The Journal of Microbiology, February 2007, p. 6-10
Vol. 45, No. 1


# Production and Biological Activity of Laidlomycin, Anti-MRSA/VRE Antibiotic from *Streptomyces* sp. CS684

**Jin Cheol Yoo[1], Jun Ho Kim[1], Jung Wan Ha[1], Nae Soo Park[1], Jae Kyung Sohng[2], June Woo Lee[3], Seong Chan Park[4], Mi Sun Kim[4], and Chi Nam Seong[4,*]**

[1]*Department of Pharmacy, Chosun University, Gwangju 501-759, Republic of Korea*
[2]*Department of Pharmaceutical Engineering, Sunmoon University, Chungnam 337-708, Republic of Korea*
[3]*Department of Environmental Engineering, College of Applied Science, Catholic University of Pusan, Pusan 607-757, Republic of Korea*
[4]*Department of Biology, College of Natural Sciences, Sunchon National University, Jeonnam 540-742, Republic of Korea*

(Received September 26, 2006 / Accepted January 1, 2007)

**Culture broth of a streptomycete isolate, *Streptomyces* sp. CS684 showed antibacterial activity on methicilin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant enterococci (VRE). Among purified substances from the organism, CSU-1, which is active against MRSA and VRE, is a $C_{37}H_{62}O_{12}Na$ ($M^+$, 721.3875), and identified as laidlomycin. The anti-MRSA and anti-VRE activity of CSU-1 was stronger than oxacillin and vancomycin. Phylogenetic analysis showed that strain CS684 is very similar to *Streptomyces ardus* NRRL 2817$^T$, whereas the ability of *Streptomyces* sp. CS684 to produce laidlomycin was shown to be unique.**

***Keywords***: Anti-MRSA, Anti-VRE, polyether antibiotic, laidlomycin, *Streptomyces* sp. CS684

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 (Yoo *et al.*, 2002).

Over the last few years, intrinsic or acquired resistance of enterococci and *Staphylococcus aureus* to many antibiotics, in particular, to β-lactams and glycopeptides, has become a major cause of concern. MRSA (methicilin resistant *Staphylococcus aureus*) and VRE (vancomycin resistant enterococci) cause a health risk, especially in patients with severe underlying disease or immunosuppression (Chadwick *et al.*, 1996; Coque *et al.*, 1996).

MRSA immerged from the time methicillin was used first in Europe, and its presence has increasingly been detected throughout the world (Franciolli *et al.*, 1991). Up to 80% of the nosocomial infections in hospital were due to MRSA (Kim, 2004). In addition to MRSA, from the first report on VRE in 1986 in Europe, their presence has also been increasingly detected worldwide (Van Horn *et al.*, 1996). In Korea, a VRE strain, *Enterococcus durans* was first isolated in 1992. Since then, the prevalence of VRE in hospitalized patients has been rising significantly (Kim *et al.*, 1995; Seong *et al.*, 2004).

Furthermore, there are cases on record of the isolation of MRSA and VRE from animals and environmental sources. Wide use of antimicrobial agents as feed additives for growth promotion in animal husbandry (Van den Boogaard and Stobberingh, 1996) was one of the reasons. The conjugative transfer of high level vancomycin resistance from *E. faecalis* to *S. aureus* (Nobel *et al.*, 1992) also supports the spreading of MRSA and VRE. Ultimately, increasing use of antimicrobial agents in human medicine and as animal growth promoters has been related to the emergence of MRSA and VRE (Murray, 1995).

To control antibiotic resistant pathogens, polyether ionophore antibiotics such as monensin, laidlomycin, and salinomycin were developed and used as animal feed (Wampler *et al.*, 1998).

This study was aimed to screen an actinomycete strain producing potent antibiotic substance active against VRE and MRSA. Hundreds of streptomycete isolates were obtained from soil samples from various locations in the province of Jeonnam, Korea. Among them, one streptomycete isolate indexed as CS684 in our culture collection was found to produce a potent antibacterial substance against VRE and MRSA.

## Materials and Methods

### *Isolation and identification of microorganism*

Actinomycete isolates were inoculated into OSYM medium consisting of 2% oatmeal, 1% dried yeast, 1% mannitol, 1% soybean meal, and 0.2% $CaCO_3$ (pH 7.0), and incubated at 30°C on a rotary shaker at 180 rpm. The anti-MRSA/VRE activity of cultured broths was assayed by the paper disc diffusion method. Strain CS684 showed highest anti-MRSA/VRE activity among the isolates screened and then selected for the study of antibiotic production.

