INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION
Lee and Jeon, *Int J Syst Evol Microbiol* 2017;67:4767–4772
DOI 10.1099/ijsem.0.002377

MICROBIOLOGY SOCIETY

Cohnella algarum sp. nov., isolated from a freshwater green alga *Paulinella chromatophora*

Yunho Lee and Che Ok Jeon*

Abstract

A Gram-stain-positive, facultatively aerobic and endospore-forming bacterium, designated strain Pch-40^T, was isolated from a freshwater green alga, *Paulinella chromatophora*. Cells were motile rods with a monotrichous polar flagellum showing catalase- and oxidase-positive reactions. Strain Pch-40^T grew at 20–50 °C (optimum, 37–40 °C), at pH 5.0–11.0 (optimum, pH 7.0) and in the presence of 0–4.0 % (w/v) NaCl (optimum, 0 %). Menaquinone-7 was detected as the sole isoprenoid quinone. The genomic DNA G+C content of strain Pch-40^T was 55.6 mol%. The major cellular fatty acids of strain Pch-40^T were $C_{16:0}$, iso-$C_{16:0}$, anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain Pch-40^T clearly belonged to the genus *Cohnella* of the family *Paenibacillaceae*. Strain Pch-40^T was most closely related to *Cohnella rhizosphaerae* CSE-5610^T with a 96.1 % 16S rRNA gene sequence similarity. The phenotypic and chemotaxonomic features and the phylogenetic inference clearly suggested that strain Pch-40^T represents a novel species of the genus *Cohnella*, for which the name *Cohnella algarum* sp. nov. is proposed. The type strain is strain Pch-40^T (=KACC 19279^T=JCM 32033^T).

The genus *Cohnella*, belonging to the family *Paenibacillaceae* of the phylum *Firmicutes*, was first proposed by Kämpfer *et al.* [1] with *Cohnella thermotolerans* as the type species. The genus *Cohnella* accommodates Gram-stain-positive, endospore-forming or non-endospore-forming, aerobic or facultatively aerobic, rod-shaped bacteria. Members of the genus *Cohnella* generally have menaquinone-7 (MK-7) as the predominant isoprenoid quinone, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine as the major polar lipids and anteiso-$C_{15:0}$, iso-$C_{16:0}$ and $C_{16:0}$ as the major cellular fatty acids [1, 2]. Members of the genus *Cohnella* have been isolated from various ecological habitats, including starch production industry [1], a volcanic pond [3], root nodules [4], soil [2, 5–8], fresh water [9], buffalo faeces [10], rhizosphere [11, 12] and coolant lubricant solution [13]. At the time of writing, the genus *Cohnella* comprises 26 validated species (www.bacterio.net/cohnella.html) and three unvalidated species '*Cohnella plataginis*', '*Cohnella humi*' and '*Cohnella capsici*' [13]. It has been reported that bacteria living in algal spheres (the surface or inside of algal cells) intimately interact with algae through their various metabolic functions, such as nitrogen fixation, vitamin and nutrient mineralization, and these algal-associated bacteria produce various useful compounds for industry [14, 15]. Therefore, algal-associated bacteria have attracted a great deal of attention in order to understand bacteria–algae interactions and to be able to exploit them in industry [16, 17]. In this study, we isolated a putative novel species belonging to the genus *Cohnella*, designated strain Pch-40^T, from the algal sphere of *Paulinella chromatophora* and characterized it further taxonomically using a polyphasic approach.

A freshwater green alga, *P. chromatophora*, that was isolated from a water reservoir located in Chungcheongnam-do, Republic of Korea (36° 33′ 39.90″ N 126° 45′ 45.13″ E) was cultivated at 25 °C for 4 weeks in DY-V medium (20 mg MES, 5 mg $MgSO_4 \cdot 7H_2O$, 0.3 mg KCl, 0.27 mg NH_4Cl, 2 mg $NaNO_3$, 0.22 mg β-Na_2glycerophosphate$\cdot 5H_2O$, 0.08 mg H_3BO_3, 0.8 mg $Na_2EDTA \cdot 2H_2O$, 1.4 mg $Na_2SiO_3 \cdot 9H_2O$, 0.1 mg $FeCl_3 \cdot 6H_2O$, 7.5 mg $CaCl_2 \cdot 2H_2O$, 0.05 µg vitamin B_{12}, 0.05 µg biotin, 10 µg thiamine hydrochloride, 20 µg $MnCl_2 \cdot 4H_2O$, 4 µg $ZnSO_4 \cdot 7H_2O$, 0.8 µg $CoCl_2 \cdot 6H_2O$, 2 µg $Na_2MoO_4 \cdot 2H_2O$, 0.2 µg Na_3VO_4, 0.2 µg H_2SeO_3, per litre, pH 6.8) under alternating light and dark conditions. After the cells of *P. chromatophora* were homogenized using a homogenizer for 1 min, the samples were serially diluted in fresh DY-V medium. The aliquots of each serial dilution were spread onto R2A agar (BD) and incubated aerobically

