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Further Finding of Antimicribial and Antifungal Activities against Human Pathegens by using the Isolates to Inhibit the Growth of *Paenibacillus lavae*, an Agent of AFB

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Abstract

More than 100 soil samples were collected from many forests in Korea during 2013. We found 43 strains from more than 2,000 isolates, which showed antimicrobial activity against *Paenibacillus larvae* as an agent of American Foulbrood (AFB) as well as common microbial human pathogens such as *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Candida albicans* and *Aspergillus niger*. On the basis of 16S rRNA gene sequence analysis, they belong to 12 genera (mostly actinobacteria): *Streptomyces* (28), *Kitasospora* (4), *Bacillus* (2), *Streptacidiphilus, Amycolatopsis, Paenibacillus, Promicromonospora, Rhodococcus, Pseudomonas, Burkholderia, Undibacterium* and *Actinomadura*. Among them, *Streptomyces* species are dominant (28/43 strains, 65.12%). *Burkholderia* and *Undibacterium* spp. were reported first as antimicrobial agents, especially against Gram-positive bacteria. In the future, they may be useful to develop some drugs against human pathogens by using their metabolites.

Key words: *Paenibacillus larvae*, American Foulbrood disease (AFB), Actinobacteria, Human pathogens, Antibiotics

INTRODUCTION

Microorganisms participate in almost all metabolic processes of life and make up 60% of the earth'sbiomass (Singh *et al*., 2009). Among them, many ones can produce lots of useful metabolic products such as antibiotics. Penicillin, discovered by Alexander Fleming in 1928, paved the way for a golden era of natural antibiotics in the 1940~1950s. However, any substrates to replace these antibiotics have not been found until now. The number of newly discovered antibiotics had decreased in the later years, but recently, advances in technology and screening methods enable the detection of previously unidentified compounds (Kurtboke, 2012). For example, some novel antibiotics were identified from different strains of the same species that shares 99.6~100% similarity of 16S rRNA sequence (Tamehiro *et al*., 2002; Nachtigall *et al*., 2011) and from new species or new genus (Clardy *et al*., 2006; Lewis, 2013; Ling *et al*., 2015).

Paenibacillus larvae (*P. larvae*) is an agent of American Foulbrood disease (AFB) and a spore-forming Gram-positive bacterium. Adult bees can be resistant to this bacterial disease, but bee larvae are very susceptible to infection (Wilson, 1971; Hitchcock *et al*., 1979). Because spores of *P. larvae* endure against heating, some antibiotics and other chemical agents (Genersch, 2010), the spores can survive for long time in environments or regional bee-keeping hives (up to 35 years) (Heyndrickx *et al*., 1996; Dobbelaere *et al*., 2001).

This study extended to screen our isolates with some

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microbial pathogens causing popular diseases such as *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Aspergillus niger*, and *Candida albicans*. *B. subtilis* is well known as an agent of endocarditis, bacteraemia, pneumonia, septicaemia (Pennington *et al*., 1976), and carcinoma of the breast (Ihde and Armstrong, 1973). Furthermore, *B. subtilis* spores seems to survive against heat treatment and amylase activity (Pepe *et al*., 2003) and to present with high amounts in some foods (10^6 c.f.u/g) (Gilbert *et al*., 1981). Like *B. subtilis, A. niger* can produce the spores that can exist for a long period, and is known as a cause of pneumonia's desease (Person *et al*., 2010). *C. albicans* is the main agent of fungal pathogens in humans, especially for immunocompromised patients (Tsai *et al*., 2013). Recently, there is an evidence that *C. albicans* may be a dangerous agent for cancer patients since they promote division and differentiation of cancer cells (Ramirez-Garcia *et al*., 2014). Unlike pathogenic microorganisms mentioned above, *Escherichia coli* species are "useful" intestinal bacteria because they participate in digesting foods. However, there are three types of desease caused by them, which are enteric/diarrhoeal diseases, urinary tract infections and sepsis/meningitis (Kaper *et al*., 2004). *S. aureus* is a common type of bacteria that lives on the skin and mucous membranes of humans, causing various human's skin diseases. The cells have the capsid that protects them from destruction of phagocytes and supports in attachment to various host tissues (Dunman and Projan, 2002).

Since antibiotics were used in the world war II for effective treatment of infectious diseases, many researchers have reported from the beginning of the 1950's that some pathogens were resistant to antibiotics known (Madigan *et al*., 2009). For example, they were methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, and vancomycin-resistant *Staphylococcus aureus* (Klevens *et al*., 2006; Moran *et al*., 2006). Further more, Candida albicans was resistant to all known antimicrobial drugs (Madigan *et al*., 2009). Therefore, researchers need to discover novel antibiotics against the resistant microbial pathogens (Donadio *et al*., 2010).