Morphological and physiological properties of strain CS684 were determined as described by 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, USA) (Shirling and Gottlieb, 1966).

* To whom correspondence should be addressed.
(Tel) 82-8-61-750-3613; (Fax) 82-2-61-750-5459
(E-mail) scnu@scnu.ac.kr

Chromosomal DNA was isolated from the test strain using a procedure by Chun and Goodfellow (1995). 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, USA) 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 (Applied Biosystems, model 373A, USA) and software provided by the manufacturer. The combined 16S rRNA gene sequence was aligned manually against the corresponding sequences of representatives of the genus *Streptomyces*. A phylogenetic tree was inferred from the regions available for all sequences (positions 28-1433; *Escherichia coli* numbering system) using the neighbour-joining (Saitou and Nei, 1987) method. Evolutionary distance matrices were generated according to Jukes and Cantor (1969). The resultant tree topology was evaluated by bootstrap analyses (Felsenstein, 1981) of the neighbour-joining data based on 1,000 resampling. The alignment and phylogenetic analyses were carried out using the jPHYDIT program (Jeon *et al.*, 2005; http://chunlab.snu.ac.kr/jphydit).

### *Isolation of antibiotics*

Fermentation was carried out at 30°C on OSYM medium using a jar fermenter (Ko Biotec, KF-7L, Korea). To isolate antibiotics, 7 day cultured broth (10 L) of *Streptomyces* sp. CS684 was centrifuged at 10,000×g for 20 min. A mycelial cake (2.2 kg, wet weight) was suspended in 80% (v/v) acetone solution, stored at -20°C for 24 h, and then centrifuged at 10,000 × g for 10 min to obtain an aqueous-acetone solution. Aqueous solution was combined with culture filtrate and extracted twice with 5 L of ethylacetate. The combined solution was evaporated and dried using a rotary evaporator at 35°C. Dried residue (4 g) was washed twice with 300 ml of n-hexane and then dissolved with 500 ml of dichloromethane. Dichloromethane fraction was evaporated, dried *in vacuo* and the residue (3 g) was dissolved with 20 ml of methanol.

Further purification of the antibiotics was carried out by gel filtration on Sephadex LH-20 (777 ml, 3.0×110 cm) and then by silica gel column chromatography (Merck Kieselgel, 68 ml, 2.2×18cm). After washing column with hexane-dichloromethane (1:4), two active principles (FI and FII) were eluted separately from the column with hexane-dichloromethane-methanol (1:45:1). FI was concentrated to dryness *in vacuo* and crystallized. After washing with ice-cold hexane, 50 mg of FI (CSU-1) was obtained as a colorless prism.

### *Instrumental analysis of the antibiotic*

The elemental analysis of the CSU-1 was performed at the Korea Basic Science Institute (KBSI, Korea). The FT-IR spectra were recorded using a Shimazu FTIR-8101 spectrometer (Shimazu, Japan). The TLC was performed using an aluminum plate coated with silica gel 60 F254 (Merck, German), and a mixture of hexane and ethylacetate (1:1, v/v) as the developing solution. All mass spectrometric analyses were performed using a JMS-HM110/110A tandem mass spectrophotometer (JEOL, Japan), a four-sector instrument with an E1B1E2B2 configuration. The ion source was operated at a 10 KeV accelerating voltage in positive-ion mode with a mass resolution of 1000 (10% valley). The NMR spectra, including homo ($^1$H-$^1$H) and hetero ($^1$H-$^{13}$C) COSY were obtained on VARIAN UNITY-300 INOVA spectrometers (Varian, USA) with $CDCl_3$ solutions at ambient temperature ($^1$H NMR at 300 MHz and $^{13}$C NMR at 75 MHz).

### *Antimicrobial activity*

Clinical strains of MRSA provided by Daewoong Pharmaceutical Co, and chicken intestinal strains of VRE provided by Wonkwang University Hospital, Korea (Seong *et al.*, 2004) were used as target microorganisms for the test of antimicrobial activity of CSU-1. In addition, 10 bacterial strains were used as reference. Antibiotics (Sigma, USA) were used to compare the antimicrobial activity. MIC's of the CSU-1 and reference antibiotics were determined using agar dilution methods.