Author affiliation: Department of Life Science, Chung-Ang University, Seoul 06974, Republic of Korea.
***Correspondence:** Che Ok Jeon, cojeon@cau.ac.kr
Keywords: *Cohnella algarum*; new taxa; Firmicutes; green alga; *Paulinella chromatophor*.
Abbreviations: LB, Luria–Bertani; MK-7, menaquinone-7; ML, maximum-likelihood; MP, maximum-parsimony; NA, nutrient agar; NJ, neighbour-joining; TSA, tryptic soy agar.
The GenBank accession number for the 16S rRNA gene sequence of strain Pch-40^T is KY864397.
One supplementary table and three supplementary figures are available with the online Supplementary Material.

at 25 °C for 3 days. The 16S rRNA genes of colonies grown on R2A agar were PCR-amplified using the universal primers, F1 and R13, and double-digested with *Hae*III and *Hha*I, as described previously [18], and then the representative PCR amplicons showing distinct fragment patterns were partially sequenced using the universal primer 340F (5′-CCT ACG GGA GGC AGC AG-3′). The resulting 16S rRNA gene sequences were compared with those of all reported type strains using the Nucleotide Similarity Search program in the EzTaxon-e server (http://www.ezbiocloud.net/identify) [19]. From the comparative 16S rRNA gene sequence analysis, a putative novel strain belonging to the genus *Cohnella*, designated strain Pch-40^T, was selected for further phenotypic and phylogenetic analyses. Strain Pch-40^T was routinely cultured aerobically on R2A agar at 40 °C for 3 days, except where indicated, and stored at −80 °C in R2A broth (BD) containing 15 % (v/v) glycerol for a long-term preservation. The type strains of *Cohnella rhizosphaerae* (DSM 28161^T), *Cohnella xylanilytica* (KCTC 22294^T), *Cohnella laeviribosi* (KCTC 3987^T), *Cohnella thermotolerans* (KACC 11643^T) and *Cohnella panacarvi* (KCTC 13060^T) were obtained from their culture collection centres for comparison of their phenotypic properties and cellular fatty acid compositions.

The 16S rRNA gene amplicon of strain Pch-40^T that was PCR-amplified using the F1 and R13 primers was further sequenced using the universal primers, 518R (5′-ATT ACC GCG GCT GCT GG-3′) and 805F (5′-GAT TAG ATA CCC TGG TAG TC-3′) at Macrogen (Republic of Korea) to obtain an almost-complete 16S rRNA gene sequence (1476 nucleotides). The 16S rRNA gene sequences of strain Pch-40^T and closely related type strains were aligned using the fast secondary-structure aware Infernal aligner in the Ribosomal Database Project (https://pyro.cme.msu.edu/login.spr) [20]. Phylogenetic relationships of strain Pch-40^T with closely related type strains were inferred using the DNADIST and DNAPARS programs based on the neighbour-joining (NJ) algorithm with the Kimura two-parameter model and the maximum-parsimony (MP) algorithm through a heuristic search, respectively, in the PHYLIP software (version 3.695) [21]. Their tree topologies were evaluated through bootstrap analyses based on a 1000-resampled dataset. A maximum-likelihood (ML) analysis with bootstrap values was performed using RAxML-HPC BlackBox (version 8.2.9) available in the Cyber-Infrastructure for Phylogenetic Research project (CIPRES, www.phylo.org) [22].

Comparative analysis based on the 16S rRNA gene sequences showed that strain Pch-40^T had the highest sequence similarities with the type strains of *C. rhizosphaerae* (96.1 %), *C. xylanilytica* (96.1 %), *Cohnella nanjingensis* (96.0 %), *Cohnella formosensis* (95.9 %) and *Cohnella lubricantis* (95.9 %). The phylogenetic analysis using the NJ algorithm indicated that strain Pch-40^T formed a distinct phylogenic lineage within the genus *Cohnella* of the family *Paenibacillaceae* (Fig. 1), which was also supported by the MP and ML algorithms (Fig. S1, available in the online Supplementary Material). The 16S rRNA gene sequence similarities between strain Pch-40^T and other type strains, and the phylogenetic analyses clearly suggested that strain Pch-40^T represented a novel species of the genus *Cohnella*.

