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Bacillus kyonggiensis sp. nov., Isolated from Soil of a Lettuce Field

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A Gram-positive, rod-shaped, motile, endospore-forming bacterial strain, designated NB22^T, was isolated from soil of a lettuce field in Kyonggi province, South Korea, and was characterized by using a polyphasic taxonomic approach. This novel isolate grew optimally at 30-37°C and pH 8-9. It grew in the presence of 0-4% NaCl (optimum, 1-2%). Comparative 16S rRNA gene sequence analysis showed that strain NB22^T was closely related to members of the genus *Bacillus* and fell within a coherent cluster comprising *B. siralis* 171544^T (98.1%) and *B. korlensis* ZLC-26^T (97.3%). The levels of 16S rRNA gene sequence similarity with respect to other *Bacillus* species with validly published names were less than 96.4%. Strain NB22^T had a genomic DNA G+C content of 36.3 mol% and the predominant respiratory quinone was MK-7. The peptidoglycan contained *meso*-diaminopimelic acid. The major cellular fatty acids were iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{14:0}$, and $C_{16:0}$. These chemotaxonomic results supported the affiliation of strain NB22^T to the genus *Bacillus*, and the low DNA-DNA relatedness values and distinguishing phenotypic characteristics allowed genotypic and phenotypic differentiation of strain NB22^T from recognized *Bacillus* species. On the basis of the evidence presented, strain NB22^T is considered to represent a novel species of the genus *Bacillus*, for which the name *Bacillus kyonggiensis* sp. nov. is proposed. The type strain is NB22^T (=KEMB 5401-267^T =JCM 17569^T).

***Keywords*:** taxonomy, 16S rRNA gene, *Bacillaceae*, *Bacillus kyonggiensis*

Bacillus species are aerobic, spore-forming, rod-shaped bacteria that are ubiquitous in nature. It was first described by Cohn in 1872, and the number of *Bacillus* species has fluctuated widely. Currently, many species originally described as *Bacillus* have been transferred to related genera. Nevertheless, the genus *Bacillus* is still considered to be one of the largest genera and currently includes more than 200 species (Euzéby, 2008). *Bacillus* species are used in many medical, pharmaceutical, and industrial processes that take advantage of their wide range of physiologic characteristics and their ability to produce a host of enzymes, antibiotics, and other metabolites. Several species are used as standards in medical and pharmaceutical assays (Baron, 1996).

During the course of a study on antifungal strains against *Fusarium oxysporum* present in a soil sample from a lettuce field in Kyonggi-do, South Korea, a large number of bacteria including NB22^T were isolated. In this study, we have characterized strain NB22^T. On the basis of phenotypic characteristics, chemotaxonomic data, phylogenetic analysis and DNA-DNA hybridization, the isolate represents a novel species of the genus *Bacillus*.

Material and Methods

Isolation of bacterial strains and culture conditions

Strain NB22^T was originally isolated from a soil sample from a lettuce field in Kyonggi-do, South Korea. The soil sample was thoroughly suspended in 50 mM phosphate buffer (pH 7.0) and the suspension, following serial dilution, was then spread onto a tryptic soy agar (TSA; Bacto^{TM}). The plates were incubated at 28°C for 1 week. Single colonies on the plates were purified by subculturing. Strain NB22^T was one of the isolates that appeared on the TSA plate under aerobic conditions. Strain NB22^T was routinely cultured on TSA at 28°C and maintained as a glycerol suspension (20%, v/v) at -70°C. The type strains of seven *Bacillus* species used as reference strains included: *Bacillus siralis* KACC 11246^T, *B. circulans* KACC 14392^T, *B. drentensis* KACC 12118^T, *B. nealsonii* KACC 12124^T, and *B. pochenonensis* KACC 14006^T, obtained from the Korean Agricultural Culture Collection (KACC); *B. korlensis* KCTC 13245^T, obtained from the Korean Collection for Type Cultures (KCTC) and *B. oceanisediminis* JCM 16506^T, obtained from the Japan Collection of Microorganisms (JCM).