Actinobacteria is Gram-positive bacteria with high DNA G+C contents (60~78 mol%), can produce a wide variety and the most important secondary metabolites that can be applied in industrial medicine and therapeutic diseases (Baltz, 2005; Rokem *et al*., 2007). Those are antibacterial, anticancer, antitumor, antiinfective, antiinflammatory and antifungal agents (Shiomi and Omura, 2004; Caffrey *et al*., 2008; Baltz, 2008; Graziani, 2009; Olano *et al*., 2009). Actinobacteria can be found in terrestrial, freshwater and marine ecosystems, but more abundant in soil (Gremion *et al*., 2003; Janssen, 2006). The genus *Streptomyces* was first described by Waksman & Henrici (1943), widely distributed in nature. In soil, *Streptomyces* species were isolated with abundant nummbers, containing 1~20% (10^4 ~ 10^7 c.f.u/g) of the total number of colonies that can be counted on petri disk (Korn-Wendisch and Kutzner, 1992). *Streptomyces* account for over 74% of the antibiotics produced by the order actinomycetales, and approximately 34% of all known antibiotics (Bérdy, 2005). *Streptomyces* species were described as the richest in the prokaryotic including over 668 species with valid names (http://www.bacterio.net/streptomycesa.html). Hence the number of *Streptomyces* species in this study was dominant.

However, according to Pham and Kim (2012), the majority of microorganisms (more than 99%) can not grow in laboratory conditions and so their phenotypic characteristics have not been detected. Therefore, we applied three methods including a new method using transwell plates for isolation of new actinobacteria and other bacteria including not-yet-cultured strains in this study, and then screened all the isolates to find the strains to have antimicribioal activity against *P. lavae* and human pathogens.

MATERIALS AND METHODS

Isolation of actinobacteria

We used three methods for isolation of soil actinobacteria and other bacteria as described previously (Nguyen *et al*., 2013). Method 1 was developed by Pham and Kim (2014) to isolate a variety of soil bacteria which may be unculturable or hardly cultivated. Some samples were treated at 40°C for 16 h to isolate common actinobacteria (Method 2: Williams and Wellington, 1982) and to isolate rare actinobacteria 30 min at 120°C (Method 3: Nonomura and Ohara, 1971).

Table 1. List of actinobacterial and other bacterial strains that showed antibacterial and antifungal activities against human pathogens by using anti-*P. larve* isolates. 1: *Paenibacillus larvae* KACC 14031; 2: *Bacillus subtilis* KEMB 51201-001; 3: *Staphylococcus aureus* KEMB 4659; 4: *Escherichia coli* KEMB 212-234; 5: *Candida albicans* KACC 30003; 6: *Aspergillus niger* KACC 40280