## Results and Discussion

### *Identification of strain CS684*

Strain CS684 (=KCTC 10631BP) formed highly branched substrate mycelia and rarely branched aerial hyphae which carried straight and white spore chains. This strain did not produce melanin and diffusible pigment. This strain could not grow below 10°C or above 45°C. The strain could grow in the presence of phenol (0.1%), potassium tellurite (0.001%), thallous acetate (0.001%), and crystal violet (0.0001%), but not sodium azide (0.01%). Strain CS684 was resistant to oleandomycin and penicillin-G, but sensitive to neomycin

**Table 1.** Phenotypic characteristics that differentiate strain CS684 from its nearest phylogenetic neighbors

| Characteristic | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Aerial mycelium color | White | Light gray | Bald | Beige |
| Melanin production | − | + | + | + |
| Hydrolysis of Xanthine | − | − | + | + |
| Growth test: | | | | |
| 10°C | − | + | + | + |
| 28°C | + | + | ND | + |
| 42°C | - | + | − | + |
| NaCl (3%, w/v) | + | + | + | ND |
| NaCl (5%, w/v) | ND | + | + | − |
| Utilization of : | | | | |
| Galactose | − | + | + | + |
| Sucrose | − | v | − | + |
| Inositol | − | + | + | + |
| Melibiose | − | v | − | − |
| Lactose | + | − | − | − |
| Acetate | − | + | + | + |
| Citrate | − | + | + | + |
| Propionate | − | + | + | + |

Species: 1, strain CS684; 2, *Streptomyces ardus* NRRL 2817 $^T$; 3, *S. blastmyceticus* NRRL B-5480 $^T$; 4, *S. parvisporogenes* NRRL B-5464$^T$. Data from Labeda *et al.* (1997). +, positive; -, negative; v, variable; ND, not determined

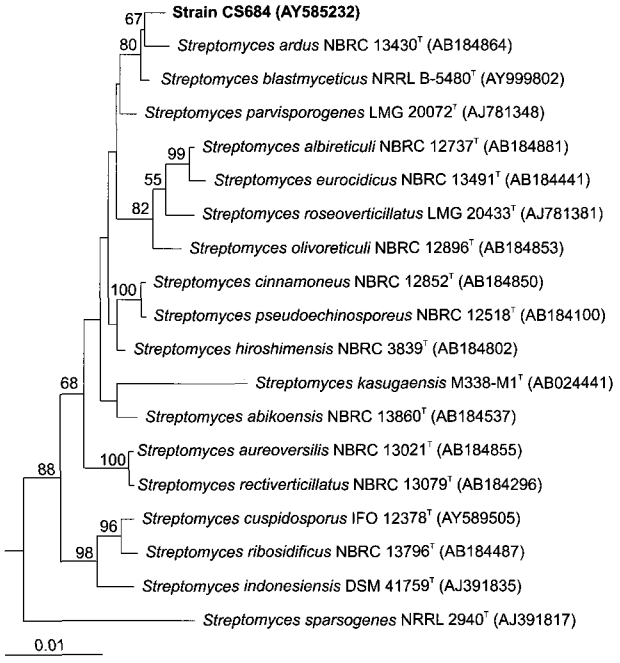


**Fig. 1.** Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationship between strain CS684 and related taxa within the genus *Streptomyces*. Numbers at the nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1,000 resampled dataset : only values more than 50% are given. *Nocardia nova* JCM 6044$^T$ (Z36930) was used as an outgroup (not shown). Bar, 0.01 nucleotide substitution per nucleotide position.