Phenotypic and biochemical characteristics

Cell morphology and size were examined by using a transmission electron microscope (JEM 1010; JEOL, Japan), with cells grown for 2 days at 28°C on TSA. Sporulation was induced in a modified Schaeffer's medium [KCl 0.1%, $MgCl_2$ 0.01%, $Ca(NO_3)_2$ 1.0 mM, $MnCl_2$ 0.01 mM, $FeSO_4$ 0.001 mM Nutrient broth 8 g/L] according to Kempf *et al*. (2005), and spore morphology was determined after 1 week of incubation by phase-contrast microscopy (BX50 microscope; Olympus, Japan) at ×1,000. Motility was tested by culturing the organism in TSB medium that contained 0.4% agar. The Gram reaction was carried out according to the classic Gram procedure described by Doetsch (1981). Growth at different temperatures (4, 10, 15, 20, 25, 30, 37, 42, 50, and 55°C) and various pH values (pH 5.0-12.0; at intervals of 1 pH unit) was assessed after incubation for up to 5 days. The effect of pH on growth was determined on tryptic soy broth media using three different buffers (final concentration, 50 mM): acetate buffer (for pH 5.0), phosphate buffer (for pH 6.0-8.0) and Tris buffer (for pH 9.0-12.0). Salt tolerance was tested

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on TSA supplemented with 0-10% (w/v) NaCl after 5-day incubation at 30°C. Growth on nutrient agar, and brain heart infusion (BHI) agar, were also evaluated at 30°C. The ability to grow under anaerobic conditions was evaluated by culturing the organisms on a TSA plate in a sealed container that contained a BBL GasPak anaerobic system envelope for 1 week at 30°C. Catalase activity was determined by bubble production in 3% (v/v) H_2O_2; oxidase activity was determined using 1% (w/v) tetramethyl-*p*-phenylenediamine. API 50 CH (with API 50 CHB/E medium) and API ZYM strips (bioMérieux, France) were used to determine the activities of constitutive enzymes and other physiological properties according to the manufacturer's instructions.

Isopernoid quinones, cellular fatty acids, and polar lipids

Isoprenoid quinone was extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum conditions and re-extracted in n-hexane/water (1:1, v/v). The crude n-hexane-quinone solution was purified using Sep-Pak Vac silica cartridges (Waters, Ireland) and subsequently analysed using HPLC, as described by Hiraishi *et al.* (1996). Cellular fatty acid profiles were determined for strains grown on TSA (for *B. pocheonensis* it was R2A agar) for 2 days at 30°C. The cellular fatty acids were saponified, methylated and extracted according to the classical protocol of the Shelock Microbial Identification System (MIDI, Sasser, 1990). The fatty acids were then analysed by gas chromatography (HP 6890 series GC system; Hewlett Packard, USA) using the Microbial Identification software package (vision 6.08, database TSBA6). The value range was obtained by duplicate experiments. The isomer type of diaminopimelic acid in the peptidoglycan was determined as described by Staneck and Roberts (1974). Polar lipids of strain NB22^T were extracted according to the procedures described by Minnikin *et al.* (1984). Extracted lipids were separated by two-dimensional TLC and identified by spraying with an appropriate detection reagent (Minnikin *et al*., 1984; Komagata and Suzuki, 1987). Ninhydrin reagent (ninhydrin reagent 0.2% solution, Sigma Life Science) was used to detect free amino-group-containing lipids, zinzadze reagent (molybdenum blue spray reagent, 1.3%, Sigma Life Science, USA) for phosphorus-containing lipids and molybdophoshpolic acid (phosphomolybdic acid reagent, 20 wt% solution in ethanol, Sigma-Aldrich, Germany) for total lipids.