Strain	Closest cultivated species/ (Genbank accession no.)	Pairwise similarity (%)	Activity against pathogens (Inhibition zone: mm)					
			1	2	3	4	5	6
T31	*Bacillus aryabhattai* B8W22^T (EF114313)	99.45	24	-	13	-	-	-
T64	*Streptomyces sporoverrucosus* NBRC 15458^T (AB184684)	99.86	9	16	4	-	5	15
T60	*Streptomyces lannensis* TA4-8^T (AB562508)	99.38	22	9	3	-	-	-
T82	*Streptomyces mauvecolor* LMG 20100^T (AJ781358)	98.9	6	10	5	1	3	8
T519	*Streptomyces ferralitis* SFOp68^T (AY262826)	99.02	20	10	10	-	11	15
T509	*Streptomyces yogyakartensis* NBRC 100779^T (AB249942)	99.86	15	-	-	-	3	-
T511	*Pseudomonas xanthomarina* KMM1447^T (AB176954)	98.9	5	-	-	-	10	-
T512	*Streptomyces xanthocidicus* NBRC 13469^T (AB184427)	99.65	21	3	14	-	13	3
T502	*Kitasatos poraviridis* 52108a^T (AY613990)	99.43	10	-	-	2	-	-
T505	*Paenibacillus peoriae* DSM 8320^T (AJ320494)	99.18	4	4	2	5	-	-
T515	*Bacillus panaciterrae* Gsoil1517^T (AB245380)	96.72	22	15	20	-	-	-
T520	*Streptacidiphilus anmyonensis* AM-11^T (DQ904546)	99.86	15	-	8	-	-	-
T528	*Promicromonospora umidemergens* 09-Be-007^T (FN293378)	99.17	5	3	9	-	-	-
T524	*Actinomadura maheshkhaliensis* 13-12-50^T (AB331731)	99.52	10	3	8	-	-	-
T525	*Streptomyces blastmyceticus* NRRL B-5480^T (AY999802)	99.18	25	20	20	-	-	15
T523	*Streptomyces scopuliridis* RB72^T (EF657884)	98.75	16	-	-	-	-	5
T811	*Streptomyces griseorubiginosus* LMG 19941^T (AJ781339)	100	9	5	5	3	-	-
T801	*Streptomyces spororaveus* LMG 20313^T (AJ781370)	99.86	12	-	-	3	-	9
T804	*Streptomyces racemochromogenes* NRRL B-5430^T (DQ026656)	100	26	-	-	30	-	10
T826	*Streptomyces cirratus* NRRL B-3250^T (AY999794)	99.86	9	-	-	2	-	-
T827	*Streptomyces cyaneofuscatus* JCM 4364^T (AY999770)	100	17	-	-	3	3	-
T806	*Streptomyces polychromogenes* NBRC 13072^T (AB184292)	100	26	2	-	30	-	12
T822	*Streptomyces cinnamonensis* NBRC 15873^T (AB184707)	99.38	8	2	-	2	5	-
T810	*Streptomyces narbonensis* NBRC 12801^T (AB184157)	99.79	8	14	12	-	-	-
T818	*Streptomyces cacaoi subsp.asoensis* NRRL B-16592^T (DQ026644)	99.93	12	2	5	-	-	-
T812	*Streptomyces filamentosus* NBRC 12767^T (AB184130)	99.24	14	-	2	-	-	-
T815	*Streptomyces drozdowiczii* NBRC 101007^T (AB249957)	99.58	7	-	3	-	-	-
T825	*Streptomyces anulatus* NRRL B-2000^T (DQ026637)	99.93	7	-	3	-	-	-
T805	*Streptomyces melanogenes*NBRC 12890^T (AB184222)	98.9	10	-	-	-	-	3
T1006	*Rhodococcus phenolicus* DSM 44812^T (AM933579)	98.89	8	-	-	5	-	-
T913	*Undibacterium pigrum* CCUG 49009^T (AM397630)	98.05	8	2	-	5	-	-
T1421	*Burkholderia dabaoshanensis* GIMN1.004^T (FJ210816)	99.44	12	10	5	-	-	-
T912	*Streptomyces griseoplanus* AS 4.1868^T (AY999894)	100	18	-	4	-	-	-
T1301	*Kitasatospora kifunensis* IFO 15206^T (AB022874)	99.86	9	2	10	-	-	-
T1303	*Kitasatospora atroaurantiaca* NRRL B-24282^T (DQ026645)	99.1	13	-	-	-	2	-
T1304	*Kitasatospora nipponensis* HKI 0315^T (AY442263)	98.99	18	-	-	-	20	3
T1300	*Streptomyces violascens* NBRC 12920^T (AB184246)	99.1	18	-	-	-	-	2
T1505	*Streptomyces antibioticus* NBRC 12838^T (AB184184)	100	20	15	-	-	-	-
T1506	*Streptomyces turgidiscabies* ATCC 700248^T (AB026221)	99.1	13	8	-	-	-	-
A43	*Streptomyces anandii* NRRL B-3590^T (AY999803)	99.79	11	4	5	-	6	12
A33	*Amycolatopsis halotolerans* N4-6^T (DQ000196)	98.9	10	-	-	-	-	8
A64	*Streptomyces resistomycificus* NBRC 12814^T (AB184166)	99.1	10	-	-	-	2	8
A19	*Streptomyces niveus* NRRL2466^T (DQ442532)	99.31	15	1	10	-	-	-

Test microorganisms

Microbial pathogens were obtained from the Korean Agricultural Culture Collection (KACC) and the Korea National Environmental Microorganisms Bank (KEMB). These bacteria included *Paenibacillus larvae* KACC 14031, *Bacillus subtilis* KEMB 51201-001, *Staphylococcus aureus* KEMB 4659, *Escherichia coli* KEMB 212-234, *Candida albicans* KACC 30003 and *Aspergillusniger* KACC 40280.

