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NOTE

Pedobacter jeongneungensis sp. nov., Isolated from Forest Soil[§]

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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°C (optimum of 25–30°C) and between pH 5–8 (optimum of pH 6–8). Its major cellular fatty acids are $C_{18:3}$ *ω6c* (6,9,12) and $C_{10:0}$. The G+C content of genomic DNA was 40.2 mol%. The major respiratory quinone system in strain BH45^T 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 were 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*

The genus *Pedobacter* belongs to the family *Sphingobacteriaceae.* The type species of this genus, *P. heparinus*, has a long taxonomic history. Korn and Payza (1956) first described strain HIM 762-3^T as *Flavobacterium heparinum* (Korn and Payza, 1956). Later, this strain, HIM 762-3^T, was successively transferred to the genera *Cytophaga* (Christensen, 1980) and *Sphingobacterium* (Takeuchi and Yokota, 1992). Finally, Steyn *et al.* (1998) proposed the genus *Pedobacter* according to a series of detailed analyses, including DNA-rRNA and DNA-DNA hybridization of heparinase-producing isolates. Four novel species-*P. heparinus*, *P. piscium*, *P. africanus*, and *P. saltans*-were the early recognized species of *Pedobacter* (Steyn *et al.*, 1998) and currently, *Pedobacter* consists of 32 species. *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°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-parametar 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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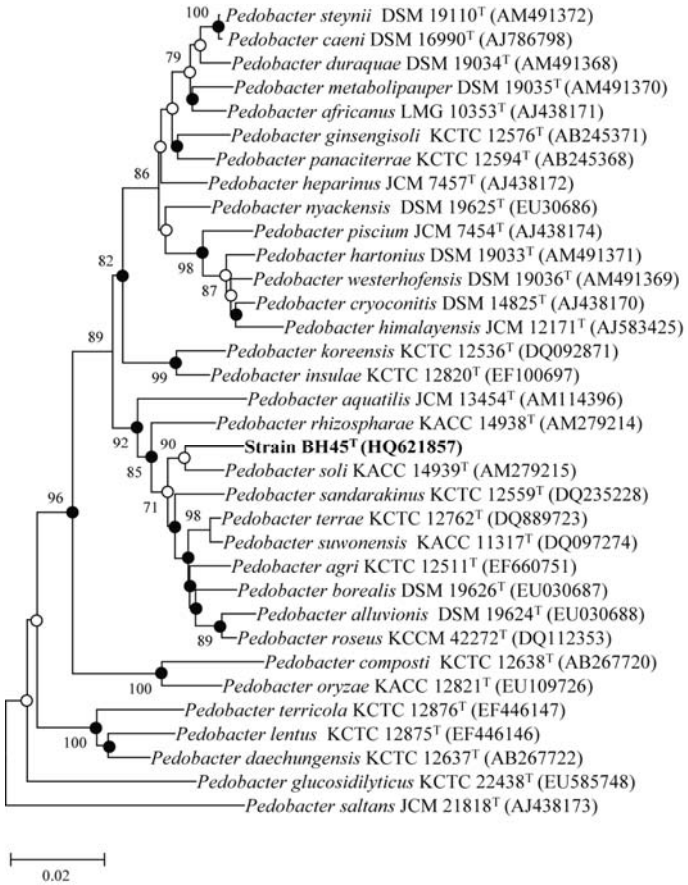

Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships of strain BH45^T and other *Pedobacter* type species. Bootstrap values (expressed as percentages of 1,000 replicates) greater than 70% are shown at branch points. Open circles and closed circles indicate that the corresponding nodes are also recovered in the maximum-likelihood only or the maximum-likelihood and the minimum evolution tree together, respectively. *Balneola alkaliphila* DSM 19538^T was used as an out-group. Bar, 0.02 changes per nucleotide position.

ology of the maximum likelihood and minimum evolution trees are essentially the same with that of the neighbor-joining tree as indicated with open and closed circles in the Fig. 1.