Determination of G+C content and DNA-DNA hybridization

For DNA-DNA hybridization experiments and determination of the DNA G+C content, genomic DNA of strain NB22^T and reference strains were prepared according to a modification of the procedure of Wilson (1987). The DNA G+C content of strain NB22^T was determined by reversed-phase HPLC according to the method of Mesbah *et al.* (1989). DNA-DNA hybridization was performed fluorometrically by the method of Ezaki *et al*. (1989), using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values were quoted as DNA-DNA relatedness values.

PCR amplification, 16S rRNA sequencing, and phylogenetic analysis

For 16S rRNA gene sequencing and phylogenetic analysis, DNA was extracted using a commercial genomic DNA Extraction kit (Solgent, Korea) and PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out using the universal bacterial primer set, 27F and 1492R (Weisburg *et al.*, 1991), according to Kim *et al.* (2005). The identification of phylogenetic neighbors and the calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon sever (http://www.eztaxon.org/; Chun *et al.*, 2007). The full sequence of the 16S rRNA gene was compiled with SeqMan software (DNASTAR Inc., USA). 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 neighbor-joining, maximum likelihood, and maximum parsimony methods in the MEGA5.03 program (Tamura *et al.*, 2011) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

The NCBI GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NB22^T (=KEMB 5401-267^T =JCM 17569^T) is JF896450.

Results and Discussion

Morphological and phenotypic characteristics

Strain NB22^T was found to be Gram-positive, facultative, non-motile, rod-shaped cells. Central and subterminal ellipsoidal endospores were observed in swollen sporangia (Fig. 1). Colonies grown on TSA plates at 30°C for 2 days were round, smooth, crateriform, light-yellow, and 1.5-3.0 mm in diameter. Strain NB22^T was able to grow within a temperature range of 15-42°C, but not at 45°C. The optimal temperature was 30-37°C. Growth occurs at pH 7.0-10.0, with optimal growth at pH 8.0-9.0. Growth occurs in the presence of 0-4% (w/v) NaCl (optimal, 1-2%). Oxidase and catalase reactions were positive. Other phenotypic features of strain NB22^T are summarized in the species description and a comparison of selective characteristics with closely related type strains is given in Table 1.

Cellular fatty acids and isoprenoid quinones

The major fatty acids of strain NB22^T were iso-$C_{15:0}$ (27.9%), anteiso-$C_{15:0}$ (24.2%), $C_{14:0}$ (11.3%), and $C_{16:0}$ (10.3%). As shown in Table 2, some qualitative and quantitative differ-

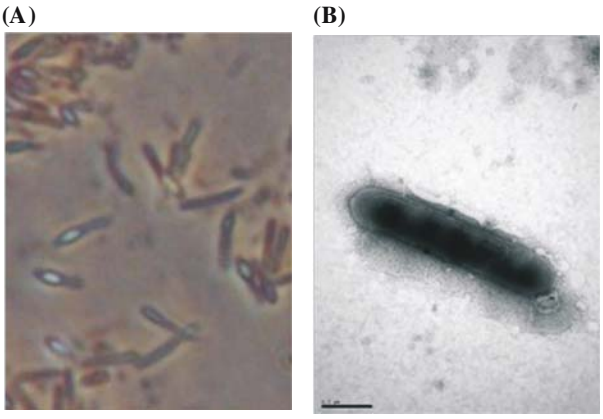

Fig. 1. A phase-contrast photomicrographs showing endospores (A) and a transmission electron microscope photo (B) of *B. kyonggiensis* strain NB22^T. Bar, 0.5 μm. Ellipsoidal endospores are formed subterminally, paracentrally (A).

