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Description of *Streptomyces neopeptinius* sp. nov., an Actinobacterium with Broad Spectrum Antifungal Activities

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A streptomycete strain producing broad-spectrum antifungal substances was taxonomically characterized. The strain, designated KNF 2047^T (= SH-09^T= KCTC 10586BP^T), was found to form extensively branching aerial and substrate mycelia, and produce spiny-ornamented spores with loose spiral chains. The whole cell hydrolyzates contained major amount of LL-diaminopimelic acid. The major fatty acids of the phospholipids were saturated and branched fatty acids containing 14~17 carbons, and the major isoprenoid quinones were hexa- and octa-hydrogenated menaquinones with 9 isoprene units. The phylogenetic analysis using the 16S rRNA gene indicated that the strain belongs to the genus *Streptomyces* but forms an independent phyletic line. These results clearly demonstrate that strain KNF2047^T forms a new center of taxonomic variation within *Streptomyces*, for which the name *Streptomyces neopeptinius* sp. nov. is proposed.

***Keywords*:** *Streptomyces neopeptinius*, neopeptin

The genus *Streptomyces* Waksman and Henrici 1943^{AL} is the largest prokaryotic genus, currently containing 490 validly described species (Euzéby, 2008, as of June 2008). Streptomycetes are mostly known as the representative antibiotic-producing prokaryotic group, while many other interesting features such as morphological differentiation including mycelial growth and formation of arthrospores, and genetic properties including linear chromosomes and genetic instability, have also been the subjects of scientific interests (Locci, 1987; Volff and Altenbuchner, 2000; Chen *et al*., 2002). The production of antifungal substances is a common phenomenon among streptomycetes, as a number of such substances have been discovered to date (Table 1).

Streptomyces sp. KNF 2047^T, an actinobacterium showing significant antifungal activity against powdery mildew fungi of cucumber, was isolated from soil and its antifungal substances were characterized in previous studies (Yoo and Kim, 2006; Kim *et al*., 2007). The substances were identified as neopeptins A and B, and their mixture exhibited good disease control activities against a number of plant pathogenic fungi (Kim *et al*., 2007). The strain itself and its use in the control of plant diseases caused by various fungi have already been patented (Yoo and Kim, 2006). In this study the taxonomic position of strain KNF 2047^T was determined by examining the morphological, physiological, chemotaxonomic and phylogenetic properties.

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Materials and Methods

Phenotypic characterization

The examination of morphological and biochemical properties of the strain followed the previously described procedures (Yoo and Kim, 2006). Modified Bennett's agar (0.5% starch, 0.1% malt extract, 0.1% yeast extract, 0.5% glucose, 0.1% N-Z amine, and 1.5% agar; pH 7.3) was used for morphological observation. Melanin production was tested in peptone-yeast extract-iron (ISP medium 6) agar, tyrosine (ISP medium 7) agar, and tryptone-yeast extract broth. The optimal growth temperature was determined using modified Bennett's agar medium.

Chemotaxonomic analysis

The major cell wall diamino acid was determined according to the method by Schaad (1985), and the composition of isoprenoid quinones by Komagata and Suzuki (1987). The fatty acid methyl esters were prepared and analyzed following the Sherlock Microbial Identification System (MIDI, Inc., USA).

Antimicrobial activities

For the test of antifungal activities against selected plant pathogens, the strain was grown in GSS medium (1% soluble starch, 2% glucose, 2.5% soybean meal, 0.4% beef extract, 0.2% NaCl, 0.025% potassium dihydrogen phosphate, and 0.2% calcium carbonate; pH 7.2). The shake flasks were cultivated at 25°C for 5 days with 250 rpm. Both the biomass and culture filtrates were tested for inhibition of fungal pathogens for selected crop plants (Yoo and Kim, 2006).

