J. Microbiol. Biotechnol. (2001), **11**(4), 677–684

JOURNAL OF
MICROBIOLOGY
AND
BIOTECHNOLOGY

Identification of Mutanase-Producing *Microbispora rosea* from the Soil of Chonnam Province

CHUNG, JIN[1], HONG-HEE KIM[2], JU-HYE SHIN[2], HYUN-CHUL LEE[3], ZANG HEE LEE[2], AND JONG-SUK OH[3]*

[1]*Department of Oral Microbiology, College of Dentistry, Pusan University, Pusan 609-735, Korea*
[2]*Department of Microbiology and Immunology, College of Dentistry, Chosun University, Kwangju 500-757, Korea*
[3]*Department of Microbiology, College of Medicine, Chonnam University, Kwangju 501-190, Korea*

Received: April 2, 2001
Accepted: July 2, 2001

Abstract To isolate mutanase-producing bacteria, soil samples were collected from several areas in Chonnam Province, South Korea. A total of 70 strains of actinomycetes were isolated from the soil samples. All isolated actinomycetes were inoculated on mutanase screening media to identify new bacterial strains producing mutanase activity. One strain in particular exhibited a strong mutanase-producing activity, and was identified as *Microbispora rosea* based on its morphological, cultural, and physiological characteristics, and also by 16S rDNA sequences.

Key words: Mutanase, *Microbispora rosea*

It has already been established that bacterial plaque is the main etiological factor in dental caries and inflammatory periodontal diseases [20, 31]. Human dental plaques are composed of closely packed bacteria and noncellular matrix material [19]. A dental plaque matrix contains polysaccharides of which glucans, including in the adherence and colonization of a large proportion of α-1,3 linkages, seem to play a significant role for oral bacteria on tooth surfaces [5].

Cariogenic mutans streptococci produce extracellular water-insoluble (mutan) and water-soluble (dextran) glucans from sucrose through the action of glucosyltransferases [5]. Water-insoluble glucan is highly adherent, promotes the film adherence and accumulation of microorganisms on tooth surfaces [37], and possesses a branched structure containing a high proportion of α-1,3 as well as α-1,6 linkages [37]. Several preparations of α-1,6-D-glucanase (dextranase) and α-1,3-D-glucanase (mutanase) have been tested for their ability to inhibit the formation of dental plaque and prevent dental caries or periodontitis in animals and humans [8-10, 14, 23].

*Corresponding author
Phone: 82-62-220-4134; Fax: 82-62-228-7294;
E-mail: joh@chonnam.ac.kr

The breakdown of the plaque matrix would presumably result in the dispersion and removal of the plaque. The enzymatic destruction of plaques using dextranase has already been attempted, yet dextranase treatment has failed to demonstrate a significant effect on the prevention of plaque formation in humans [24]. Dextranase-producing microorganisms appear to be abundant in samples of dental plaques. Oral bacteria capable of demonstrating dextranase activity include many streptococci [32], *Actinomyces israelii* [33], and *Bacteroides ochraceus* [34]. Furthermore, a variety of microorganisms, belonging to the genera *Trichoderma* [7], *Aspergillus* [39], *Streptomyces* [1], *Flavobacterium* [2], and *Actinomycetes* [18], produce cell-bound as well as cell-free hydrolases active against α-1,3-glucans. Some of these enzymes have been purified, and their substrate specificities and modes of action identified. These enzyme preparations may provide a useful tool for eliminating dental plaques, thereby preventing the initiation of dental caries.

The current study isolated a bacterial strain from soil microorganisms that produce mutanase. Based on the morphological, cultural, and physiological characteristics and 16S rDNA sequences, the strain was identified as *Microbispora rosea*.