Table 1. Different phenotypic characteristics of strain NB22^T and some related *Bacillus* species
Strains: 1, *B. kyonggiensis* NB22^T; 2, *B. siralis* KACC 11246^T; 3, *B. korlensis* KCTC 13245^T; 4, *B. circulansis* KACC 14392^T; 5, *B. drentensis* KACC 12118^T; 6, *B. oceanisediminis* JCM 16506^T; 7, *B. nealsonii* KACC 12124^T; 8, *B. pocheonensis* KACC 14006^T. +, positive; -, negative; w, weakly positive; v, viriable.

Characteristic	1	2	3	4	5	6	7	8
Motility	-	-	+	+	+	+	+	-
Gram staining	+	+	+	+	+/v	+	+	+
Anaerobic growth	+	-	-	-	+	-	+	-
Optimal growth temperature (°C)	30-37	30	30-37	30	30	37	30-35	25
Growth on								
TSA	+	+	+	+	+	+	+	-
50°C	-	+	-	+	+	-	+	-
NaCl 5%	-	+	+	+	+	+	+	+
pH 6	-	+	+	+	+	+	+	+
Acid production from								
D-Arabinose	+	-	-	-	-	-	-	-
L-Arabinose	-	w	+	+	-	-	+	w
Galactose	+	-	+	+	+	-	+	+
Amygdalin	+	-	+	+	-	-	+	-
Melibiose	-	-	+	+	+	-	+	-
Starch	+	-	+	+	+	+	w	-
Gentiobiose	+	-	+	+	-	-	+	+
Gluconate	+	-	w	+	-	w	+	-
DNA G+C content (mol%)	36.3	37.7	38.2	35.7	39.4	44.8	ND	44.9
Isolation source	Lettuce field soil	Silage	Sand soil	Sewage	Hay field soil	Marine sediment	Spacecraft-assembly facility	Ginseng field soil

Table 2. Fatty acid composition (%) of strain NB22^T and related species of the genus *Bacillus*
Strains: 1, *B. kyonggiensis* NB22^T; 2, *B. circulansis* KACC 14392^T; 3, *B. drentensis* KACC 12118^T; 4, *B. korlensis* KCTC 13245^T; 5, *B. nealsonii* KACC 12124^T; 6, *B. oceanisediminis* JCM 16506^T; 7, *B. pocheonensis* KACC 14006^T; 8, *B. siralis* KACC 11246^T. -, Not detected; Tr, Trace.

Fatty acid	1	2	3	4	5	6	7*	8
Straingt-chain saturated								
$C_{14:0}$	11.3	3.6	Tr	1.2	8.7	6.6	1.5	3.5
$C_{16:0}$	10.3	8.1	2.2	3.7	15.9	12.7	2.8	25.4
$C_{18:0}$	-	Tr	Tr	Tr	Tr	Tr	-	1.2
Branched saturated								
iso-$C_{13:0}$	Tr	Tr	1.1	Tr	1.4	Tr	-	Tr
iso-$C_{14:0}$	6.4	2.1	14.1	9.9	4.1	3	24.5	5.3
iso-$C_{15:0}$	27.9	13	27	40.2	24.5	23.8	15.4	28.7
iso-$C_{16:0}$	6.2	5.5	4.7	7.3	2.8	2.9	12.9	5.3
iso-$C_{17:0}$	3.4	2.4	2.5	5.4	3.6	1.1	Tr	4
anteiso-$C_{13:0}$	Tr	-	Tr	-	Tr	-	-	-
anteiso-$C_{15:0}$	24.2	46.6	29.6	8	28.5	22.6	34.8	17.6
anteiso-$C_{17:0}$	6.1	13.3	2	2.1	6.8	5	1.8	3.5
$C_{15:0}$-2OH	-	1.8	-	-	-	-	-	-
Monounsaturated								
$C_{16:1}$ ω11*c*	1.9	Tr	3.3	6.5	2.4	11.6	1.5	3.4
$C_{16:1}$ *ω7c* alcohol	Tr	Tr	5.4	9.8	Tr	3.4	4.4	Tr
iso-$C_{17:1}$ *ω10c*	-	-	3.7	2.3	-	1.1	-	Tr
$C_{16:1}$ *ω5c*	-	-	-	-	-	1	-	-
Summed features*								
4	-	Tr	1	1	-	3.6	-	Tr

* All the strains were grown on TSA plates and incubated at 30°C for 24 h except strain 7, *B. pocheonensis* KACC 14006^T, which was grown on the R2A agar.
* Summed features repersent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 4 contained iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B.