Table 1. Examples of antifungal compounds produced by species of *Streptoymces*

Antifungal substance	Species	Reference
Amphotericin A	*S. nodosus*	Trejo and Bennett (1963)
Antimycin A	*S. kitasawaensis*	Kluepfel *et al*. (1970)
Azalomycin	*Streptomyces* sp.	Arai and Hamano (1970)
Bafilomycin A2	*Streptomyces* sp.	Werner *et al*. (1984)
Cycloheximide	*S. griseus*	Abou Zeid (1972)
Demethoxyrapamycin	*S. hygroscopicus*	Sehgal *et al*. (1983)
Furanone	*Streptomyces* sp.	Schiewe and Zeeck (1999)
Kasugamycin	*S. kasugaensis*	Umezawa *et al*. (1965)
Leptomycins A and B	*Streptomyces* sp.	Hamamoto *et al*. (1983)
Meroparamycin	*Streptomyces* sp.	El-Naggar *et al*. (2006)
Nanaomycin E	*S. rosa*	Kasai *et al*. (1979)
Nikkomycins	*S. tendae*	Delzer *et al*. (1984)
Nystatin	*S. noursei*	Cohen and Webb (1952)
Phenamide	*S. albospinus*	Makkar *et al*. (1995)
Pimaricin	*S. natalensis*	Struyk *et al*. (1957~58)
Rapamycin	*Streptomyces* sp.	Vésina *et al*. (1975)
Sordarin	*S. avermitilis*, *S. capreolus*, and *S. fradiae*	Hall *et al*. (2001)
Trichomycin	*S. hachijoensis*	Hosoya *et al*. (1953)
Trichostatin	*Streptomyces* sp.	Tsuji *et al*. (1976)
Trichostatin C	*Streptomyces* sp.	Tsuji and Kobayashi (1978)
Venturicidins A, B	*S. aureo*	Brufani *et al*. (1971)

Phylogenetic analysis

The sequencing and phylogenetic analysis using 16S rRNA gene sequences followed the procedures previously described by Cho *et al*. (2006). From the BLAST search, 21 mostly related valid species of *Streptomyces* were selected, and the phylogenetic tree was inferred based on the 1,390 nucleotide positions (Fig. 1). The topology of the tree was evaluated using the maximum-likelihood method and bootstrap analysis (Felsenstein, 1993).

Results

Phenotypic properties

The strain formed extensively branching aerial and substrate mycelia, and grey spore mass on modified Bennett's agar. No characteristic color or mycelial fragmentation was observed for substrate mycelia. Production of any diffusible pigment with obvious color into the media was not observed. However, melanin was produced in both peptone-yeast extract-iron and tyrosine agar media. Melanoid pigments were also produced in tryptone-yeast extract broth. The aerial arthrospores formed open spiral-shaped chains, and the surface of individual spores was spiny-ornamented. The optimal growth temperature was in the range between 27 and 35°C.

The main biochemical and physiological properties of KNF 2047^T are presented in Table 2. The strain hydrolyzed pectin and hippuric acid, and also exhibited β-lactamase activity on YPG (2% peptone, 1% yeast extract, and 2% agar) and Beecham's Fs agars. Hydrogen sulfide was produced. However, lecithinase, protease, lipase or chitinase activity was not detected.

Chemotaxonomic analysis

The major cell wall diamino acid was found to be LL-diaminopimelic acid. MK-9[H_8] was the predominant isoprenoid quinone, and smaller amounts of MK-9[H_6], and MK-9[H_4] were also present. The major fatty acids were *iso*-$C_{14:0}$ (8.4±2.1%), *iso*-$C_{15:0}$ (12.0±1.1%), *anteiso*-$C_{15:0}$ (26.6±2.7%), *iso*-$C_{16:0}$ (23.8±2.5%), *iso*-$C_{17:0}$ (6.9±1.8%), and *anteiso*-$C_{17:0}$ (8.5±2.0%), and smaller amounts of $C_{18:3\omega6c}$ (1.8±0.4%), and *anteiso*-$C_{17:1}$ (1.3±0.1%) were also present.