Growth of strain Pch-40^T was assessed at 40 °C for 3 days on several bacteriological agar media: R2A agar (BD), Luria-Bertani (LB; MP Biomedicals) agar, nutrient agar (NA; BD) and tryptic soy agar (TSA; BD). Growth of strain Pch-40^T at different temperatures (5–55 °C at 5 °C intervals) was tested on R2A agar at 40 °C. Growth of strain Pch-40^T at different pH values was evaluated in R2A broth with different pH values (4.0–12.0 at 1.0 pH unit intervals) for 3 days. R2A broth media with pH values below 8.0 and pH 8.5–12.0 were prepared using Na_2HPO_4-NaH_2PO_4 and Tris-HCl buffers, respectively, and were adjusted again if necessary after sterilization (121 °C for 15 min). Salt tolerance of strain Pch-40^T was assessed in R2A broth with different NaCl concentrations (0–5 % at 1 % intervals), which was prepared in the laboratory according to the BD formula. Gram-staining was conducted using the Gram stain kit (bioMérieux) according to the manufacturer's instructions.

Anaerobic growth was assessed on R2A agar at 40 °C for 3 weeks under an anaerobic condition (with 4–10 % CO_2) using the GasPak Plus system (BBL). Cell morphology and motility were observed using transmission electron microscopy (JEM-1010, JEOL) and phase-contract microscopy with cells grown in R2A broth at 40 °C for 3 days. Endospore formation of strain Pch-40^T was determined by malachite green spore-staining of cells grown on R2A agar at 40 °C for 3 days. Catalase and oxidase activities were tested by observing the production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution and the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck), respectively [23]. The following properties of strain Pch-40^T were investigated with reference strains under the same conditions. Hydrolysis of Tweens 20 and 80, casein, starch, tyrosine, and aesculin was checked on R2A agar, according to the methods of Smibert and Krieg [23] and Lányí [24]. Additional enzymatic activities, biochemical features and oxidations of carbon compounds were tested using the API ZYM and API 20NE kits (bioMérieux) and the GN2 MicroPlate system (Biolog), respectively, according to the manufacturers' instructions. Cells of strain Pch-40^T and reference strains resuspended in 0.85 % (w/v) saline were used as inocula for the tests.

Strain Pch-40^T grew well on R2A agar, TSA and NA, but did not grow on LB agar (optimum, R2A agar). Cells of strain Pch-40^T were Gram-stain-positive, motile rods with a monotrichous polar flagellum (Fig. S2). Cells of strain Pch-40^T were approximately 0.9–1.0 µm wide and 2.5–2.7 µm long. Colonies of strain Pch-40^T were 1.0–1.5 mm in diameter on R2A agar after 3 days of incubation. Anaerobic growth of Pch-40^T was observed after incubation on R2A agar at 40 °C for 3 weeks. In the Biolog GN2 MicroPlate, strain Pch-40^T oxidized D-fructose, D-galactose, centiobiose, α-D-glucose, Tween 40, Tween 80, lactose, maltose, D-mannose, melibiose, β-methyl-D-