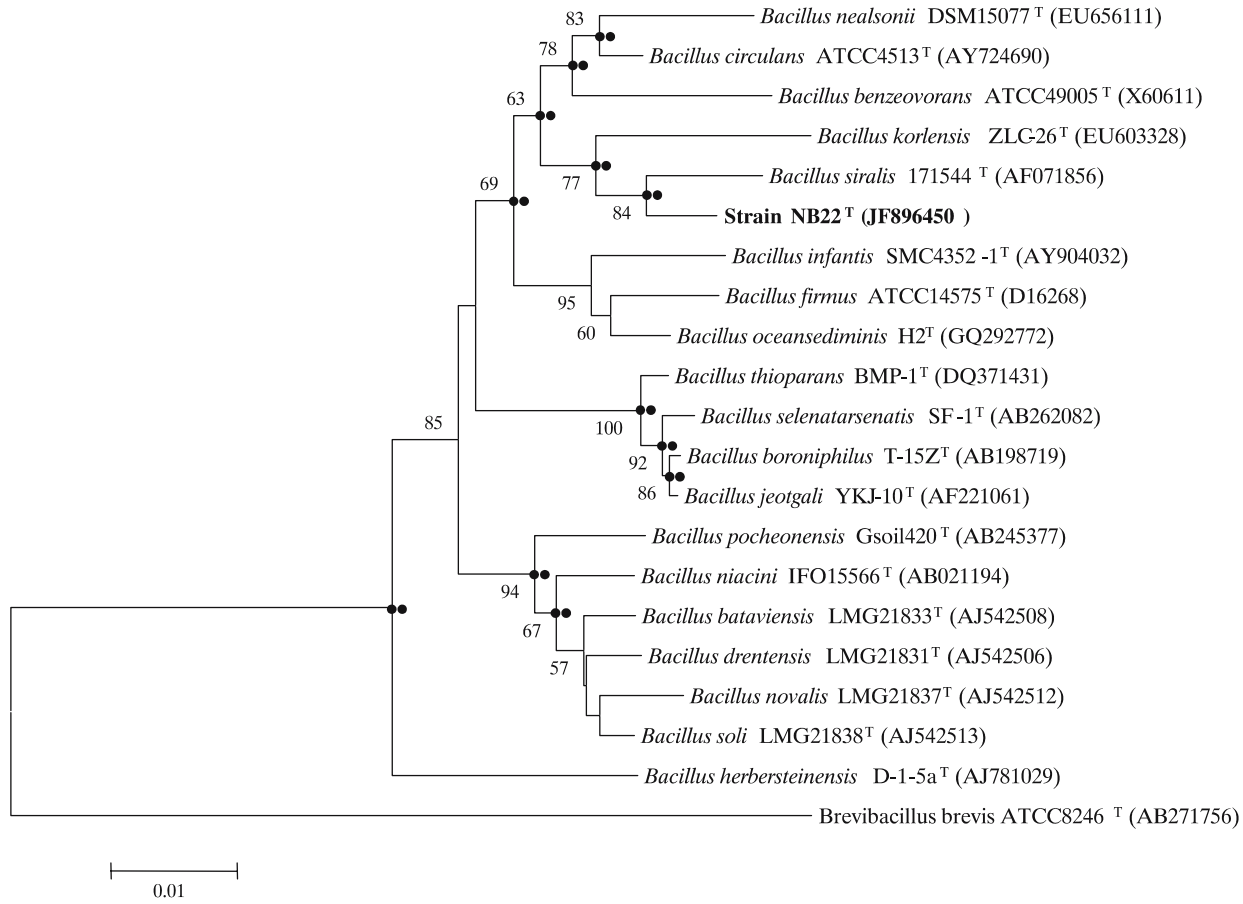

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships of strain NB22^T and related taxa. Percentage bootstrap values based on 1,000 replications are given at branch points. *B. brevis* ATCC 8246^T (AB2717456) was used as the outgroup. Bar, 0.01 substitutions per nucleotide position. A filled circle indicates the common nodes recovered from either the maximum-parsimony tree or the maximum-likelihood tree. Filled double circles indicate that, the corresponding nodes were recovered in both the maximum-parsimony tree and maximum-likelihood tree.

ences in fatty acid contents could be observed between strain NB22^T and closely related species. In particular, in comparison with *B. siralis* KACC 11246^T, *B. korlensis* KCTC 13245^T, *B. circulans* KACC 14392^T, *B. drentensis* KACC 12118^T, and *B. pocheonensis* KACC 14006^T, strain NB22^T contained larger amounts of $C_{14:0}$.

The predominant isoprenoid quinone was MK-7, in agreement with those of numerous species of genus *Bacillus* (Claus and Berkeley, 1986).

Phylogenetic analysis

For strain NB22^T, 1,463 bp of the 16S rRNA gene sequence were determined. Comparative 16S rRNA gene sequence analysis showed that strain NB22^T was most closely related to members of the genus *Bacillus*. Strain NB22^T exhibited the highest 16S rRNA gene sequence similarities to *B. siralis* KACC 