
D-Melibiose	+	
Dextran	-	
Growth on nitrogen sources (0.1%):		
DL-α-Amino-n-butyrate	+	
L-Cysteine	+	
L-Valine	+	+
L-Hydroxyproline	+	+
Antibiotic activity against:		
Bacillus subtilis	-	
Escherichia coli	-	
Micrococcus luteus	-	
Candida albicans	-	
Saccharomyces cerevisiae	-	

*data from Lambert and Loria (1989).

Strain M0137 had similar 16S rDNA sequences with *S. scabiei* ATCC49173^T, and only 7 out of 1342 nucleotides differed from each other (99.48% similarity; Fig. 1). But, this value was lower than the levels between strains belonging to the species *S. scabiei* which were more than 99.9% (Takeuchi *et al.*, 1996). The G+C content of strain M0137 was 76.13 mol%, while *S. scabiei* ATCC49173^T was 71% (Lambert and Loria, 1989). Moreover, genomic fingerprinting using repetitive sequence-based PCR showed that strain M0137 differed from *S. scabiei* (Fig. 2). The

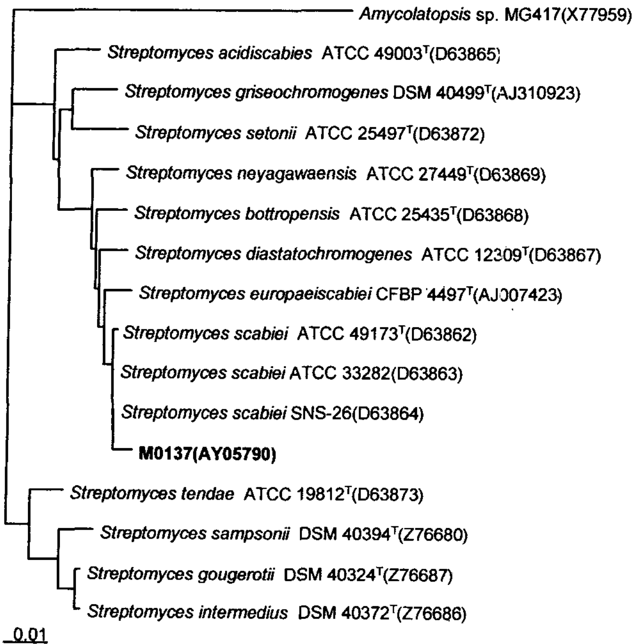

Fig. 1. Neighbor-joining tree based on nearly complete 16S rRNA sequences of strain M0137 and representative species of related taxa. The scale bar indicates 0.01 substitutions per nucleotide position.

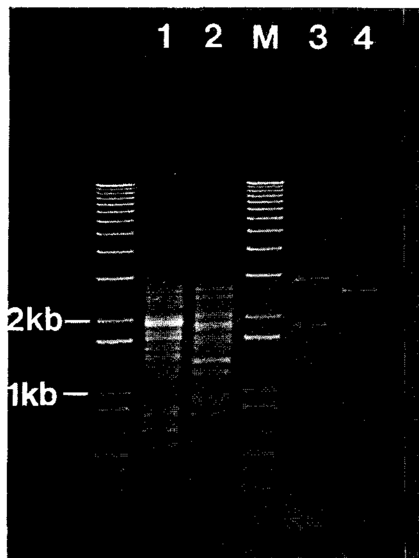

Fig. 2. Band patterns obtained from repetitive sequence-based PCR product. Lanes: 1, M0137 (BOXA1R); 2, *S. scabiei* ATCC 49173^T (BOXA1R); 3, M0137(REP-); 4, *S. scabiei* ATCC 49173^T(REP-); M, Ladder.

size of amplified DNA fragments were significantly different when the two REP primer sets were used.

The taxonomic definition of *Streptomyces scabiei* has been confused for many years. Strain IMRU 3018 (= ISP 5078) represented *S. scabiei* in the International *Streptomyces* project (ISP) of the 1960s (Shirling and Gottlieb, 1966; Gordon and Horan, 1968). A disproportionate number of "*S. scabies*" reference strains isolated from potatoes have subsequently been placed in other species such as *S. griseus*, *S. olivaceous*, and *S. aureofaciens* (Lambert and Loria, 1989; Takeuchi *et al.*, 1996). Thus, the species was considered invalid, and was listed in Bergey's Manual of Determinative Bacteriology, 8th ed. as species incertae sedis (Buchann and Gibbons, 1974). In 1989, Lambert and Loria (1989) proposed that the name *Streptomyces scabies* should be revived. They demonstrated that *S. scabies* strains were not closely related to any of the other strains phenotypically which caused potato scabs. Takeuchi *et al.* (1996) determined the phylogenetic relationship among *Streptomyces* spp. that caused potato scabs, and confirmed the previous study (Lambert and Loria, 1989).

Although strain M0137 is very similar to *S. scabies*, phenotypically and phylogenetically, it can be distinguished from the strains belonging to *S. scabiei* when genomic fingerprinting and G+C content are compared. Moreover, most of the strains of *S. scabiei* that were isolated from diverse geographical areas are pathogenic (Lambert and Loria, 1989; Takeuchi *et al.*, 1996). In contrast, strain M0137 shows weak pathogenicity on potato and produces a potent cytotoxic substance.