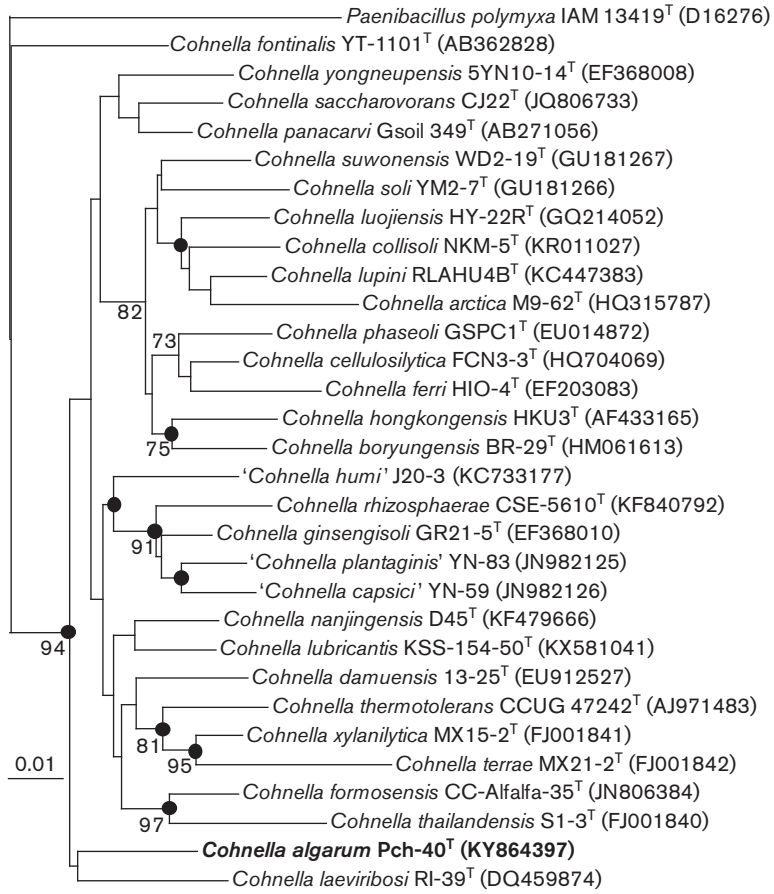

Fig. 1. A neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain Pch-40^T and related taxa. Bootstrap values with more than 70 % are shown on the nodes as percentages of 1000 replicates. Filled circles indicate that the corresponding nodes were also recovered in the trees reconstructed by the ML and MP algorithms. *Paenibacillus polymyxa* IAM 13419^T (D16276) was used as an outgroup. Bar, 0.01 changes per nucleotide position.

glucoside, D-psicose, raffinose, D-sorbitol, sucrose, trehalose, turanose, methyl-pyruvate, *cis*-aconitic acid, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, dextrin, *N*-acetyl-D-galactosamine, L-arabinose, D,L-lactic acid, D-alanine, hydroxy-L-proline, L-serine, glycerol, D, L-α-glycerol phosphate, *N*-acetyl-D-glucosamine, adonitol, L-fucose, *m*-inositol, D,L-carnitine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, γ-hydroxy butyric acid, D-arabitol, cellobiose, D-mannitol, D-serine, α-cyclodextrin, glycogen, lactulose, L-rhamnose, mono-methyl-succinate, acetic acid, formic acid, D-glucosaminic acid, β-hydroxy butyric acid, *p*-hydroxy phenylacetic acid, α-keto glutaric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, L-histidine, L-leucine, L-ornithine, L-proline, L-pyroglutamic acid, γ-amino butyric acid, urocanic acid and inosine, but did not oxidize *i*-erythritol, xylitol, α-keto valeric acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, succinamic acid, L-phenylalanine, glucuronamide, L-alaninamide, L-threonine, α-hydroxy butyric acid, itaconic acid and α-keto butyric acid. Many characteristics of Pch-40^T such as colony colour, Gram reaction, morphology, endospore formation and nitrate reduction were in good agreement with those of the reference strains of the genus *Cohnella*, whereas other properties such as facultatively aerobic growth, NaCl tolerance, motility and other many phenotypic properties allowed the differentiation of strain Pch-40^T from other closely related *Cohnella* species (Table 1). Phenotypic characteristics of strain Pch-40^T are presented in the species description and compared with those of the closely related type strains in Tables 1 and S1.

Isoprenoid quinone of strain Pch-40^T was analysed by using a high-performance liquid chromatography (model LC-20A, Shimadzu) system equipped with a reversed-phase column (250 Í 4.6 mm, Kromasil, AkzoNobel) and a diode array detector (SPD-M20A, Shimadzu) using methanol–isopropanol (2 : 1, v/v) as an eluent (1 ml min^{-1}), as described by Komagata and Suzuki [25]. For analysis of cellular fatty acids, strain Pch-40^T and the reference strains were cultivated in R2A broth at 40 °C and cells were harvested at the

Table 1. Phenotypic comparisons of strain Pch-40^T and the type strains of closely related *Cohnellla* species

Taxa: 1, strain Pch-40^T (this study); 2, *C. rhizosphaerae* DSM 28161^T [13]; 3, *C. xylanilytica* KCTC 22294^T [9]; 4, *C. laeviribosi* KCTC 3987^T [3]; 5, *C. thermotolerans* KACC 11643^T [1]; 6, *C. panacarvi* KCTC 13060^T [5]. All strains were positive for the following characteristics: Gram reaction, activity* of alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phospatase, naphtol-AS-BI-phosphohydrolase and β-galactosidase; assimilation* of 4-nitrophenyl-β-D-galactopyranoside, D-glucose, L-arabinose, D-mannose and maltose, and hydrolysis* of aesculin ferric citrate. All strains were negative for the following characteristics: nitrate reduction*, indole production*, activity* of arginine dihydrolase, cystine arylamidase, trypsin, α-chymotrypsin, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase; assimilation* of capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid and hydrolysis* of casein, tyrosine, Tween 20 and Tween 80. +, Positive; –, negative; W, weakly positive; ND, not determined.