11246^T (98.1%), *B. korlensis* KCTC 13245^T (97.3%), *B. circulans* KACC 14392^T (97.0%), *B. drentensis* KACC 12118^T (96.9%), *B. ocenisediminis* JCM 16506^T (96.8%), *B. nealsonii* KACC 12124^T (96.8%), and *B. pocheonensis* KACC 14006^T (96.5%). The 16S rRNA gene sequence similarities to the type strains of all other *Bacillus* species with validly published names were below 96.4%. These data indicate that strain NB22^T is separate from the recognized *Bacillus* species other than the seven mentioned above (Stackebrandt and Goeble, 1994). In the phylogenetic tree based on the neighbor-joining algorithm, strain NB22^T fell within a coherent cluster comprising *B. siralis* KACC 11246^T and *B. korlensis* KCTC 13245^T. The topologies of the phylogenetic trees generated using the maximum-parsimony and maximum-likelihood algorithms were somewhat different from that of the tree constructed using the neighbor-joining method. Nevertheless, strain NB22^T formed a branch with *B. siralis* KACC 11246^T and *B. korlensis* KCTC 13245^T in these three phylogenetic trees. (Fig. 2)

DNA G+C content and DNA-DNA hybridization

The DNA G+C content of strain NB22^T was 36.3 mol%, which lies within the range observed for members of the genus *Bacillus* (Shida *et al*., 1997).

To differentiate strain NB22^T from closely related species, DNA-DNA hybridization was performed. Levels between