Materials and Methods

Isolation of Mutanase-Producing Bacteria

Soil samples collected from several sites in Chonnam Province, Korea, were dispersed in sterile water and spread onto actinomycetes isolation agar plates. The plates were incubated at 27°C for 10–15 days, and the resulting colonies isolated. The isolates were transferred every month onto the slope of a Bennett's agar medium (Difco Lab, Detroit, MI, U.S.A.) and kept in a refrigerator. To identify those strains producing mutanase, the isolates were inoculated

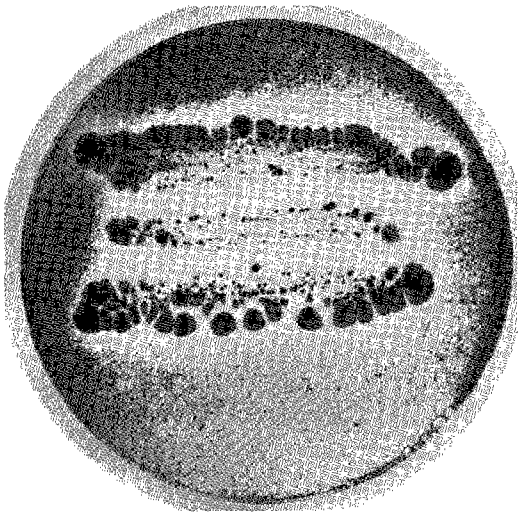

Fig. 1. Clear zone detected around microorganisms producing mutanase on mutanase screening medium.

on mutanase screening media and incubated at 27°C for 5-7 days. The mutanase-producing strains displayed clear zones around each colony, resulting from mutan-hydrolyzing activity (Fig. 1).

Media

The mutan and blue mutan for the mutanase screening medium were prepared by the methods of Takehara *et al.* [36] and Yang and Chung [38]. The mutanase screening medium was made of a minimal essential agar containing 0.5% ammonium sulfate, 0.05% K_2HPO_4, 0.03% KOH, 0.02% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$, 0.0001% glucose, and 1.5% agar, and overlaid with a 0.6% agar containing blue mutan.

Morphology

The morphological characteristics of the isolate were observed under a light microscope. In addition, the mycelia were grown for 14 days on an ISP2 agar for examination by scanning electron microscopy. The specimens for electron microscopy were prepared by the method of Itoh *et al.* [12].

Cultural and Physiological Characteristics

To determine the cultural characteristics, the isolated strains were cultured on an ISP medium (Difco Lab, Detroit, MI, U.S.A.) and Bennett's agar medium for 7, 14, or 21 days. After each incubation period, the growth status, aerial mycelium color, reverse color, and production of soluble pigment were recorded [6]. Analysis of cell wall was performed using the method of Lechevalier and Lechevalier [17]. Diaminopimelic acid in the cell wall peptidoglycan was detected by cellulose TLC. The temperature range for growth was observed on an agar medium containing 1% starch, 0.2% yeast extract, and 2% agar (pH 7.0). The method by Shirling and Gottlieb [27] was applied to assess optimal temperature for growth and to determine whether carbohydrates were utilized as the sole carbon source.

16S rDNA Sequencing

Chromosomal DNA extraction: The extraction of chromosomal DNA from an isolated strain was carried out as described previously [30]. Two-hundred ml of the culture was harvested, resuspended in 0.3 ml of a TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 7.5), incubated at 65°C for 20 min, and added to a mixture of lysozyme-mutanolysin (40 mg/ml lysozyme, 200 U/ml mutanolysin) for 1 h at 37°C prior to the addition of 10% sodium dodecyl sulfate and proteinase K (20 mg/ml). The mixture was added to a CTAB (hexadecyltrimethyl ammonium bromide)/NaCl solution, incubated for 10 min at 65°C, and extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) mixture. The crude DNA fraction was recovered using a 0.6-fold volume of isopropanol. The precipitated DNA was washed, dried, and resuspended in the TE buffer.

PCR amplification of 16S rDNA and cloning of amplified 16S rDNA: The 16S rDNAs were amplified from the chromosomal DNA, as prepared above, using a PCR. The oligonucleotide primers for PCR amplification were designed to include sequences of a region known to be highly conserved between species [15, 26]. The sequences of the PCR primers used were 5'-AGAGTTTGATCCTGGCTC-AG-3' (nucleotide positions 8 to 27 of the *Escherichia coli* 16S rRNA gene) and 5'-TACGG(C/T)TACCTTGTTAC-ACTT-3' (nucleotide positions 1,492 to 1,513 of the *Escherichia coli* 16S rRNA gene). The PCR reaction was performed using a thermal cycler (Perkin-Elmer 480) for 35 cycles. The amplification program was as follows: denaturation at 94°C for 1 min, primer annealing at 60°C for 1 min, and extension at 72°C for 2 min. The amplified PCR product was recovered, purified using a GENECLEAN kit (Bio101), and ligated to a TA cloning vector (Invitrogen). After transformation and selection in ampicillin, the plasmid DNA was isolated and purified from cultures of the ampicillin-resistant colonies.