Assay of antibacterial and antifungal activities

For preparing microbial pathogens, the bacteria in glycerol stock were refreshed in 20mL of LB medium (except *P. larvae* with R2A) at optimal temperature overnight and then streaked on agar plates. Single colonies were picked and adjusted to 10^4 c.f.u/mL with R2A broth. Spores of *A. niger* were prepared at 10^4 spores/mL, and spores were taken from Sabaroud dextrose agar. *C. albicans* was grown on YM medium (ATCC medium 200) at 10^4 c.f.u/mL. The absorbance of the suspensions was measured at 550 nm for fungal spores and 600 nm for bacterial cells. Then, 100μL of bacterial suspension or spore suspension was spread on R2A agar plates (petri dish size, 90×15mm). Pure colonies of isolates were picked off and spotted on the plates on which the human pathogens were spread in advance. These agar plates were incubated at 28°C and then the diameter of inhibition zone was measured after incubation for 3~4 days.

Identification and phylogenetic anlaysis by 16S rRNA gene sequence comparison

The almost-complete 16S rRNA gene sequences of our strain were identified by using the EzTaxon-e server (http://www.ezbiocloud.net/eztaxon). The 16S rRNA gene sequences of related taxa were obtained from GenBank and edited using the BioEdit program (Hall, 1999). Multiple alignments were performed with the CLUSTAL_X program (Thompson *et al*., 1997). Evolutionary distances were calculated using the Kimura two parameter model (Kimura, 1983). Phylogenetic trees were constructed using the neighbour-joining method with the MEGA5.03 program (Tamura *et al*., 2011); bootstrap values were based on 1,000 replicates (Felsenstein, 1985).

RESULTS AND DISCUSSION

In this study, we found 43 strains that inhibited the growth of *Paenibacillus larvae*. Those strains were obtained through screening more than 2,000 isolates which were isolated from 100 different soil samples collected from all around South Korea. The number of highly active strains (the diameter of inhibition zone ≥ 20mm) were 9 (20.93%) (Table 1). Especially, *Bacillus* sp. T515 (96.72% similarity) proven to be a new species (Nguyen and Kim, 2015) can be a strong candidate to discover new antibiotics. Although others have the values between 99.18 to 100% in similarity levels of 16S rRNA gene sequences with the closest species (except strain *Bacillus sp*. T515), they were still potential candidates for discovering new antibiotics as mentioned before. Besides, there were two other strains that may be new species [*Streptomyces* sp. T523 (98.75% similarity) and *Undibacterium* sp. T913 (98.05% similarity)] which can be candidates to discover new antibiotics, in spite of less active strains (12 and 8 mm

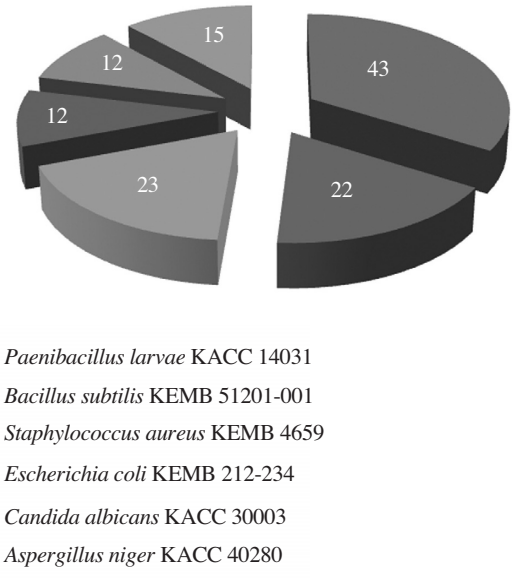

Fig. 1. Distribution (number among total 43 strains) of the strains showing activities against microbial pathogens, isolated from soil samples collected in South Korea.

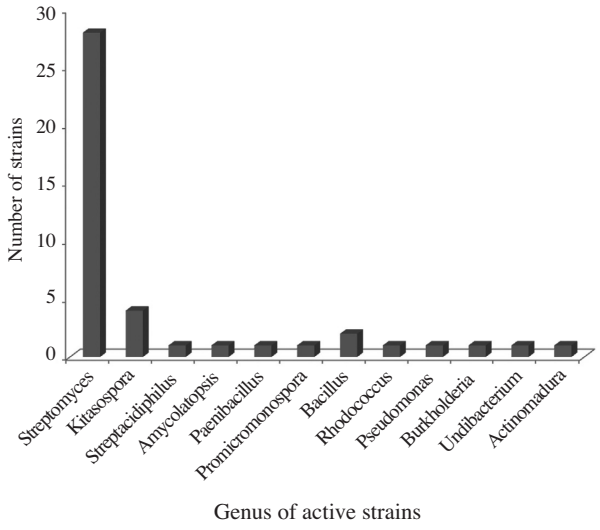

Fig. 2. Relative distribution of the active strains in the level of bacterial genus, showing antimicrobial and antifungal activities against microbial pathogens.

in inhibition zone, respectively).