Antimicrobial activities

Strain KNF 2047^T was found to be active against powdery mildew of tobacco (*Erisiphe tabacina*), cucumber (*Sphaerotheca fusca*), strawberry (*Sphaerotheca aphanis*), tomato (*Golocinomyces cichoracearum*), and Chinese matrimony vine (*Arthrocladiella mougeotii*). The strain was also active against a number of fungi-mediated plant diseases, including leaf blast of rice (*Magnaporte grisea*), fungal disease of tomato leaf (*Cladosporium fulvum*), anthracnose of cucumber (*Colletotrichum lagenarium*) and grape (*Glomerella cingulata*), wilt of cucumber (*Fusarium oxysporum*), leaf spot of apple (*Alternaria mali*), and brown spot of rice (*Cochliobolus miyabeanus*). However, no antagonistic activities were observed against the yeast or bacteria such as *Candida albicans*, *Bacillus subtilis*, and *Salmonella typhimurium*.

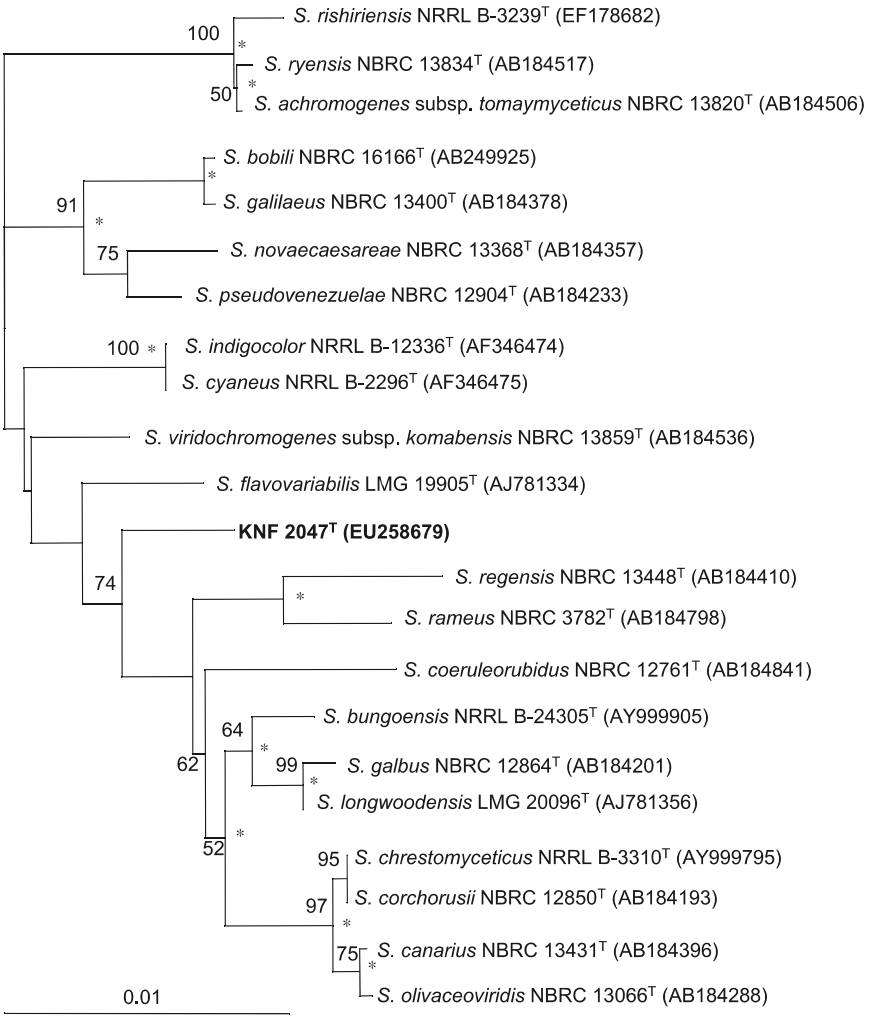

Fig. 1. A neighbor-joining tree based on 16S rRNA gene sequences of KNF 2047^T and related species of *Streptomyces*. Jukes-Cantor model was used for the estimation of evolutionary distances. Asterisks indicate the branches also recovered in maximum-likelihood trees, and numbers at nodes are the bootstrap values (%) based on 1,000 resamplings. The scale bar corresponds to the 0.01 substitutions per nucleotide position.