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^T was grown at 4, 20, 25, 30, and 37°C. To determine the pH range of growth, strain BH45^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^T were tested on the following 23 carbon sources: glucose, gluconate, fructose, succinate, pyruvate, citrate, acetate, sucrose, galactose, corn oil, phenylacetic acid, gentisate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, 3,4-dihydroxybenzoate, salicylate, catechol, naphthalene, toluene, 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^T, *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. heparinus* JCM 7457^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, N9, N9*-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^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 (~10^5 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

μg/ml of chloramphenicol, gentamicin, and norfloxacin. Increase of OD_{600} more than 0.1 after 7 days incubation at 30°C was cutoff value to determine antibiotics resistance. The enzymatic characteristics were determined using a VITEK 2 system (bioMérieux, France) as described in the manufacturer's instructions. In brief, strain BH45^T, *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. heparinus* JCM 7457^T were grown on nutrient agar plates for 24 h. Colonies were suspended in 3 ml of 0.45% NaCl solution. The prepared solutions were then loaded onto VITEK2 GN cards and the data were analyzed using the AES parameter version of VITEK2 (03.01). DNA G+C content was determined as described previously (Gonzalez and Saiz-Jimenez, 2002).

Phenotypic characteristics of strain BH45^T and reference species are shown in Table 1. Colonies of strain BH45^T are circular, 1–2 mm in diameter, smooth, convex, and pink after 2 days. The cells are rod-shaped and 1.8×0.5 μm in size. Strain BH45^T exhibited growth at 4, 20, 25, and 30°C. No significant differences in growth were noted between 25°C and 30°C, and no growth was noted at 37°C. Strain BH45^T grows between pH 4 and 8, but optical density did not increase more than 0.4 at pH 4. No differences in growth were noted at pH 6–8. Therefore, the optimal growth conditions were 25–30°C and pH 6–8. Strain BH45^T exhibited positive oxidase and catalase activity, as was also noted with other *Pedobacter* species (Steyn *et al.*, 1998). Flexirubin-type pigment is absence. The results of casein, Tween (20, 40, 60, 80), carboxymethylcellulose hydrolysis, indole production, nitrate reduction are shown in Table 1. Antibiotics resistance is also shown in Table 1 with the maximum concentration that the strains grow. Strain BH45^T and reference strains grew on the following carbon sources within 3 days under the test conditions: glucose, fructose, citrate, sucrose, and 3-hydroxybenzoate.

In order to evaluate the membrane fatty acids composition, fatty acid methyl esters (FAME) were prepared in accord-

Table 1. Enzymatic characteristics determined with the VITEK 2 system and other phenotypic features. 1, Strain BH45^T; 2, *P. terrae* KCTC 12762^T; 3, *P. suwonensis* KACC 11317^T; 4, *P. soli* KACC 14939^T; 5, *P. alluvionis* DSM 19624^T; 6, *P. roseus* KCCM 42272^T; 7, *P. yonginense* KCTC 22721^T; 8, *P. heparinus* JCM 7457^T. All strains are positive for the followings: Tween 20, 40, 60, 80 and starch hydrolysis, enzyme presence of Ala-Phe-Pro-arylamidase, lipase, and tyrosine arylamidase. All strains are negative for the followings: enzyme presence of γ-glutamyl transferase, urease, glycine arylamidase, β-xylosidase, β-alanine arylamidase, and β-glucuronidase; acidification of adonitol, L-arabitol, D-mannitol, D-sorbitol, palatinose, tagatose, trehalose, citrate, malonate, 5-ketogluconate, coumarate, and ellman; assimilation of L-lactate, succinate, L-histidine, L-malate and production of H_2S; presence of flexirubin-type pigment; carboxymethylcellulose hydrolysis, and indole production; nitrate reduction; resistance to norfloxacin and rifampicin. All strains were grown under the same conditions and analyzed from this study except cell shape, maximum growth temperature, DNA G+C content.

	1	2	3	4	5	6	7	8
Colony color	Pink	Pinkish yellow	Orange	Pink	Reddish Pink	Pink	Red	Transluent yellow
Cell shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Short Rod/Rod
Maximum growth temperature	30	35	37	35	30	33	37	37
DNA G+C content	40.2	39.7	44.2	38.6	34.4	41.3	41.0	42-43
Gliding motility	+	-	-	-	-	-	-	+
Gelatin hydrolysis	-	+	+	+	+	+	-	+
Casein hydrolysis	+	+	+	+	+	-	-	-
Enzyme presence								
L-Pyroglutamic acid arylamidase (Pyrase)	+	+	+	+	-	-	-	-
β-Galactosidase	-	+	+	+	+	-	-	-
β-N-Acetylglucosaminidase	+	+	+	+	+	-	-	-
Glutamyl arylamidase	+	+	+	-	+	-	-	-
β-Glucosidase	+	+	+	+	+	-	-	-
α-Glucosidase	+	+	+	+	+	-	+	-
β-N-acetyl-galactosaminidase	-	+	+	-	+	-	-	-
α-Galactosidase	-	+	+	+	+	-	-	-
Phosphatase	-	-	+	+	+	+	-	-
Proline arylamidase	+	-	-	+	-	-	-	-
Glu-Gly-Arg-Arylamidase	-	+	-	-	+	-	-	-
Acidification								
D-Cellobiose	-	+	+	-	+	-	-	-
D-Glucose	-	+	-	+	+	+	-	-
D-Maltose	-	+	-	+	+	-	-	-
D-Mannose	-	+	+	+	+	+	-	-
Sucrose	-	+	+	-	+	-	+	-
Antibiotics resistance (μg/ml)								
Ampicillin	40	10	10	10	10	10	10	-
Chloramphenicol	-	-	-	-	-	-	-	5
Gentamicin	1	30	30	30	30	10	10	-
Kanamycin	-	20	20	20	20	30	20	-
Tetracycline	-	-	-	-	-	-	-	2