Among the effective 43 strains against *P. larvae*, 23 strains (53.49%) showed inhibitory activity against *Staphylococcus aureus*, 22 strains (51.16%) against *Bacillus subtilis*, 15 strains (34.88%), against *Aspergillus niger*, 12 strains (27.91%) against *Escherichia coli*, and 12 strains against *Candida albicans* (Fig. 1). The number of strains showing high activity ($\geq$ 20mm in inhibition zone) against at least one of human pathogens was 5 among 43 (11.63%). They are *Bacillus* sp. T515 against *S. aureus*, *Streptomyces* sp. T525 against *B. subtilis* and *S. aureus*, *Streptomyces* sp. T804 against *E. coli, Streptomyces* sp. T806 against *E. coli*, and *Kitasatospora* sp. T1305 against *C. albicans* (Table 1). It is considered to be potential strains for producing useful compounds as effective antibiotics than any other strains. Althogh many other strains showed low activity, they may be increased in acitivity by better expression of their functional genes through optimal culture conditions. For example, during the screening process, *Bacillus* sp. T515 was effective on only *P. larvae, B. subtilis* and *S. aureus* (all Gram-positive), but the spectrum of control was extended further by choosing an optimal medium. Its crude extractant could kill both Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Paenibacillus larvae*) (Nguyen and Kim, 2015).

However, it seems to be very hard to find bacterial strains to control a broad spectrum of infectious human pathogens including prokaryotic Gram-negative and Gram-positive bacteria and eukaryotic fungi. Only three strains among 43 can control three different groups (G^+-bacteria, G^--bacteria and fungi) such as *Streptomyces* spp. T82, T806 and T822. In particular, strain T82 showed inhibitory effect on all the tested pathogens (Table 1).

On the basis of 16S rRNA sequence analysis, the 43 strains belong to 12 genera: 7 genera in actinobacteria (*Streptomyces, Kitasospora, Streptacidiphilus, Amycolatopsis, Promicromonospora, Rhodococcus* and *Actinomadura*) and 5 genera in other bacteria (*Bacillus, Pseudomonas, Burkholderia, Undibacterium* and *Paenibacillus*) (Fig. 2). Among 43 strains, 28 (65.12%) were identified as the genus *Streptomyces*, a most well-known antibiotic-producing genus (Bérdy, 2005). Although they were proven to have a little possibility of new species (98.75~100% similarity to type species, most of which were discovered in the 1950s), they are still potential candiates to produce new secondary active metabolites because they were not discovered during that period mentioned above. Among the rest 11 genera, *Kitasospora* and *Bacillus* had 4 and 2 strains, respectively, and other genera had 1 each. Non-actinobacterial genera can be divided into two groups, Gram-positive (*Bacillus* and *Paenibacillus*) and Gram-negative (*Pseudomonas*, *Burkholderia* and *Undibacterium*). Phylogenetic analysis of our strains were constructed to establish their relationship. Based on the analysis of 16S rRNA gene sequences, they were divided into three groups in the level of phylum such as actinobacteria (major), fermicutes and proteobacteria (Fig. 3). Actinobacteria and fermicutes can be bound in Gram-positive bacteria, separating from Gram-negative proteobacter.

In conclusion, natural balances are always in a state of activity, and so effect of climate change and others factors increase the evolution of microorganisms. Since antibiotics had discovered and used up to today, lots of microbial pathogens have developed their resistancy to various antibiotics. Hence we have conducted to find new candid-