Phylogenetic analysis

Strain KNF 2047^T formed an independent phyletic line of its own, and the topology was supported by the maximum-likelihood method and high bootstrap value (74%). In the comparison of 16S rRNA gene sequences, KNF 2047^T was mostly related with *S. flavorariabilis* LMG 19905^T (99.2% sequence similarity, corresponding to 11 nucleotide difference), *S. bungoensis* NRRL B-24305^T (99.1%, 12 nucleotide difference), and *S. longwoodensis* LMG 20096^T (99.0%, 14 nucleotide difference). Seven other species shared 98.9% sequence similarity with KNF 2047^T.

Discussion

The main morphological and chemotaxonomic properties of strain KNF2047^T were consistent with those of *Streptomyces* (Locci, 1987). KNF2047^T could be distinguished from the three mostly related species by the comparison of phenotypic and genotypic properties. *S. flavovariabilis*, the closest relative in terms of 16S rRNA gene sequence similarity, belongs to red series, which is comparable to the grey spore mass of KNF 2047^T (Pridham and Tresner, 1974). In addition, KNF 2047^T was not the only closest strain to *S. flavorariabilis* LMG 19905^T, as the latter also shared 99.2% 16S rRNA gene sequence similarity with both *S. indigocolor* NRRL B-12336^T and *S. cyaneus* NRRL B-2296^T. *S. bungoensis* NRRL B-24305^T shared 99.6% similarity with *S. longwoodensis* LMG 20096^T, and 99.4% with *S. galbus* NBRC 12864^T. *S. longwoodensis* LMG 20096^T shared over 99.5% with *S. chrestomyceticus* NRRL B-3310^T, *S. corchorusii* NBRC 12850^T and *S. galbus* NBRC 12864^T. The expanded phylogenetic tree also showed that KNF 2047^T stably formed an independent phylogenetic line (data not shown).

It is evident from the above results that KNF 2047^T should represent a new center of taxonomic variation within *Streptomyces*, for which the name *Streptomyces neopeptinius* sp. nov. is proposed.

Table 2. Major phenotypic properties of KNF 2047^T

Character	Description
Cultural properties	
Aerial mycelium	Abundant
Sporulation	Good
Spore mass	Grey
Color of substrate mycelium	–
Fragmentation of substrate mycelium	–
Motility of spore	–
Spore chain	Open spiral
Spore surface ornamentation	Spiny
Melanin production on ISP 6	+
Melanin production on ISP 7	+
Melanoid pigment on tryptone-yeast extract broth	+
Biochemical properties	
Lecithinase	–
Protease	–
Lipase	–
Pectinase	–
Chtinase	–
H_2S production	+
Hippurate hydrolysis	+
β-Lactamase on YPG agar	+
β-Lactamase on Bee cham's Fs agar	+
Klebsiella β-lactamase inhibitor	–

Description of *Streptomyces neopeptinius* sp. nov.

Streptomyces neopeptinius (neo.pep.ti'ni.us. N.L. adj. *neopeptinius* producing neopeptins, antifungal substances).

Extensively branching aerial and substrate mycelia, and grey spore mass are produced on modified Bennett's agar. No fragmentation of substrate mycelia is observed. Melanin is produced, but not colored diffusible pigment. The aerial arthrospores with spiny surfaces form open spiral-shaped chains. The optimal growth temperatures are 27~35°C. The major cell wall diamino acid is LL-diaminopimelic acid, and the major isoprenoid quinone MK-9[H_8]. The main fatty acids are *iso*-$C_{14:0}$, iso-$C_{15:0}$, *anteiso*-$C_{15:0}$, *iso*-$C_{16:0}$, iso-$C_{17:0}$, and *anteiso*-$C_{17:0}$.