Table 2. Cellular fatty acid compositions (%) of strain BH45^T and related *Pedobacter* species. 1, Strain BH45^T; 2, *P. terrae* KCTC 12762^T; 3, *P. suwonensis* KACC 11317^T; 4, *P. soli* KACC 14939^T; 5, *P. alluvionis* DSM 19624^T; 6, *P. roseus* KCCM 42272^T; 7, *P. yonginense* KCTC 22721^T; 8, *P. heparinus* JCM 7457^T Values are expressed as the percentage of total fatty acids. ND, not detected. All strains were grown at the same condition and analyzed from this study.

Fatty acids	1	2	3	4	5	6	7	8
$C_{10:0}$	29.70	34.80	30.87	32.35	25.53	29.35	27.16	32.06
$C_{11:0}$ 2OH	5.28	14.40	7.12	9.16	5.61	7.47	5.49	5.62
$C_{16:0}$ iso 3OH	ND	ND	ND	ND	24.63	ND	16.17	ND
$C_{18:3}$ *ω6c* (6, 9, 12)	46.82	43.00	48.14	43.78	44.24	43.07	44.40	48.08
$C_{20:2}$ *ω*6,9*c*	11.55	ND	13.86	5.53	ND	12.74	ND	ND
Summed feature 1[a]	6.65	7.94	ND	9.18	ND	7.37	6.78	7.92
Summed feature 3[a]	ND	ND	ND	ND	ND	ND	ND	6.32

[a] Summed features are groups of two or three fatty acids that could not be separated by gas liquid chromatography using MIDI system. Summed feature 1 contains $C_{15:1}$ isoH/13:0 3OH. Summed feature 3 contains $C_{16:1}$ *ω7c*/C16:1 *ω6c*.

ance with MIDI technical note #101 (Sasser, 1990) after 48 h of cultivation in TSBA at 28°C. GC-FID (Agilent 7890GC) and a Sherlock Microbial Identification System (version 6.0B, Aerobe library) were used to analyze the FAME results.

For polar lipids analysis, strain BH45^T and reference strains were cultured in nutrient broth at 30°C with shaking at 220 rpm for 1 days. Cells were harvested by centrifugation at 5,000×g and lyophilized. Polar lipids and sphingolipids were extraction and TLC analysis were performed as described by Tindall (1990) and Kato *et al.* (1995). Five microliter of extracts was spotted on a TLC plate (HPTLC silica gel 60F254, Merck, Germany). Mobile phases were chloroform-methanol-water (65:25:4, v/v/v) and chloroform-acetic acid-methanol-water (40:7.5:6:2) for the first and second dimension. Total lipids were visualized by spraying 5% phosphomolybdic acid and charring at 180°C for 15 min. Sphingolipids were detected with 0.5% ninhydrin and charred at 110°C for 15 min. Modified Dittmer-Lester reagent visualizing phospholipids was applied to the TLC plates used for sphingolipid analysis (Ryu and MacCoss, 1979). Glycolipid were visualized with 0.5% (w/v) 1-naphthol dissolved in methanol-water (1:1, v/v) and sulfuric acid followed by charring at 120°C until color appeared.