Sequencing of double-stranded 16S rDNA and phylogenetic analysis: The isolated plasmid DNA was sequenced with an automatic DNA sequencer (ABI 310, Perkin-Elmer) using the primers T7 and M13 reverse. The 16S rDNA sequences were manually aligned with selected sequences (Fig. 4) obtained from the Ribosomal Database Project release 4 [16]. A phylogenetic tree was constructed using the neighbor-joining method [13, 25]. In order to determine the stability of the resultant phylogenetic tree, the sequence data were repeatedly sampled 1,000 times for a bootstrap analysis [4].

Fig. 2. Light micrograph of spore chains of *Microbispora rosea* on ISP2 agar incubated at 28°C for 2 weeks (×400).

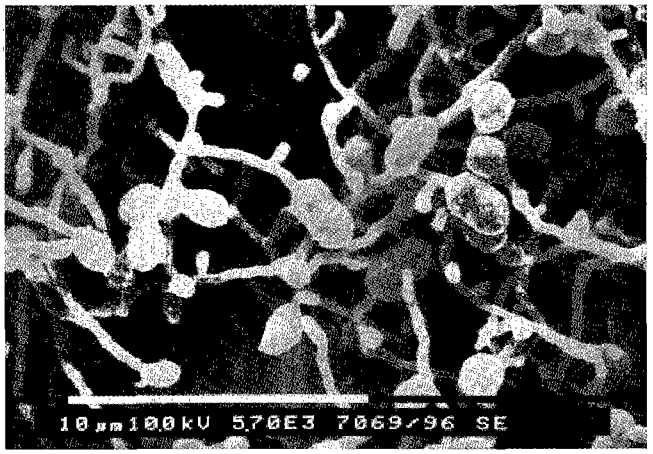

Fig. 3. Scanning electron micrograph of spore chains of *Microbispora rosea* on ISP2 agar incubated at 37°C for 2 weeks (×57,000).

RESULTS

Isolation of Mutanase-Producing Strain

To isolate strains producing mutan hydrolytic activity, soils from the Chonnam Province area were collected. The soil bacteria were isolated from the collected samples and the isolates screened on a blue mutan agar for their ability to create a clear zone. Among the 70 strains isolated, one strain named OJ-05 displayed a clear zone on the blue mutan media (Fig. 1). After selecting this strain, common procedures for identification were taken following *Bergey's Manual of Systemic Bacteriology.*

Table 2. Physiological characteristics of isolated strain (OJ-05).

General	
Liquefaction of gelatin	Negative
Peptonization of milk	Negative
Reduction of nitrate	Positive
Hydrolysis of starch	Negative
Hydrolysis of skim milk	Positive
Cell chemistry	
Diaminopimelic acid	Meso-isomers
Menaquinones	**MK-9**(H4)
Phospholipids	Containing glucosamine
Growth temperature	
Growth range	20–40°C
Optimal growth	37°C
Carbohydrate utilization	
D-Glucose	+
L-Arabinose	+
D-Xylose	+/–
Inositol	–
D-Mannitol	+/–
D-Fructose	+/–
L-Rhamnose	+
Sucrose	+/–
Raffinose	–
D-Galactose	–
Cellobiose	–
Cellulose	–
Inulin	+
Melibiose	+/–

[a]+, utilized; +/–, doubtful; –, not utilized.

Morphological Observations

Morphological observation of the isolate cultured for 28 days on an ISP2 agar under light and electron microscopes showed two spores per chain (Figs. 2 and 3). The spores of

Table 1. Cultural characteristics of isolated strain (OJ-05).

Media	Growth[a]	Aerial mycelium	Reverse color
Yeast extract-malt extract (ISP No. 2)	++	Poor	Yellowish pink
Oatmeal agar (ISP No. 3)	++	Moderate (Pinkish white)	Yellowish pink
Inorganic salts-starch agar (ISP No. 4)	+	Moderate (Pale yellow)	Pinkish yellow
Glycerol-asparagine agar (ISP No. 5)	–	Poor	Pale yellow
Peptone-yeast extract-iron agar (ISP No. 6)	–	Poor	Pale yellow
Tyrosine agar (ISP No. 7)	–	Poor	Pale yellow
Glucose-asparagine agar	++	Moderate (Pinkish yellow)	Yellowish pink
Bennett's agar	++	Moderate (Pinkish yellow)	Yellowish pink

[a]++, good growth; +, growth; –, no growth.

the isolate were spherical (1.2–1.6 μm in diameter) and nonmotile. The surface of the spores was smooth.