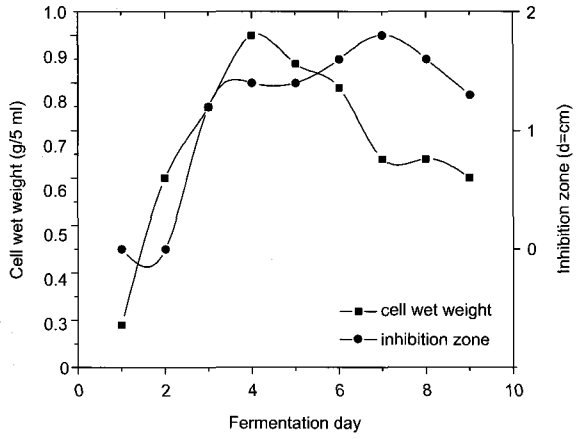


**Fig. 2.** Production profiles of anti-MRSA substances from culture broth of *Streptomyces* sp. CS684.

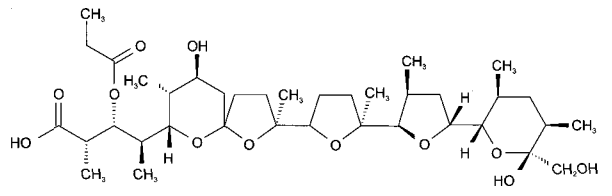

**Fig. 3.** Structure of CSU-1 (laidlomycin), isolated from *Streptomyces* sp. CS684.

**Table 2.** Physicochemical properties of CSU-1 (laidlomycin)

| | |
|---|---|
| Appearance | white powder |
| Molecular formula | $C_{37}H_{62}O_{12}$ |
| FAB-MASS (positive) | m/z=721.3875 (M sodium salt) |
| Elemental analysis | |
| Found: | C 63.65%, H 8.88% 65-67℃ |
| FT-IR (in KBr pellet) | 2982, 2930, 2877, 1730, 1570, 1469 and 1448 $cm^{-1}$ |

**Table 3.** $^{13}C$ NMR data of CSU-1 and authentic laidlomycin in $CDCl_3$

| Carbon | | CSU-1 | laidomycin |
|---|---|---|---|
| 1 | | 180.1 | 179.98 |
| 2 | | 43.6 | 43.54 |
| 2' | (-$CH_3$) | 16.6 | 16.45 |
| 3 | | 75.8 | 75.70 |
| 3' | (C=O) | 173.7 | 173.51 |
| 3" | (-$CH_2$-) | 27.8 | 27.86 |
| 3"' | (-$CH_3$) | 9.3 | 9.33 |
| 4 | | 40.4 | 40.38 |
| 4' | (-$CH_3$) | 11.1 | 11.06 |
| 5 | | 68.4 | 68.29 |
| 6 | | 35.2 | 35.25 |
| 6' | (-$CH_3$) | 10.2 | 10.18 |
| 7 | | 70.6 | 70.58 |
| 8 | | 33.4 | 33.34 |
| 9 | | 107.0 | 106.99 |
| 10 | | 39.3 | 39.25 |
| 11 | | 33.2 | 33.16 |
| 12 | | 85.2 | 85.23 |
| 12' | (-$CH_3$) | 27.6 | 27.60 |
| 13 | | 81.5 | 81.52 |
| 14 | | 27.4 | 27.39 |
| 15 | | 30.4 | 30.33 |
| 16 | | 83.9 | 83.88 |
| 16' | (-$CH_3$) | 23.9 | 23.92 |
| 17 | | 86.4 | 86.46 |
| 18 | | 34.5 | 34.54 |
| 18' | (-$CH_3$) | 14.1 | 14.09 |
| 19 | | 33.0 | 33.02 |
| 20 | | 76.6 | 76.60 |
| 21 | | 74.5 | 74.54 |
| 22 | | 31.8 | 31.76 |
| 22' | (-$CH_3$) | 16.8 | 16.77 |
| 23 | | 35.6 | 35.63 |
| 24 | | 36.5 | 36.45 |
| 24' | (-$CH_3$) | 16.0 | 16.04 |
| 25 | | 98.2 | 98.14 |
| 26 | | 64.9 | 64.86 |

and rifampicin. The strain could degrade pectin, but not allantoin, urea, guanine, arbutine, and xylan. The strain produced lipase but not nitrate reductase. Strain CS684 could utilize fructose and lactose as sole carbon sources, but not manitol, L-rhamnose, raffinose, D-melezitose, adonitol, dextran, xylitol, L-arabinose, D-xlose, salicin, and sodium pyruvate. Strain

CS684 could utilize DL-α-amino-n-butryric acid, L-threonine, L-cysteine, L-valine, L-phenylalanine, L-histidine, L-hydroxyproline, and potassium nitrate as sole nitrogen sources. Culture broth of strain CS684 inhibited the growth of *Bacillus subtilis*, *S. auresus*, and *E. faecium*, but did not inhibit that of *Escherichia coli*, *Pseudomonas fluorescens*, and *Saccharomyces cerevisiae*. Other characteristics of the strain were described in Table 1.

Almost-complete 16S rRNA gene sequence (1,436 nt) of strain CS684 has been deposited in the GenBank database under accession number AY585232. Strain CS684 had similar 16S rDNA sequences with *Streptomyces ardus* NRRL $2817^T$ (99.2%), *S. blastmyceticus* NRRL B-$5480^T$ (99.4%), and *S. parvisporogenes* NRRL B-$5464^T$ (99.3%).