The major fatty acids were identified as $C_{18:3}$ *ω6c* (6, 9, 12) (46.8%), and $C_{10:0}$ (29.7%). Although detection of several fatty acids including $C_{11:0}$ 2OH and $C_{20:2}$ *ω*6,7*c* are not consistent with the results of the genus *Pedobacter* (Steyn *et al.*, 1998), the type species of the genus *Pedobacter*, *P. heparinus* JCM 7457^T also has those fatty acids under the condition we tested. Therefore variation in the fatty acids composition could be attributed to the culture condition of strains. Polar lipids profile of strain BH45^T was shown in Supplementary data Fig. S1. Phosphatidylethanolamine and an unidentified polar lipid were the major polar lipids in strain BH45^T. Five aminolipids, minor amount of unidentified polar lipids and a sphingolipid were detected. Glycolipid was not detected. All tested *Pedobacter* species showed similar results of polar lipid analysis (data not shown). The respiratory quinone system is determined via thin-layer chromatography (Hu *et al.*, 1999). DNA-DNA hybridization was performed according to Kang *et al.* (2007). Melting curve analysis results indicate that the G+C content of strain BH45^T is 40.2 mol% and the respiratory quinone system in strain BH45^T is menaquinone-7 (MK-7), which is consistent with the genus identification of *Pedobacter* (Steyn *et al.*, 1998). According to Wayne *et al.* (1987), a DDH value of 70% is the cutoff value for defining a novel species, and *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. heparinus* JCM 7457^T had DDH values of 13.0±0.9, 7.1±0.5, 2.5±0.7, 6.6±0.8, 7.2±0.4, 16.3±0.9, and 1.2±0.2, respectively. In conclusion, polyphasic approaches including the phylogenetic analysis with the sequence of 16S rRNA gene, FAME analysis, G+C content, the composition of quinone, and polar lipids analysis indicated that strain BH45^T is confidently affiliated with the genus *Pedobacter* while significant differences such as maximum growth temperature, antibiotic resistance, biochemical characteristics, and DNA-DNA hybridization were evidenced. Therefore, the phenotypic and molecular evidence support the notion that strain BH45^T is a novel species of the genus *Pedobacter*. The name *Pedobacter jeongneungensis* sp. nov. is proposed for this strain.

Description of *Pedobacter jeongneungensis* sp. nov.

Pedobacter jeongneungensis (jeong.neung.en'sis. N. L. masc. adj. *jeongneungensis* of Jeongneung, where the type strain was isolated. Mt. Bukhan is located in Jeongneung-dong).

Cells are Gram-staining-negative, 1.8×0.5 um in size, motile (gliding) and rod-shaped. Colonies are 1–2 mm in diameter, pink-color, convex, and round shape on nutrient agar plates at 2 days. Growth occurs aerobically at 4–30°C and between pH 5–8. Grows optimally at 25–30°C and pH 6–8. Oxidase and catalase teat are positive. Antibiotic resistance is observed in nutrient broth containing ampicillin (40 μg/ml) and gentamycin (1 μg/ml). Gelatin and carboxymethylcellulose is not hydrolyzed. Tween 20, 40, 60, 80, casein, and starch are hydrolyzed. Flexirubin-type pigment is absent. Indole and hydrogen sulfide are not produced. Enzyme presence determined via the VITEK 2 system is positive in Ala-Phe-Pro-arylamidase, L-pyroglutamic acid arylamidase, β-N-acetylglucosaminidase, glutamyl arylamidase, β-glucosidase, lipase, tyrosine arylamidase, α-glucosidase, proline arylamidase; negative in β-galactosidase, γ-glutamyl transferase, urease, β-N-acetyl-galactosaminidase, α-galactosidase, phosphatase, glycine arylamidase, β-xylosidase, β-alanine arylamidase, β-glucoronidase, and Glu-gly-Arg-arylamidase. Acidification is negative in adonitol, L-arabitol, D-cellobiose, D-glucose, D-maltose, D-mannitol, D-mannose, D-sorbitol, palatinose, sucrose, tagatose, trehalose, citrate, malonate, 5-ketogluconate, coumarate, and ellman. Assimilation is negative in L-lactate, succinate, L-histidine, and L-malate.

Sensitive to vibriostatic agent O129. Hydrogen sulfide is not produced. Glucose is not fermented. The major polar lipids are phosphatidylethanolamine and an unidentified polar lipid. Five unidentified aminolipids, minor amount of an unidentified polar lipid and a sphingolipid are present. The major fatty acids are $C_{18:3}$ *ω6c* (6, 9, 12) and $C_{10:0}$. Major respiratory quinone system is menaquinone-7. G+C content of DNA is 40.2 mol%. The type strain, BH45^T (=KACC 15514^T =JCM 17626^T), was isolated from forest soils of Mt. Bukhan (Seoul, Korea).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BH45^T is HQ621857.

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