Pectin and hippuric acid are hydrolyzed, and hydrogen sulfide is produced. The β-lactamase activity is detected, but not lecithinase, protease, lipase or chitinase activity. The type strain produces neopeptins A and B, and is antagonistic against a wide range of fungi, in particular those causing powdery mildew, but not against yeasts or bacteria.

The type and only strain was isolated from soil at Daejeon City, Republic of Korea (KNF 2047^T= SH-09^T= KCTC 10586BP^T).

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References

Abou Zeid, A.Z. 1972. Production of cycloheximide by *Streptomyces* sp. *Acta Microbiol. Pol.* B4, 83-88.

Arai, M. and K. Hamano. 1970. Isolation of three main components. F3, F4 and F5, from azalomycin F-complex. *J. Antibiot.* 23, 107-112.

Brufani, M., S. Cerrini, W. Fedeli, C. Musu, L. Cellai, and W. Keller-Schierlein. 1971. Structures of the venturicidins A and B. *Experientia* 27, 604-606.

Chen, C.W., C.H. Huang, H.H. Lee, H.H. Tsai, and R. Kirby. 2002. Once the circle has been broken: dynamics and evolution of *Streptomyces* chromosomes. *Trends Genet.* 18, 522-529.

Cho, S.H., J.H. Han, C.N. Seong, and S.B. Kim. 2006. Phylogenetic diversity of acidophilic sporoactinobacteria isolated from various soils. *J. Microbiol.* 44, 600-606.

Cohen, R. and P.A. Webb. 1952. Nystatin, a coccidioidocidal antibiotic. *Arch. Pediatr.* 69, 414-416.

Delzer, J., H.P. Fiedler, H. Müller, H. Zähner, R. Rathmann, K. Ernst, and W.A. König. 1984. New nikkomycins by mutasynthesis and directed fermentation. *J. Antibiot.* 37, 80-82.

El-Naggar, M.Y., S.A. El-Assar, and S.M. Abdul-Gawad. 2006. Meroparamycin production by newly isolated *Streptomyces* sp. strain MAR01: taxonomy, fermentation, purification and structural elucidation. *J. Microbiol.* 44, 432-438.

Euzéby, J.P. 2008. List of prokaryotic names with standing in nomenclature. Last full update: June 04, 2008 (URL: http://www.bacterio.net).

Felsenstein, J. 1993. PHYLIP (phylogenetic inference package), version 3.5c. Department of Genetics, University of Washington, Seattle, USA.

Hall, R.M., M.J. Dawson, C.A. Jones, A.D. Roberts, P.J. Sidebottom, P. Stead, and N.L. Taylor. 2001. The production of novel sordarin analogues by biotransformation. *J. Antibiot.* 54, 948-957.

Hamamoto, T., S. Gunji, H. Tsuji, and T. Beppu. 1983. Leptomycins A and B, new antifungal antibiotics. I. Taxonomy of the producing strain and their fermentation, purification and characterization. *J. Antibiot.* 36, 639-645.

Hosoya, S., M. Soeda, N. Komatsu, K. Okada, and S. Watanabe. 1953. Studies on trichomycin. II. Antibiotic activities against *Trichomonas*, *Candida*, and *Treponema pallidum*. *J. Antibiot.* 6, 92-97.

Kasai, M., K. Shirahata, S. Ishii, K. Mineura, H. Marumo, H. Tanaka, and S. Omura. 1979. Structure of nanaomycin E, a new nanaomycin. *J. Antibiot.* 32, 442-445.

Kim, Y.S., H.M. Kim, C. Chang, I.C. Hwang, H. Oh, J.S. Ahn, K.D. Kim, B.K. Hwang, and B.S. Kim. 2007. Biological evaluation of neopeptins isolated from a *Streptomyces* strain. *Pest Manag. Sci.* 63, 1208-1214.