Kitame *et al.* (1974) identified new antibiotic, laidlomycin produced from *Streptomyces* S-822, which was very similar to *S. eurocidicus* var. *asterocidicus* (unpublished strain). However, *S. eurocidicus* showed relatively low similarity (98.6%) with strain CS684, comparable that of with *S. ardus* NRRL $2817^T$, *S. blastmyceticus* NRRL B-$5480^T$ and *S. parvisporogenes* NRRL B-$5464^T$, respectively.

Our isolate *Streptomyces* sp. CS684 is secondly known microorganism producing laidlomycin. Phylogenetically, this strain is very similar to the known species of the genus *Streptomyces* such as *S. ardus*, *S. blastmyceticus*, and *S. parvisporogenes* (Fig. 1). However, the physiological and morphological properties of strain CS684 are different from those of above species (Labeda *et al.*, 1997). Moreover, laidlomycin-producing ability of the isolate can be a unique character compared to those three neighbor species. For the recognition of *Streptomyces* sp. CS684 as new species or subspecies, DNA-DNA hybridization between the strain and the neighbors should be carried out.

### *Production of anti-MRSA antibiotic*

Growth of strain CS684 reached maximum after the incubation of 4 days, and then growth decreased. Strain CS684 secreted antibiotic substances after 48 h of cultivation, and its productivity continued to increase for 7 days of fermentation (Fig. 2). The pH of the medium was 6.0-6.5 at the time of maximum activity.

### *Physicochemical properties and chemical structure of CSU-1*

CSU-1 was obtained as a white solid with a melting point of 151-153°C. All the physico-chemical properties including FTIR data were summarized in Table 2. The positive FAB-MS spectra, $^1H$ NMR, $^{13}C$ NMR (Table 3), $^1H$-$^1H$ and $^1H$-$^{13}C$ COSY (HMQC and HMBC) NMR spectra suggest that the molecular formula of CSU-1 is $C_{37}H_{62}O_{12}Na$ ($M^+$, 721.3875), revealing that the substance CSU-1 is laidlomycin (Fig. 3).

### *Antimicrobial activity of CSU-1*

The antimicrobial activity of CSU-1 was determined against various kinds of microorganisms including MRSA and VRE. As shown in Table 4, CSU-1 (laidlomycin) showed antimicrobial activity against Gram-positive bacteria including MRSA and VRE. In contrast, it did not have antimicrobial activity against Gram-negative bacteria. These anti-microbial patterns of CSU-1 correlate that of polyether antibiotics, such as monensin, salinomycin, and narasin. Especially, CSU-1 and polyether antibiotics showed stronger anti-MRSA activity than oxacillin, which is clinically used against penicillin resistant bacteria. Also, CSU-1 and polyether antibiotics showed stronger anti-VRE activity than vancomycin, which is clinically used against MRSA.

The anti-MRSA/VRE activity of CSU-1 was similar to salinomycin, higher than monensin, and lower than narasin.

**Table 4.** MIC of CSU-1 (laidlomycin) against bacteria including MRSA and VRE (μg/ml)

| Compounds / Strains | CSU-1 | Monensin | Salinomycin | Narasin | Oxacillin | Vancomycin |
|---|---|---|---|---|---|---|
| *Enterococcus faecalis* ATCC 29212 | 2 | 8 | 2 | 0.25 | 0.25 | <2 |
| *Bacillus subtilis* ATCC 6633 | 4 | 8 | 2 | 0.25 | 0.25 | <2 |
| *Staphylococcus aureus* KCTC 1928 | 4 | 8 | 4 | 0.25 | 0.25 | <2 |
| *Micrococcus luteus* ATCC 9341 | 8 | 16 | 8 | 0.25 | 0.25 | <2 |
| *Mycobacterium smegmatis* ATCC9341 | 4 | 8 | 8 | 0.5 | 32 | <2 |
| *Salmonella typhimirium* KCTC 1925 | >128 | >128 | >128 | >128 | >128 | >64 |
| *Escherichia coli* KCTC 1923 | >128 | >128 | >128 | >128 | >128 | >64 |
| *Pseudomonas aeruginosa* KCTC 1637 | >128 | >128 | >128 | >128 | >128 | >64 |
| *Alcaligenes faecalis* ATCC 1004 | >128 | >128 | >128 | >128 | >128 | >64 |
| MRSA 694E* | 1 | 4 | 1 | 0.5 | 32 | <2 |
| MRSA 703E* | 1 | 4 | 1 | 0.5 | >32 | <2 |
| MRSA 693E* | 1 | 4 | 1 | 0.5 | >32 | <2 |
| MRSA 2-32* | 1 | 4 | 1 | 0.5 | >32 | <2 |
| MRSA 4-5* | 1 | 4 | 1 | 0.5 | 32 | <2 |
| VRE 82* | 16 | 16 | 16 | 8 | >32 | 64 |
| VRE 94* | 16 | 16 | 16 | 8 | >32 | 64 |
| VRE 98* | 16 | 16 | 16 | 8 | >32 | 64 |