Cultural and Physiological Characteristics

The cultural characteristics of the isolate are shown in Table 1. The isolate exhibited good growth on the ISP No. 2, 3, and 4 media, glucose-asparagine agar, and Bennett's agar, and produced yellow to pink colonies on most of the media tested. Aerial mycelia were formed on the ISP Nos. 3 and 4 media, glucose-asparagine agar, and Bennett's agar. The appearance of the colonies of the isolate was a pinkish aerial mass, but no soluble pigment was produced.

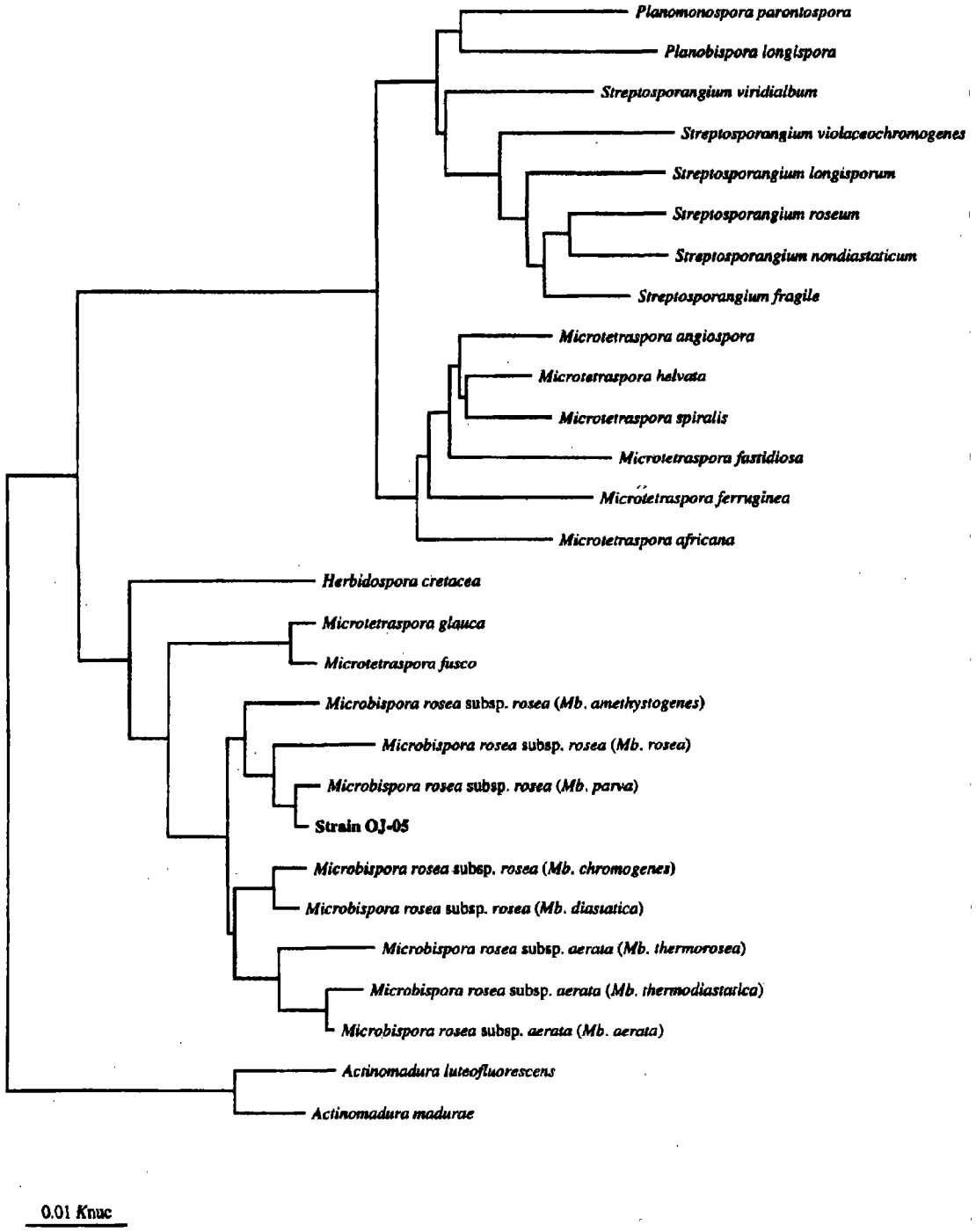

Fig. 4. Rooted tree showing phylogenetic positions of OJ-05, representatives of family *Streptosporangiaceae*, and two *Actinomadura* species as outgroup actinomycetes, based on 16S rDNA sequences.

Table 3. Levels of 16S rRNA gene similarity for strain OJ-05, representatives of genera *Microbispora*, *Microtetraspora*, and *Streptosporangium*, and related taxa.

Species	16S rRNA gene similarity to:																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
(1) Strain OJ-05																		
(2) *Mb.*[a] *rosea* subsp. *aerataa* (*Mb. aerata*)	98.9																	
(3) *Mb. rosea* subsp. *aerata* (*Mb. thermorosea*)	97.7	98.4																
(4) *Mb. rosea* subsp. *aerata* (*Mb. thermodiastatic*)	98.5	99.6	98.4															
(5) *Mb. rosea* subsp. *rosea* (*Mb. diastatica*)	98.2	98.0	98.2	97.7														
(6) *Mb. rosea* subsp. *rosea* (*Mb. rosea*)	98.9	97.9	97.0	98.2	97.7													
(7) *Mb. rosea* subsp. *rosea* (*Mb. amethystogenes*)	98.8	98.5	97.2	98.2	98.6	98.1												
(8) *Mb. rosea* subsp. *rosea* (*Mb. chromogenes*)	98.0	97.7	98.0	97.4	99.4	97.4	98.5											
(9) *Mb. rosea* subsp. *rosea* (*Mb. parva*)	99.6	98.9	97.8	98.9	98.2	98.9	98.9	98.0										