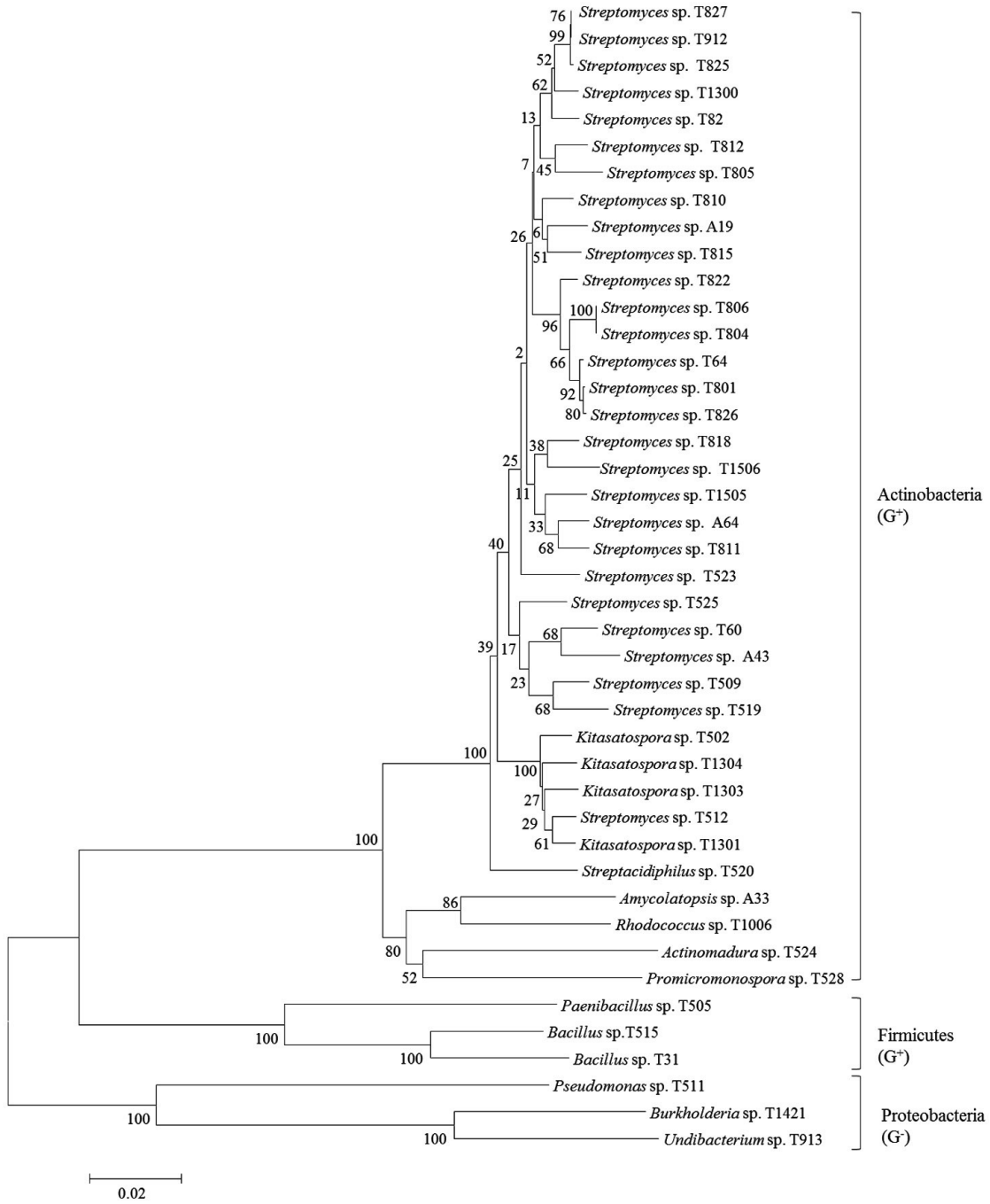

Fig. 3. A phylogenetic tree of selected antimicrobial strains isolated from various soil samples based on 16S rRNA gene sequence analysis.

ates that may be the sources to control antibiotic-resistant *P. larvae* and human pathogens in this study. As a result, we have recruited a number of species including Gram-positive and Gram-negative bacteria, which are the candidates for a research of novel antibiotics/secondary metabolites. A continuous study will be performed for isolation and purification of new compounds, and examination of their activity against antibiotic-resistant pathogens.

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LITERATURE CITED

Baltz, R. H. 2005. Antibiotic discovery from actinomycetes: will a renaissance follow thedecline and fall?. Sim News. 55: 186-96.

Baltz, R. H. 2008. Renaissance in antibacterial discovery from actinomycetes. Curr. Opin. Pharmacol. 8: 557-563.

Bérdy, J. 2005. Bioactive microbial metabolites. J. Antibiot. 58: 1-26.

Caffrey, P., J. F. Aparicio, F. Malpartida and S. B. Zotchev. 2008. Biosynthetic engineering of polyene macrolides towards generation of improved antifungal and antiparasitic agents. Curr. Top. Med. Chem. 8: 639-653.

Clardy, J., M. A. Fischbach and C. T. Walsh. 2006. New antibiotics from bacterial natural products. Nature biotechnology. 24: 1541-1550.

Dobbelaere, W., D. C. De Graaf, W. Reybroeck, E. Desmedt, J. E. Peeters, and F. J. Jacobs. 2001. Disinfection of wooden structures contaminated with Paenibacillus larvae subs *P. larvae* spores. J. Appl. Microbiol. 91: 212-216.

Donadio, S., S. Maffioli, P. Monciardini, M. Sosio and D. Jabes. 2010. Antibiotic discovery in the twenty-first century: current trends and future perspectives. J. Antibiot. 63: 423-430.