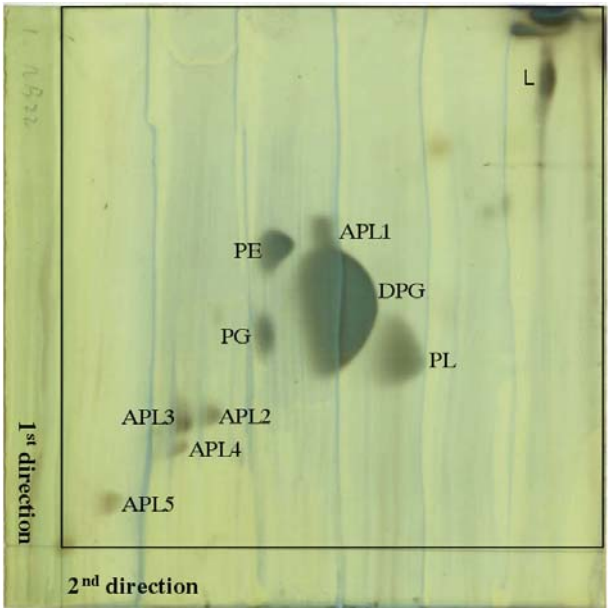

Fig. 3. Two dimensional thin-layer chromatogram of the polar lipids of *B. kyonggiensis* nov. sp NB22^T. Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PL, unknown phospholipid; APL1-APL5, unknown aminophospholipid; L, unknown polar lipid.

strain NB22^T and *B. siralis* KACC 11246^T and *B. korlensis* KCTC 13245^T were 18% and 9%, respectively. These values indicate that strain NB22^T is not related to them at the species level (Wayne *et al*., 1987).

Taxonomic conclusion

The phenotypic and phylogenetic characterizations indicated that strain NB22^T belongs to the genus *Bacillus*. The phylogenetic distinctiveness, together with DNA-DNA hybridization data, confirmed that this strain represents a species that differs from the recognized *Bacillus* species. There are some phenotypic differences between strain NB22^T and phylogenetically related *Bacillus* species. Therefore, on the basis of the data presented, strain NB22^T should be classified within the genus *Bacillus* as the type strain of a novel species, for which the name *Bacillus kyonggiensis* sp. nov., is proposed.

Description of *Bacillus kyonggiensis* sp. nov.

Bacillus kyonggiensis (*ky.ong.gi.en'sis*. N.L. masc. adj. *kyonggiensis*, pertainging to Kyonggi Province, South Korea from where the type strain was isolated).

Cells are Gram-positive, rod-shaped, facultative, spore-forming, non-motile, 1.8-3.0 μm long, and 0.5-0.7 μm wide. Endospores are oval, lie subterminally, occasionally para-centrally, and usually cause the sporangia to swell. Growth occurs at 15-42°C (optimal, 30-37°C), at pH 7.0-10.0 (optimal, pH 8.0-9.0), and in the presence of 0-4% (w/v) NaCl (optimal, 1-2%). Oxidase- and catalase-positive. Acid is produced from D-arabinose, amygdalin, arbutin, celiobiose, esculin, ribose, fructose, galactose, gentiobiose, gluconate, glucose, mannose, mannitol, maltose, salicin, starch, trehalose, and D-xylose, and weakly from glycerol, methyl-α-D-glucose, N-acetyl-glucosamine, lactose, rhamnose, and D-turanose, but not from L-arabinose, D-arabitol, L-arabitol, L-adobitol, dulcitol, erythritol, D-fucose, L-fucose, 2-keto-gluconate, 5-keto-gluconate, glycogen, inositol, inulin, D-lyxose, methyl-α-D-mannopyranside, melezitose, melibiose, raffinose, sorbitol, sucrose, sorbose, D-tagatose, xylitol, xylose or methyl-B-D-xylopyranside. According to the API ZYM gallery, strain NB22^T produces alkaline phosphatase, α-chymotrypsin, esterase (C4), esterase lipase (C8), α-glucosidase, β-glucuronidase, naphtol-AS-BI-phosphohydrolase, but not acid phosphatase, cystine arylamidase, α-fucosidase, α-galactosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, lipase (C14), leucine arylamidase, α-mannosidase, trypsin or valine arylamidase. The cell wall peptidoglycan contains *meso*-diaminopimelic acid. The polar lipid profile is composed of the major compounds phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an unkown phospholipid, five kinds of unknown aminophospholipid and a unknown polar lipid. The predominatant menaquinone is MK-7. Major fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{14:0}$, and $C_{16:0}$. The DNA G+C content of the type strain is 36.3 mol%. The type strain, NB22^T (=KEMB 5401-267^T =JCM 17569^T), was isolated from soil of a lettuce field in Kyonggi province, South Korea.