(10) *Microtetraspora africana*	95.7	95.2	94.6	94.8	95.6	95.2	95.7	95.5	95.5									
(11) *Microtetraspora angiospora*	96.1	95.7	95.8	95.4	96.8	95.3	96.1	96.5	96.1	97.7								
(12) *Microtetraspora glauca*	97.1	96.7	96.2	96.5	97.4	96.6	97.4	97.2	97.0	95.6	96.0							
(13) *Streptosporangium roseum*	95.2	94.8	94.0	94.5	94.8	95.3	95.0	95.0	94.9	96.4	95.4	94.6						
(14) *Streptosporangium violaceochromogenes*	94.8	94.7	94.7	94.7	94.8	94.7	94.7	94.8	94.6	95.7	95.5	94.9	97.1					
(15) *Streptosporangium viridialbum*	95.7	95.1	95.5	94.7	96.5	95.3	95.5	96.3	95.4	97.0	96.9	95.5	96.6	96.0				
(16) *Herbidospora cretacea*	97.3	97.1	96.3	96.7	96.7	97.1	97.0	96.7	97.0	95.7	96.2	96.5	95.3	95.5	96.0			
(17) *Planobispora longispora*	94.7	94.4	94.4	94.3	94.9	94.6	94.7	94.9	94.3	95.4	96.2	94.6	95.6	95.3	96.4	95.4		
(18) *Planomonospora parontospora*	95.2	95.0	95.1	94.6	95.3	95.1	94.8	95.3	94.6	95.7	95.9	95.0	97.1	96.3	96.9	95.5	96.8	
(19) *Actinomadura madurae*	94.8	94.4	94.0	94.4	94.4	94.0	94.7	94.4	94.6	95.4	94.8	94.6	93.7	93.9	94.1	94.7	93.2	93.7

[a]*Mb*, *Microbispora*.

The results of the analyses of the physiological characteristics are summarized in Table 2. The isolate was able to reduce nitrate and to hydrolyze skim milk. However, it could not liquefy gelatin, peptonize milk, or hydrolyze starch. The TLC analysis of the cell wall revealed that most of the diaminopimelic acid (DAP) was in the form of meso-isomers. The isolate contained MK-9(H4) as the predominant menaquinones along with glucosamine-containing phospholipids. The growth permissive temperature was between 20 and 40°C. As regards to the ability of carbohydrate utilization, the isolate could utilize glucose, arabinose, rhamnose, galactose, and inulin, yet not inositol, raffinose, cellobiose, and cellulose.