In conclusion, it is proposed to name M0137 as *S. scabiei* subsp. *chosunensis*. The description of *Streptomyces scabiei* subsp. *chosunensis* is as follows: *Streptomyces scabiei* subsp. *chosunensis* (M.L. adj. *chosunensis* from Chosun University). Aerial mycelium produces open spiral and grey spore chains, produces melanin and pectinase, does not grow at 45°C and is sensitive to rifampicin and penicillin G. Other phenotypic properties are given in Table 1. The G+C content of the DNA is 76.1 mol% (*Tm* method). Isolated from a soil collected in Chonnam, Korea. The type strain is M0137 (KCTC 9927).

This study was supported by research funds from Chosun University, 1998.

References

Buchann, R.E. and N. E. Gibbons (ed.). 1974. Bergey's Manual of Determinative Bacteriology, 8th ed. The Williams and Wilkins Co., Baltimore.

Chun, J. and M. Coodfellow. 1995. A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int. J. Syst. Bacteriol.* 45, 240-245.

Gordon, R.E. and C. Horan. 1968. A piecemeal description of *Streptomyces griseus* (Krainsky) Waksman and Henrichi. *J. Gen. Microbiol.* 50, 223-233.

Iwai, H. and Y. Takahashi. 1992. Selection of microbial sources of bioactive compounds. p. 281-302, *In* S. Oumra (ed.), The Search for bioactive compounds from microorganisms. Springer-Verlag, New York.

Kanzaki H., S. Yanagisawa, and T. Nitoda. 2000. Biosynthetic intermediates of the tetradehydro cyclic dipeptide albonoursin produced by Streptomyces albulus KO-23. *J. Antibiot.* 53, 1257-

1264.

Kawashiri, N., K. Toriizuka, I. Adachi, M. Ueno, K. Terasawa, and I. Jorikoshi. 1986. Effects of traditional crude drugs on fibrinolysis by plasmin: antiplasmin principles in Eupolyphaga. *Chem. Pharm. Bull.* 34, 2512-2517.

Kim, D., J. Chun, N. Sahin, Y.C. Hah, and M. Goodfellow. 1996. Analysis of thermophilic clades within the genus streptomyces by 16S ribosomal DNA sequence comparisons. *Int. J. Syst. Bacteriol.* 46, 581-587.

Lambert D.H. and R. Loria. 1989. *Streptomyces scabies* sp. nov., nom. rev. *Int. J. Syst. Bacteriol.* 39, 387-392.

Mandel, M. and J. Marmur. 1968. Use of ultrviolet absorbance temperature profile for determining the guanine plus cytosine content of DNA. *Methods Enzymol.* 12B, 195-206.

Matsushima Y., T. Nakayama, M. Fujita, R. Bhandari, T. Eguchi, K. Shindo, and K. Kakinuma. 2001. Isolation and structure elucidation of vicenistatin M, and importance of the vicenisamine aminosugar for exerting cytotoxicity of vicenistatin. *J. Antibiot.* 54, 211-219.

Mirabelli, C.K., H. Bartus, J.O.L. Bartus, R. Johnson, S.M. Mong, S.P. Sung, and S.T. Crooke. 1985. Application of tissue culture micrtitre test for the detection of cytotoxic agents from natural products. *J. Antibiot.* 38, 758-766.

Nakae K., Y. Yoshimoto, T. Sawa, Y. Homma, M. Hamada, T. Takeuchi, and M. Imoto. 2000. Migrastatin, a new inhibitor of tumor cell migration from *Streptomyces* sp. MK929-43F1. Taxonomy, fermentation, isolation and biological activities. *J. Antibiot.* 53, 1130-1136.

Nam, S.K. 2002. Isolation and characterization of nonadecanoic acid produced by *Streptomyces scabies* subsp. *chosunensis*. M.S. thesis, Chosun University.

O'Donnell, A.G. 1985. Numerical analysis of chemotaxanomic data, p. 403-414. *In* M. Goodfellow, D. Jones and F. G. Priest (eds.), Computer assisted bacterial systematics, Academic Press, London.

Paananen A., R. Mikkola, T. Sareneva, S. Matikainen, M. Andersson, I. Julkunen, M.S. Salkinoja-Salonen, and T. Timonen. 2000. Inhibition of human NK cell function by valinomycin, a toxin from Streptomyces griseus in indoor air. *Infect. Immun.* 68, 165-169.

Pitcher, D.G., N.A. Saunders, and R.J. Owen. 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett. Appl. Microbiol.* 8, 151-156.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4, 406-425.

Shirling, E.B. and D. Gottlieb. 1966. Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.* 16, 313-340.

Stackebrandt, E., D. Witt, C. Kemmerling, R.M. Kroppenstedt, and W. Liesack. 1991. Designation of streptomycete 16S and 23S rRNA-based target regions of oligonucleotide probes. *Appl. Environ. Microbiol.* 57, 1468-1477.

Takeuchi, T., H. Sawada, F. Tanaka, and I. Matsuda. 1996. Phylogenetic analysis of *Streptomyces* spp. causing potato scab based on 16S rRNA sequences. *Int. J. Syst. Bacteriol.* 46, 476-479.

Van Wezel, G.P., E. Vijgenboom, and L. Bosch. 1991. A comparative study of the ribosomal RNA operons of *Streptomyces coelicolor* A3(2) and sequence analysis of rRNA. *Nucleic Acids Res.* 19, 4399-4403.

Versalovic, J., M. Schneider, F.J. Bruijn, and J.R. Lupski. 1994. Genomic fingerprinting of bacteria using repitative sequence-based polymerase chain reaction. *Methods Mol. Cell. Biol.* 5, 25-40.

Williams, S.T., M. Goodfellow, G. Alderson, E.M.H. Wellington, P.H.A. Sneath, and M.J. Sackin. 1983. Numerical classification of *Streptomyces* and related genera. *J. Gen. Microbiol.* 129, 1743-1813.