Dunman. P. M. and S. J. Projan. 2002. The Regulation of virulence in the *Staphylococci*. p. 4 in *Staphylococcus aureus* infection and disease, eds. by A. L. Honeyman, H. Friedman and M. Bendinelli. New York, Boston, Dordrecht, London, Moscow.

Felsenstein, J. 1985. Confidence limit on phylogenies: an approach using the bootstrap. Evolution. 39: 783-791.

Genersch E. 2010. American foulbrood in honeybees and its causative agent, *Paenibacillus larvae*. J. Invertebr. Pathol. 103: 510-519.

Gilbert, R. J., P. C. B. Turnbull, J. M. Parry, J. M. Kramer. 1981. *Bacillus cereus* and other *Bacillus* species : their part in food poisoning and other clinical infections. pp 297-314. in The aerobic endospore-forming bacteria ; classification and identification, eds. by R. C. W. Berkeley and M. Goodfellow. Academic Press, London.

Graziani, E. I. 2009. Recent advances in the chemistry, biosynthesis and pharmacology of Rapamycin analogs. Nat. Prod. Rep. 26: 602-609.

Gremion, F., A. Chatzinotas, and H. Harms. 2003. Comparative 16S rDNA and 16S rRNA sequence analysis indicates that Actinobacteria might be a dominant part of the metabolically active bacteria in heavy metal-contaminated bulk and rhizosphere soil. Environ. Microbiol. 5: 896-907.

Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic. Acids. Symp. Ser. 41: 95-98.

Heyndrickx, M., K. Vandemeulebroecke and B. Hoste *et al*. 1996. "Reclassification of *Paenibacillus* (formerly *Bacillus*) *pulvifaciens* (Nakamura 1984) Ash *et al*. 1994, a later subjective synonym of Paenibacillus (formerly Bacillus) larvae (White 1906) Ash *et al*. 1994, as a subspecies of *P. larvae*, with emended descriptions of *P. larvae* as *P. larvae* subs *P. larvae* and *P. larvae* subsp. *pulvifaciens*. Int. J. Syst. Bacteriol. 46: 270-279.

Hitchcock, J. D., A. Stoner, W. T. Wilson and D. M. Menapace. 1979. Pathogenicity of *Bacillus pulvifaciens* to honeybee larvae of various ages (Hymenoptera: Apidae). J. Kansas Entomol. Soc. 52: 238-246.

Ihde, D. C. and D, Armstrong. 1973. Clinical spectrum of infection due to *Bacillus* species. American Journal of Medicine. 55: 839-845.

Janssen, P. H. 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Appl. Environ. Microbiol. 72: 1719-1728.

Kaper, J. B., J. P. Nataro and H. L. T. Mobley. 2004. Pathogenic Escherichia coli. Nature reviews. 2: 123-140.

Kimura, M. 1983. The Neutral Theory of Molecular Evolution. Cambridge University Press. Cambridge, UK.

Klevens, R. M., J. R. Edwards, F. C. Tenover, L. C. McDonald, T. Horan, R. Gaynes, R and National Nosocomial Infections Surveillance System. (2006). Changes in the epidemiology of methicillin-resistant *Staphylococcus aureus* in intensive care units in US hospitals, 1992-2003. Clin. Infect. Dis. 42: 389-391.

Korn-Wendisch, F and H. J. Kutzner. 1992. The family *Streptomycetaceae*. pp. 921-995. in The Prokaryotes: a Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, eds. by Balows, Truper, Dworkin, Harder and Schleifer. 2nd ed. Springer, New York, pp.

Kurtboke, D. I. 2012. Biodiscovery from rare actinomycetes: an eco-taxomical perspective. Appl. Microbiol. Biotechnol. 93: 1843-1852.

Lewis. K. 2013. Platforms for antibiotic discovery. Nature reviews 12: 371-387.

Ling, L. L., T. Schneider, A. J. Peoples, A. L. Spoering, I. Engels, B. P. Conlon, A. Mueller and other. 2015. A new

antibiotic kills pathogens without detectable resistance. Nature. 517: 455-459.

Madigan, M. T., J. M. Martinko, P. V. Dunlap and D. P. Clark. 2009. Metabolic diverity and microbial ecology. pp. 805-806. in Brock Biology of Microorganisms (12th ed). Pearson international edition.

Moran, G. J., A. Krishnadasan, R. J. Gorwitz, G. E. Fosheim, L. K. McDougal and others. 2006. Methicillin-resistant *Staphylococcus aureus* infections among patients in the emergency department. N. Engl. J. Med. 355: 666-674.