Phylogenetic Analyses (or 16S rRNA Gene Sequences)
The near complete 16S rDNA gene sequences of the isolate was consisted of 1,476 nucleotides. The result of the phylogenetic analysis of the isolate and some strains of the *Streptosporangiaceae* family are shown in Fig. 4. The 16S rDNA sequences of the isolated strain OJ-05 showed nearly identical sequence (97.7–99.6%) identity with those of *Microbispora rosea* (Table 3).

Accordingly, based on the morphological, cultural, and physiological characteristics, in addition to the 16S rDNA gene sequences, the mutanase-producing strain was identified as *Microbispora rosea*.

DISCUSSION

The cariogenic mutans streptococci secrete insoluble glucans in which the 1,3-linked glucose chain is predominant. Insoluble extracellular glucans have also been identified as an etiologic factor for dental plaque-induced oral diseases, such as dental caries and periodontal diseases. Efforts have been made to identify a mutan-hydrolyzing enzyme (mutanase) as a chemical method, rather than mechanical means, for dissolving insoluble glucans. A few mutanase-producing strains such as *Trichoderma harzianum* and *Pseudomonas* have been reported [7, 28]. Most of the studies reported to date have shown the efficacy of mutanases to reduce dental caries in rodents [7, 28-29]. Inoue *et al.* [11] showed that *Pseudomonas* mutanase also inhibits the formation of dental plaques in humans. Therefore, to find new bacterial strains producing mutanase activity, we have screened microorganisms from soil samples collected in the Chonnam province of Korea and identified a strain belonging to the *Microbispora* genus.

Nonomura and Ohara [22] described the genus *Microbispora* as an actinomycete that produces paired spores on aerial hyphae. *Microbipora rosea* exhibits aerial mycelia which was initially white and then become pale pink with the formation of spores on an oatmeal-yeast extract agar. Strain OJ-05 isolated in the present study also displayed the characteristic pinkish color of aerial mycelia (Table 2). The isolate also showed the typical morphological characteristics of *Microbispora rosea*; smooth spores and a pinkish aerial mass. The genus *Microbispora* can be separated into two groups based on the temperature range for growth [21]; either mesophilic or thermophilic (growth permissive at 55°C). The *Microbispora* strain OJ-05 grew well between 20–40°C and the optimal growth temperature was 37°C. Most species of the genus *Microbispora* are characterized by smooth-surfaced spores (smooth-spored species). As shown in Fig. 3, the strain isolated in the current study also exhibited smooth-surfaced spores. In some species of *Microbispora* the number of spores per chain is three, whereas smooth-spored species never have three spores per chain. The isolated strain OJ-05 had two spores per chain (Figs. 2 and 3).

The analysis of 16S rDNA sequences is well-accepted for studying the evolutionary relationships among microorganisms. This phylogenetic method has also been used successfully to resolve relationships among microorganisms at virtually all taxonomic levels [3, 35]. Therefore, the sequencing of the 16S rDNA of the isolated strain was also performed in the current study. The result of comparing the 16S rDNA sequences of strain OJ-05 with those of other species led to identifying the isolate as *Microbispora rosea*.

Despite the extensive development of dentistry, the major dental diseases, dental caries and periodontal diseases, still occur frequently. Recently, prevention rather than the treatment of diseases is being emphasized, therefore, methods to prevent diseases by removing etiological factors are being sought. To prevent dental caries and periodontal diseases, a variety of research on inhibiting dental plaque formation and dissolving already-formed plaques has been performed. Early efforts to regulate plaques using dextranase, based on the concept that the major component of dental plaques is dextran composed of dominant α-1,6-linked glucose chains, did not result in significant effects. Later, the focus of research extended to mutanase that can destroy insoluble mutan, since evidence was accumulated for the notion that mutan, in which α-1,3-linked glucose chains are dominant, is the major component of a dental plaque matrix.

In this study, a *Microbispora rosea* strain isolated from soil microorganisms exhibited a mutanase activity. The protein responsible for the mutanase was purified from a culture medium of the isolated strain (data not shown) and the characterization of the purified mutanase protein is currently underway. This protein may be applicable in the future to control dental plaques.