Characteristics	1	2	3	4	5	6
Isolation source	Green alga	*Zea mays*	Soil	Volcanic pond	Starch-producing company	Soil
Endospore formation	+	–	+	+	+	+
Catalase	+	–	+	+	ND	+
Oxidase	+	+	+	–	+	+
Temperature range (°C)	20–50	15–45	20–45	37–52	20–55	18–45
Aerobic growth	Facultatively aerobic	Aerobic	Facultatively aerobic	Aerobic	Aerobic	Aerobic
Motility	+	–	+	–	–	–
NaCl tolerance (%)	<4	<1	<3	>1	<2	<2
Glucose fermentation	W	–	–	+	+	–
Hydrolysis of starch*	+	+	+	+	+	–
Assimilation (API 20NE)* of:						
D-Mannitol	–	+	+	+	+	+
N-acetyl-glucosamine	–	+	–	–	–	–
Potassium gluconate	–	–	+	+	+	+
Enzyme activity (API ZYM)* of:						
Urease	+	–	+	–	–	–
Gelatin hydrolysis	–	–	+	+	–	–
Lipase (C14)	W	W	+	–	–	–
Leucine arylamidase	W	W	+	W	+	–
Valine arylamidase	W	–	+	–	W	–
α-Galactosidase	+	+	+	+	+	–
β-Glucuronidase	+	–	+	+	+	–
α-Glucosidase	+	–	+	+	+	+
β-Glucosidase	+	–	+	+	+	–
DNA G+C content (mol%)	55.6	60	63.0	51.0	59.0	53.4

*These analyses were conducted under the same conditions in this study.

same growth phase (exponential phase, OD=0.8 at 600 nm). The fatty acids were saponified, methylated, extracted and washed using the standard MIDI protocol and the fatty acid methyl esters were analysed using a gas chromatography system (model 6890; Hewlett Packard) based on the TSBA6 database in the Microbial Identification System (Sherlock version 6.2B) [26]. The DNA G+C content of Pch-40^T was determined by a fluorometric method using SYBR Green I and a real-time PCR thermocycler (Bio-Rad) [27]. The polar lipids were analysed by thin-layer chromatography using cells harvested at the exponential growth phase according to the method described previously [28]. The following reagents were used to detect different polar lipids: 10 % ethanolic molybdatophosphoric acid (for total polar lipids); ninhydrin (for aminolipids); Dittmer–Lester reagent (for phospholipids); and α-naphthol (for glycolipids).

The only detected isoprenoid quinone of strain Pch-40^{T}was menaquinone-7 (MK-7), which was in line with all other members of the genus *Cohnella* [1, 2]. The major fatty acids (>5.0 %) of strain Pch-40^T were iso-$C_{16:0}$ (44.5 %), anteiso-$C_{15:0}$ (30.8 %), $C_{16:0}$ (6.5 %) and anteiso-$C_{17:0}$ (6.0 %) (Table 2). The overall fatty acid profile of strain Pch-40^T was similar to those of members of the genus *Cohnella* [1, 2], but there were some differences in the respective compositions of some fatty acid components, as shown in Table 2. The DNA G+C content of strain Pch-40^T was 55.6 mol%, which was in the range of those of the reference strains (Table 1) [1, 3, 5]. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine (Fig. S3). An unknown phospholipid and five unknown lipids were also detected as minor polar lipids. The polar lipid profile of strain Pch-40^T was similar to those of members of the genus *Cohnella* [1, 3, 5]. In conclusion, the phenotypic and chemotaxonomic features, and the phylogenetic inference support the proposition that strain Pch-40^T represents a novel species of the genus *Cohnella,* for which the name *Cohnella algarum* sp. nov. is proposed.