Nachtigall, J., K. Schneider, C. Bruntner, A. T. Bull, M. Goodfellow, H. Zinecker, J. F. Imhoff, G. Nicholson, E. Irran, R. D. Süssmuth and H. P. Fiedler. 2011. Benzoxacystol, a benzoxazine-type enzyme inhibitor from the deep-sea strain *Streptomyces* sp. NTK 935. J. Antibiot. 64: 453-457.

Nguyen, T. M and J. Kim. 2015. *Bacillus polymachus* sp. nov., with a broad range of antibacterial activity, isolated from forest topsoil samples by using a modified culture method. Int. J. Syst. Evol. Microbiol. 65: 704-709.

Nguyen, T. M., H. Lee and J. Kim. 2013. Selective isolation of actinobacteria showing antibacterial activity against *Paenibacillus larvae* from soil samples collected in South Korea. J. Apiculture. 28: 265-272.

Nonomura, H. and Y. Ohara. 1971. Distribution of actinomycetes in soil. A new genus and species of monosporic actinomycetes in soil. J. Ferment. Technol. 49: 895-903.

Olano, C., C. Mendez and J.A. Salas. 2009. Antitumor compounds from marine *actinomycetes*. Mar. Drugs. 7: 210-248.

Pennington, J. E, N. D. Gibbons, J. E. Strobeck, G. L. Simpson and R. L. Myerowitz. 1976. *Bacillus* species infection in patients with hematologic neoplasia. Journal of the American Medical Association 235: 1473-1474.

Pepe, O., G. Blaiotta, G. Moschetti, T. Greco and F. Villan. 2003. Rope-Producing strains of *Bacillus* spp. from wheat bread and strategy for their control by lactic acid bacteria. Appl. Environ. Microbiol. 69: 2321-2329.

Person, A. K., S. M. Chudgar, B. L. Norton, B. C. Tong and J. E. Stout. 2010. *Aspergillus niger*: an unusual cause of invasive pulmonary aspergillosis. Journal of Medical Microbiology. 59: 834-838.

Pham, V. H. T. and J. Kim. (2014). *Bacillus thaonhiensis* sp. nov., a new species, was isolated from the forest soil of Kyonggi University by using a modified culture method. Curr. Microbiol. 68: 88-95.

Pham, V. H. T. and J. Kim. 2012. Cultivation of unculturable soil bacteria. Trends. Biotechnol. 30: 475-484.

Ramirez-Garcia, A., A. Rementeria, J. M. Aguirre-Urizar, M. D. Moragues, A. Antoran, A. Pellon, A. Abad-Diaz-de-Cerio and F. L. Hernando. 2014. Candida albicans and cancer: Can this yeast induce cancer development or progression?. Crit. Rev. Microbiol. 1-13, DOI: 10.3-109/1040841X.2014.913004.

Rokem, J. S., A. E. Lantz and J. Nielsen. 2007. Systems biology of antibiotic production by microorganisms. Nat. Prod. Rep. 24: 1262-1287.

Shiomi, K. and S. Omura. 2004. Antiparasitic agents produced by microorganisms. Proc. Jpn. Acad. Ser. B. Phys. Biol. Sci. 80: 245-258.

Singh, B. K., C. D. Campbell, S. J. Sorenson and J. Zhou. 2009. Soil genomics. Nat. Rev. Microbiol. 7: 756.

Tamehiro, N., Y. O. Hosoya, S. Okamoto, M. Ubukata, M. Hamada, H. Naganawa and K. Ochi. 2002. Bacilysocin, a Novel Phospholipid Antibiotic Produced by *Bacillus subtilis* 168. Antimicrob. Agents. Chemother. 46: 315-320.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar. 2011. MEGA 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. 28: 2731-2739.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin and D. G. Higgins. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic. Acids. Res. 25: 4876-4882.

Tsai, P. W., Y. T. Chen, P. C. Hsu and C. Y. Lan. 2013. Study of Candida albicans and its interactions with the host: A mini review. Bio Medicine. 3: 51-64.

Waksman, S. A. and A. T. Herici. 1943. The nomenclature and classification of the Actinomycetes. J. Bacteriol. 46: 337-341.

Williams, S. T. and E. M. H. Wellington. 1982. Actinomycetes. pp. 969-987. in Methods of soil analysis, part 2: Chemical and Microbiological Properties, eds. by A.L. Page, R. H. Miller and O. R. Keency. 2nd ed. American Society of Agronomy/Soil Science Society of America, Madison.

Wilson, W. T. 1971. Resistance to American foulbrood in honey bees XI. Fate of *Bacillus larvae* spores ingested by adults. J. Invertebr. Pathol. 17: 247-255.