Acknowledgment

This study was supported by a grant from the 1998 Good Health Research and Development Project, Ministry of Health and Welfare, Republic of Korea.

REFERENCES

1. Bacon, J. S., D. Jones, V. C. Farmer, and D. M. Webley. 1968. The occurrence of α(1-3)glucan in *Cryptococcus*, *Schizosaccharomyces* and *Polyporus* species, and its hydrolysis by a *Streptomyces* culture filtrate lysing cell walls of *Cryptococcus*. *Biochim. Biophys. Acta* **158:** 313–315.
2. Ebisu, S., K. Kato, S. Kotani, and A. Misaki. 1975. Isolation and purification of *Flavobacterium* α-1,3-glucanase-hydrolyzing, insoluble, sticky glucan of *Streptococcus mutans*. *J. Bacteriol.* **124:** 1489–1501.
3. Embley, T. M. and E. Stackebrandt. 1994. The molecular phylogeny and systematics of the actinomycetes. *Annu. Rev. Microbiol.* **48:** 257–289.
4. Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39:** 783–791.
5. Gibbons, R. J. and S. B. Banghart. 1967. Synthesis of extracellular dextran by cariogenic bacteria and its presence in human dental plaque. *Arch. Oral Biol.* **12:** 11–23.
6. Goodfellow, M. 1989. Maduromycetes. pp. 2509–2551. *In* Williams, S. T., M. E. Sharpe, and J. G. Holt (eds.), *Bergey's Manual of Systematic Bacteriology.* Williams & Wilkins, Baltimore, MD, U.S.A.
7. Guggenheim, B. and R. Haller. 1972. Purification and properties of an α-(1-3) glucanohydrolase from *Trichoderma harzianum*. *J. Dent. Res.* **51:** 394–402.
8. Guggenheim, B., B. Regolati, and H. R. Muhlemann. 1972. Caries and plaque inhibition by mutanase in rats. *Caries Res.* **6:** 289–297.
9. Guggenheim, B., B. Regolati, R. Schmid, and H. R. Muhlemann. 1980. Effects of the topical application of mutanase on rat caries. *Caries Res.* **14:** 128–135.
10. Hull, P. S. 1980. Chemical inhibition of plaque. *J. Clin. Periodontol.* **7:** 431–442.
11. Inoue, M., T. Yakushiji, J. Mizuno, Y. Yamamoto, and S. Tanii. 1990. Inhibition of dental plaque formation by mouthwash containing an endo-α-1,3 glucanase. *Clin. Prev. Dent.* **12:** 10–14.
12. Itoh, T., T. Kudo, F. Parenti, and A. Seino. 1989. Amended description of the genus *Kineosporia*, based on chemotaxonomic and morphological studies. *Int. J. Syst. Bacteriol.* **39:** 168–173.
13. Kahng, H.-Y., J. J. Kukor, and K.-H. Oh. 2000. Physiological and phylogenetic analysis of *Burkholderia* sp. HY1 capable of aniline degradation. *J. Microbiol. Biotechnol.* **10:** 643–650.
14. Kelstrup, J., P. Holm-Pedersen, and S. Poulsen. 1978. Reduction of the formation of dental plaque and gingivitis in humans by crude mutanase. *Scand. J. Dent. Res.* **86:** 93–102.
15. Lane, D. J., B. Pace, G. J. Olsen, D. A. Stahl, M. L. Sogin, and N. R. Pace. 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc. Natl. Acad. Sci. USA* **82:** 6955–6959.
16. Larsen, N., G. J. Olsen, B. L. Maidak, M. J. McCaughey, R. Overbeek, T. J. Macke, T. L. Marsh, and C. R. Woese. 1993. The ribosomal database project. *Nucleic Acids Res.* **21:** 3021–3023.
17. Lechevalier, M. P. and H. Lechevalier. 1970. Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int. J. Syst. Bacteriol.* **20:** 435–443.
18. Lee, Z. H. and C. H. Chung. 1993. Isolation and preliminary studies on mutanase-producing Actinomycetes. *J. Oral Biol. (Seoul)* **17:** 45–49.
19. Listgarten, M. A. 1976. Structure of surface coatings on teeth. A review. *J. Periodontol.* **47:** 139–147.
20. Loe, H., F. R. Von der Fehr, and C. R. Schiott. 1972. Inhibition of experimental caries by plaque prevention. The effect of chlorhexidine mouthrinses. *Scand. J. Dent. Res.* **80:** 1–9.
21. Miyadoh, S., S. Amano, H. Tohyama, and T. Shomura. 1990. A taxonomic review of the genus *Microbispora* and a proposal to transfer two species to the genus *Actinomadura* and to combine ten species into *Microbispora rosea*. *J. Gen. Microbiol.* **136:** 1905–1913.
22. Nonomura, H. and Y. Ohara. 1957. Distribution of actinomycetes in soil. II. *Microbispora*, a new genus of *Streptomycetaceae*. *J. Ferment. Technol.* **35:** 307–311.
23. Nyman, S., J. Lindhe, and J. C. Janson. 1972. The effect of a bacterial dextranase on human dental plaque formation and gingivitis development. *Odontol. Rev.* **23:** 243–252.
24. Poulsen, S., P. Holm-Pedersen, and J. Kelstrup. 1979. Comparison of different measurements of development of plaque and gingivitis in man. *Scand. J. Dent. Res.* **87:** 178–183.
25. Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4:** 406–425.
26. Seo, W.-T., G.-G. Kahng, S.-H. Nam, S.-D. Choi, H.-H. Suh, S.-W. Kim, and Y.-H. Park. 1999. Isolation and characterization of a novel exopolysaccharide-producing *Paenibacillus* sp. WN9 KCTC 8951P. *J. Microbiol. Biotechnol.* **9:** 820–825.
27. Shirling, E. B. and D. Gottlieb. 1966. Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.* **16:** 313–340.
28. Simonson, L. G., R. W. Gaugler, B. L. Lamberts, and D. A. Reiher. 1982. Purification and properties of endo-1,3-α-D-glucanase from *Pseudomonas*. *Biochim. Biophys. Acta* **715:** 189–195.
29. Simonson, L. G., B. L. Lamberts, D. A. Reiher, and R. G. Walter. 1983. Prevention of dental caries in hamsters by an endo-1,3--D-glucanase. *J. Dent. Res.* **62:** 395–397.
30. Shin, H.-S., J.-R. Ryu, Y.-S. Han, Y.-J. Choi, and Y. G. Yu. 1999. Random sequence analysis of the genomic DNA of *Methanopyrus kandleri* and molecular cloning of the gene encoding a homologue of the catalytic subunit of carbon monoxide dehydrogenase. *J. Microbiol. Biotechnol.* **9:** 404–413.
31. Socransky, S. S. 1970. Relationship of bacteria to the etiology of periodontal disease. *J. Dent. Res.* **49:** 203–222.
32. Staat, R. H. and C. F. Schachtele. 1974. Evaluation of dextranase production by the cariogenic bacterium *Streptococcus mutans*. *Infect. Immun.* **9:** 467–469.