* Sources of the Clinical isolates were described in Seong *et al.* (2004)

## Acknowledgements

This study was supported by the research grant of Chosun University, 2003.

## References

Chadwick, P.R., N. Woodford, E.B. Kacmarski, S. Gray, R.A. Barrell, and B.A. Oppenheim. 1996. Glycopeptide resistant enterococci isolated from uncooked meat. *J. Antimicrob. Chemother.* 38, 908-909.

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

Coque, T.M., J.F. Tomayko, S.C. Ricke, P.S. Okhyunsen, and B.E. Murray. 1996. Vancomycin-resistant enterococci from nosocomial, community, and animal sources in the United States. *Antimicrob. Agents Chemother.* 40, 2605-2609.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* 17, 368-76.

Franciolli, M., J. Bille, M. Glauser, and P. Moreill. 1991. Beta-lactam resistance mechanism of methicillin-resistance *Staphylococcus aureus. J. Infect. Dis.* 163, 514-523.

Jeon, Y.S., H. Chung, S. Park, L. Hur, J.H. Lee, and J. Chun. 2005. jPHYDIT: a JAVA-based integrated environment for molecular phylogeny of ribosomal RNA sequences. *Bioinformatics* 21, 3171-3173.

Jukes, T.H. and C.R. Cantor. 1969. Evolution of protein molecules. In *mammalian Protein Metabolism*, vol. 3, p. 21-132. *In* H.N. Munro. Academic Press, New York, USA

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.

Kim, S.A. 2004. Molecular genotyping and antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* isolated from patients specimen. Ms. D. thesis, Sunchon Nat. Univ. p. 10-15.

Kim, S.H., S.C. Kim, S.B. Gu, J.H. Shin, S.P. Shu, and D.W. Ryang. 1995. Four cases of vancomycin-resistant enterococci infection. *Kor. J. Clin. Pathol.* 15, S265.

Kitame, F., K. Utsushikawa, T. Koama, T. Saito, and M. Kikuchi. 1974. Laidlomycin, a new antimycoplasmal polyether antibiotic. *J. Antibiot.* 7, 884-888.

Labeda, D.P., M.P. Lechevalier, and R.T. Testa. 1997. *Streptomyces stramineus* sp. nov., a new species of the verticillate streptomycetes. *In. J. Syst. Bacteriol.* 47, 747-753.

Murray, B. 1995. What can we do about vancomycin-resistant enterococci? *Clin. Infect. Dis.* 20, 1134-1136.

Noble, W.C., Z. Virani, and R.G.A. Cree. 1992. Co-transfer of vancomycin and other resistance genes from *Enerococcus faecalis* NCTC 12201 to *Staphylococcus aureus. FEMS Microbiol. Lett.* 93, 195-198.

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

Seong, C.N., E.S. Shim, S.M. Kim, and J.C. Yoo. 2004. Prevalence and characterization of vancomycin-resistant enterococci in chicken intestines and humans of Korea. *Arch. Pharm. Res.* 27, 246-253.

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

Van den Boogaard, A.E. and E.E. Stobberingh. 1996. Time to ban all antibiotics as animal growth-promoting agents? *Lancet* 31, 619

Van Horn, K.G., C.A. Gedris, and K.M. Rodney. 1996. Selective isolation of vancomycin-resistant enterococci. *J. Clin. Microbiol.* 34, 924-927.

Wampler, J.L., S.A. Martin, and G.M. Hill. 1998. Effects of laidlomycin propionate and monensin on glucose utilization and nutrient transport by *Streptococcus bovis* and *Selenomonas ruminantium. J. Anim. Sci.* 76, 2730-2736.

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.

Yoo, J.C., J.M. Han, S.K. Nam, K.S. Baik, J.S. Jo, and C.N. Seong. 2002. Characterization of a streptomycete isolate producing the potent cytotoxic substance, nonadecanoic acid. *J. Microbiol.* 40, 178-181.