Strain BH45\(^T\) was isolated from forest soil of Mt. Bukhan in Jeongneung, Seoul, Korea. The Gram-staining-negative strain BH45\(^T\) grows at 4–30\(^\circ\)C (optimum of 25–30\(^\circ\)C) and between pH 5–8 (optimum of pH 6–8). Its major cellular fatty acids are C\(_{18:1}\)ω6c (6,9,12) and C\(_{16:0}\). The G+C content of genomic DNA was 40.2 mol%. The major respiratory quinone system is menaquinone-7. Phylogenetic analysis based on 16S rRNA gene sequences indicates that strain BH45\(^T\) is closely related to the genus Pedobacter. Sequence similarities with P. terrae KCTC 12762\(^T\), P. suwonensis KACC 11317\(^T\), P. soli KACC 14939\(^T\), P. alluvionis DSM 19624\(^T\), P. roseus KCCM 42272\(^T\), P. yonginense KCTC 22721\(^T\), and P. africanus KCTC 12762\(^T\) evidenced 97.5, 97.1, 97.0, 97.0, 97.0, and 96.0%, respectively. DNA-DNA hybridization results distinguish strain BH45\(^T\) from two Pedobacter species with high 16S rRNA gene sequence similarities. According to the phenotypic and molecular data, the strain BH45\(^T\) clearly represents a novel species within the genus Pedobacter; thus, the name Pedobacter jeongneungensis sp. nov. is proposed for this strain. The type strain is BH45\(^T\) (=KACC 15514\(^T\) =JCM 17626\(^T\)).

**Keywords:** soil, sphingolipid, Bacteroidetes

Pedobacter species have been isolated from diverse environments, including sediments (Gordon et al., 2009), compost (Lee et al., 2009), glaciers (Shivaji et al., 2005), rice paddies (Jeon et al., 2009), and soils (Yoon et al., 2006). Pedobacter’s diverse sources of isolation and frequent detection by bacterial community analysis (Leung and Topp, 2001; Sun et al., 2004) are reflective of its ubiquity and excellent adaptability. Isolation of a novel bacterial species was performed in our effort to study bacterial communities and nitrogen biogeochemical cycles in the forest soils (Jung et al., 2012). Strain BH45\(^T\) was isolated from forest soils of Mt. Bukhan (Seoul, Korea). One gram of soil was vigorously agitated in MSB media (Stanier et al., 1966) containing 0.1% (v/v) methanol for three days. The supernatant of the methanol enrichment was cultured for 7 days on MSB agar plates containing 0.1% (v/v) methanol at room temperature. One of the colonies was transferred to a nutrient agar plate and designated as strain BH45\(^T\). Phenotypic, biochemical and morphological characteristics were tested using routine culture in nutrient broth at 30\(^\circ\)C, unless stated.

The 16S ribosomal RNA gene was amplified using universal 27f and 1492r primers. PCR product was cloned into pGEM-T easy vector (Promega, USA) and sequenced with T7 primer. Sequence data was manually checked to guarantee high quality of sequences. Sequence similarity was calculated on EzTaxon (Chun et al., 2007). To construct the phylogenetic trees, the 16S rRNA gene sequences of strain BH45\(^T\) and all Pedobacter type species were aligned with ClustalX (Larkin et al., 2007). Phylogenetic trees were drawn using Phylip software (Felsenstein, 1989) (100–105). Neighbor-joining tree was drawn based on the distance matrix calculated with Kimura 2-parameter model. Bootstrapping was performed with 1,000 iteration and bootstrap values greater than 70 were shown.