Kluepfel, D., S.N. Sehgal, and C. Vézina. 1970. Antimycin A components. I. Isolation and biological activity. *J. Antibiot.* 23, 75-80.

Komagata, K. and K.I. Suzuki. 1987. Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol.* 19, 161-207.

Locci, R. 1987. Streptomycetes and related genera, p. 2451-2508. *In* S.T. Williams, M.E. Sharpe, and J.G. Holt (eds.), Bergey's Manual of the Systematic Bacteriology, vol. 4. The Williams and Wilkins Co., Baltimore, Maryland, USA.

Makkar, N.S., T.E. Nickson, M. Tran, N. Biest, M. Miller-Wideman, J. Lawson, C. McGary, and R. Stonard. 1995. Phenamide, a fungicidal metabolite from *Streptomyces albospinus* A19301. Taxonomy, fermentation, isolation, physico-chemical and biological properties. *J. Antibiot.* 48, 369-374.

Pridham, T.G. and H.D. Tresner. 1974. Genus I. *Streptomyces* Waksman and Henrici 1943, 339, p. 748-829. *In* R.E. Buchanan and N.E. Gibbons (eds.), Bergey's Manual of Determinative Bacteriology, 8th ed. The Williams and Wilkins Co., Baltimore, Maryland, USA.

Schaad, K.P. 1985. Identification of clinically significant actinomycetes and related bacteria using chemical techniques. p. 173-199. *In* M. Goodfellow and D.E. Minnikin (eds.), Chemical Methods in Bacterial Systematics, Academic Press, London, UK.

Schiewe, H.J. and A. Zeeck. 1999. Cineromycins, gamma-butyrolactones and ansamycins by analysis of the secondary metabolite pattern created by a single strain of *Streptomyces*. *J. Antibiot.* 52, 635-642.

Sehgal, S.N., H. Baker, C.P. Eng, K. Singh, and C. Vézina. 1983. Demethoxyrapamycin (AY-24,668), a new antifungal antibiotic. *J. Antibiot.* 36, 351-354.

Struyk, A.P., I. Hoette, G. Drost, J.M. Waisvisz, T. Van Eek, and J. C. Hoogerheide. 1957-58. Pimaricin, a new antifungal antibiotic. *Antibiot. Annu.* 5, 878-885.

Trejo, W.H. and R.E. Bennett. 1963. *Streptomyces nodosus* sp. n., the amphotericin-producing organism. *J. Bacteriol.* 85, 436-439.

Tsuji, N. and M. Kobayashi. 1978. Trichostatin C, a glucopyranosyl hydroxamate. *J. Antibiot.* 31, 939-944.

Tsuji, N., M. Kobayashi, K. Nagashima, Y. Wakisaka, and K. Koizumi. 1976. A new antifungal antibiotic, trichostatin. *J. Antibiot.* 29, 1-6.

Umezawa, H., Y. Okami, T. Hashimoto, Y. Suhara, M. Hamada, and T. Takeuchi. 1965. A new antibiotic, kasungsmycin. *J. Antibiot.* 18, 101-103.

Vézina, C., A. Kudelski, and S.N. Sehgal. 1975. Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *J. Antibiot.* 28, 721-726.

Volff, J.N. and J. Altenbuchner. 2000. A new beginning with new ends: linearisation of circular chromosomes during bacterial evolution. *FEMS Microbiol. Lett.* 186, 143-150.

Werner, G., H. Hagenmaier, H. Drautz, A. Baumgartner, and H. Zähner. 1984. Metabolic products of microorganisms. 224. Bafilomycins, a new group of macrolide antibiotics. Production, isolation, chemical structure and biological activity. *J. Antibiot.* 37, 110-117.

Yoo, S.H. and Y.S. Kim. 2006. The noble *Streptomyces* sp. SH09 and control of plant fungal diseases including powdery mildews using this microorganism. KR Patent No. 10-0634874.