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References

Baron, S. 1996. Chapter 15, *Bacillus*. *In* Medical Microbiology 4th edition. University of Texas Medical Branch at Galveston, Galveston, USA.

Chun, J., J.H. Lee, Y. Jung, M. Kim, S. Kim, B.K. Kim, and Y.W. Lim. 2007. EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int. J. Syst. Evol. Microbiol.* 57, 2259-2261.

Claus, D. and R.C.W. Berkeley. 1986. Genus *Bacillus* Cohn 1872. *In* Bergey's Manual of Systematic Bacteriology, vol. 2, pp. 1105-1140. Williams and Wilkins, Baltimore, USA.

Doetsch, R.N. 1981. Determinative methods of light microscopy. *In* Manual of Methods for General Bacteriology, pp. 21-33. American Society for Microbiology, Washington, D.C., USA.

Euzéby, J.P. 2008. List of Prokaryotic Names with Standing in Nomenclature. http://www.bacterio.cict.fr/

Ezaki, T., Y. Hashimoto, and E. Yabuuchi. 1989. Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Bacteriol.* 39, 224-229.

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

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.

Hiraishi, A., Y. Ueda, J. Ishihara, and T. Mori. 1996. Comparative lipoquinone analysis of influent sewage and activated sludge by highperformance liquid chromatography and photodiode array detection. *J. Gen. Appl. Microbiol.* 42, 457-469.

Kempf, M.J., F. Chen, R. Kern, and K. Venkateswaran. 2005. Recurrent isolation of hydrogen peroxide-resistant spores of *Bacillus pumilus* from a spacecraft assembly facility. *Astrobiology* 5, 391-405.

Kim, M.K., W.T. Im, H. Ohta, M. Lee, and S.T. Lee. 2005. *Sphingopyxis granuli* sp. nov., a β-glucosidase producing bacterium in the family *Sphingomonadaceae* in a-4 subclass of the Proteobacteria. *J. Microbiol.* 43, 152-157.

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

Komagata, K. and K. Suzuki. 1987. Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol*. 19, 161-203.

Mesbah, M., U. Premachandran, and W.B. Whitman. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by highperformance liquid chromatography. *Int. J. Syst. Bacteriol.* 39, 159-167.

Minnikin, D.E., A.G. O'Donnell, M. Goodfellow, G. Anderson, M. Athalye, A. Schaal, and J.H. Parlett. 1984. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J. Microbiol. Methods* 2, 233-241.

Sasser, M. 1990. Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids, *MIDI Technical Note* 101. MIDI Inc., Newark, DE, USA.

Shida, O., H. Takagi, K. Kadowaki, L.K. Nakamura, and K. Komagata. 1997. Transfer of *Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdlanolyticus*, *Bacillus glucanolyticus*, *Bacillus kobensis*, and *Bacillus thiaminolyticus* to the genus *Paenibacillus* and emended description of the genus. *Int. J. Syst. Bacteriol.* 47, 289-298.

Stackebrandt, E. and B.M. Goebel. 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* 44, 846-849.

Staneck, J.L. and G.D. Roberts. 1974. Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl. Microbiol.* 28, 226-231.

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. Evol.* 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.

Wayne, L.G., D.J. Brenner, R.R. Colwell, P.A.D. Grimont, O. Kandler, M.I. Krichevsky, L.H. Moore, and *et al.* 1987. International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.* 37, 463-464.

Weisburg, W.G., S.M. Barns, D.A. Pelletier, and D.J. Lane. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol*. 173, 697-703.

Wilson, K. 1987. Preparation of genomic DNA from bacteria. *In* Current Protocols in Molecular Biology, pp. 2.4.1-2.4.5. Green Publishing and Wiley-Interscience, New York, NY, USA.