The phylogenetic relationship of strain BH45\(^T\) was evaluated using 16S ribosomal RNA gene sequences. Nucleotide sequence similarity showed that strain BH45\(^T\) is closely related to the genus Pedobacter. Stackebrandt and Goebel (1994) suggested that sequence similarity of less than 97% could be considered a cutoff value for novel species identification. P. terrae KCTC 12762\(^T\), P. suwonensis KACC 11317\(^T\), P. soli KACC 14939\(^T\), P. alluvionis DSM 19624\(^T\), P. roseus KCCM 42272\(^T\), and P. yonginense KCTC 22721\(^T\) evidenced 97.5, 97.1, 97.0, 97.0, 97.0, and 96.0% of 16S rRNA gene sequence similarities with the strain BH45\(^T\). The neighbor-joining tree shows that strain BH45\(^T\) is most closely related to P. terrae KCTC 12762\(^T\) and P. suwonensis KACC 11317\(^T\), as suggested by the measured gene sequence similarities (Fig. 1). The top-
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Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships of strain BH45\textsuperscript{T} and other Pedobacter type species. Bootstrap values (expressed as percentages of 1,000 replicates) greater than 70% are shown at branch points. Open circles and closed circles indicate that the corresponding nodes are also recovered in the maximum-likelihood only or the maximum-likelihood and the minimum evolution tree together, respectively. Balneola alkaliiphila DSM 19538\textsuperscript{T} was used as an out-group. Bar, 0.02 changes per nucleotide position.

Cell shape and size was observed with a phase-contrast microscope (Zeiss Axio Imager 2.0). Growth was tested by measuring the optical density at 600 nm. To determine temperature range, strain BH45\textsuperscript{T} was grown at 4, 20, 25, 30, and 37°C. To determine the pH range of growth, strain BH45\textsuperscript{T} was grown at pH values of 4, 5, 6, 7, 8, and 9 in nutrient broth. Mono- and disodium phosphate buffer were used for pH adjustment before autoclaving and pH of culture media was verified after autoclaving. For anaerobic growth, strains were incubated in the vial that the gas was exchanged with nitrogen gas and sealed with silicon. Nitrate reduction test was performed according to Lányi (1987). The growth of strains BH45\textsuperscript{T} were tested on the following 23 carbon sources: glucose, gluconate, fructose, sucinate, pyruvate, acetate, succinate, citrate, acetate, succrose, galactose, corn oil, phenylactic acid, gentisate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, 3,4-dihydroxybenzoate, salicylate, catechol, naphthalene, tolenuene, paraffin, hexadecane, and diesel fuel. Insoluble liquid compounds and naphthalene were used at 1% (v/v) and 1% (w/v), respectively. The other soluble compounds were added to MSB media at 5 mM. Strain BH45\textsuperscript{T}, P. terrae KCTC 12762\textsuperscript{T}, P. suwonensis KACC 11317\textsuperscript{T}, P. soli KACC 14939\textsuperscript{T}, P. alvillanis DSM 19624\textsuperscript{T}, P. roseus KCCM 42272\textsuperscript{T}, and P. yonginense KCTC 22721\textsuperscript{T}, and P. heparinus JCM 7457\textsuperscript{T} were cultured at room temperature (~25°C) for carbon source utilization. For oxidase activity, the overnight cultures were allowed to react with 1% (w/v) N, N, N', N'-tetramethyl-p-phenylenediamine dihydrochloride on a slide glass. A color change to violet within 20 sec is considered an oxidase-positive result. To determine catalase activity, 3% (v/v) hydrogen peroxide solution was dropped onto the overnight cultures. Bubble production was regarded as a catalase-positive result. For the gelatin hydrolysis test, strain BH45\textsuperscript{T} was grown in nutrient broth containing 12% (w/v) gelatin for 2 days and 5 days at 30°C. Liquefaction of culture media at 4°C is considered as gelatin hydrolysis-positive. Indole production and hydrolysis of carboxymethylcellulose and casein were determined as described by Smibert and Krieg (1994). Hydrolysis of starch, Tween 20, 40, 60, and 80 was evaluated as described by Cowan and Steel (1965). Gliding motility was determined under the phase-contrast microscope (Zeiss Axio Imager 2.0) via hanging drop technique (Bernardet et al., 2002). The presence of flexirubin-type pigments was determined as described by Reichenbach (1992). To determine antibiotics resistance, cells were grown in the nutrient broth and washed twice with sterile PBS buffer. Washed cells (~10\textsuperscript{5} CFU/ml) were inoculated into the fresh nutrient broth containing antibiotics. Antibiotics resistance was tested in the nutrient broth containing 1, 2, 5, 10, 20, 40 μg/ml of ampicillin, kanamycin, rifampicin, and tetracyclin; 0.1, 0.5, 1, 2, 5, 10, 30