33. Staat, R. H. and C. F. Schachtele. 1975. Characterization of a dextranase produced by an oral strain of *Actinomyces israelii*. *Infect. Immun.* **12:** 556–563.
34. Staat, R. H. and C. F. Schachtele. 1976. Analysis of the dextranase activity produced by an oral strain of *Bacteroides ochraceus*. *J. Dent. Res.* **55:** 1103–1110.
35. Stackebrandt, E., W. Liesack, and D. Witt. 1992. Ribosomal RNA and rDNA sequence analyses. *Gene* **115:** 255–260.
36. Takehara, T., M. Inoue, T. Morioka, and K. Yokogawa. 1981. Purification and properties of endo-α-1,3-glucanase from a *Streptomyces chartreusis* strain. *J. Bacteriol.* **145:** 729–735.
37. Yakushiji, T., M. Inoue, and T. Koga. 1984. Inter-serotype comparison of polysaccharides produced by extracellular enzymes from *Streptococcus mutans*. *Carbohydr. Res.* **127:** 253–266.
38. Yang, K. H. and J. Chung. 1996. A study about the induction from *Streptomyces*. *J. Korean Acad. Ped. Dent.* **23:** 764–773.
39. Zonneveld, B. J. 1972. A new type of enzyme, and exo-splitting-1,3 glucanase from non-induced cultures of *Aspergillus nidulans*. *Biochim. Biophys. Acta* **258:** 541–547.