Table 2. Cellular fatty acid compositions (%) of strain Pch-40^T and the type strains of closely related *Cohnella* species

Taxa: 1, strain Pch-40^T; 2, *C. rhizosphaerae* DSM 28161^T; 3, *C. xylanilytica* KCTC 22294^T; 4, *C. laeviribosi* KCTC 3987^T; 5, *C. thermotolerans* KACC 11643^T; 6, *C. panacarvi* KCTC 13060^T. All data were obtained from this study. The data are expressed as percentages of the total fatty acids. Major fatty acid components (>5.0 %) are highlighted in bold. –, Not detected; TR, trace amount (<1.0 %).

	1	2	3	4	5	6
Saturated fatty acid:						
$C_{9:0}$	TR	TR	TR	TR	TR	1.3
$C_{10:0}$	TR	–	–	TR	TR	1.5
$C_{14:0}$	TR	TR	TR	TR	1.7	2.0
$C_{16:0}$	**6.5**	**5.9**	**7.3**	**9.2**	**17.8**	–
$C_{18:0}$	TR	TR	1.9	–	–	2.4
Branched fatty acid:						
iso-$C_{14:0}$	2.4	2.1	2.2	1.4	2.3	3.4
iso-$C_{15:0}$	3.6	**13.8**	3.0	2.0	3.3	2.3
iso-$C_{16:0}$	**44.5**	**18.7**	**36.2**	**30.4**	–	**23.2**
iso-$C_{17:0}$	1.1	3.8	TR	1.0	1.3	TR
anteiso-$C_{15:0}$	**30.8**	**40.3**	**34.7**	**25.3**	**44.1**	**42.3**
anteiso-$C_{17:0}$	**6.0**	**5.9**	**7.1**	**7.3**	**11.4**	**8.2**
Unsaturated fatty acid:						
$C_{14:1}\omega5c$	TR	–	–	TR	TR	2.4
$C_{17:1}\omega6c$	–	–	–	**6.3**	**5.7**	–
$C_{18:1}\omega9c$	TR	TR	TR	–	–	1.7
Hydroxy fatty acid:						
$C_{8:0}$ 3-OH	TR	TR	TR	TR	TR	2.5
$C_{13:0}$ 2-OH	TR	TR	TR	TR	TR	1.0
iso-$C_{17:0}$ 3-OH	TR	TR	–	TR	TR	1.1
Summed feature*:						
8	TR	TR	TR	**12.5**	**7.1**	–
9	TR	–	–	TR	TR	1.3

*Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 8, $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$; summed feature 9, iso-$C_{17:1}\omega9c$ and/or 10-methyl $C_{16:0}$.

DESCRIPTION OF *COHNELLA ALGARUM* SP. NOV.

Cohnella algarum (al.ga′rum. L. gen. pl. n. *algarum* of/from algae).

Cells are Gram-stain-positive, facultatively aerobic, oxidase- and catalase-positive, motile rods with a monotrichous polar flagellum. Colonies on R2A agar are creamy white, circular, convex, semi-translucent, smooth and slimy. Subterminal ellipsoidal endospores are observed in swollen sporangia. Growth occurs at 20–50 °C (optimum, 37–40 °C), at pH 5.0–11.0 (optimum, pH 7.0) and in the presence of 0–4.0 % (w/v) NaCl (optimum, 0 %). Starch and aesculin are hydrolysed, but casein, tyrosine, Tween 20 and Tween 80 are not. Does not reduce nitrate. Alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), acid phospatase, naphtol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, leucine arylamidase, valine arylamidase, α-galactosidase, β-glucuronidase and β-glucosidase activities are positive, but cystine arylamidase, trypsin, α-chymotrypsin, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are negative. Assimilation of aesculin ferric citrate, 4-nitrophenyl-β-D-galactopyranoside, D-glucose, L-arabinose, D-mannose, maltose, D-glucose and urea is positive, but assimilation of L-tryptophane, L-arginine, *N*-acetyl-glucosamine, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, gelatin, D-mannitol and potassium gluconate is negative. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, an unknown phospholipid and five unknown lipids. The major fatty acids are iso-$C_{16:0}$, anteiso-$C_{15:0}$, $C_{16:0}$ and anteiso-$C_{17:0}$. MK-7 is detected as the sole isoprenoid quinone.

The type strain is Pch-40^T (=KACC 19279^T=JCM 32033^T), isolated from a green alga *Paulinella chromatophora* in the Republic of Korea. The DNA G+C content of the type strain is 55.6 mol%.

Funding information
This work was supported by the Program for Collection of Domestic Biological Resources from the National Institute of Biological Resources (NIBR No. 2017-02-001) of Ministry of Environment (MOE) and the Strategic Initiative for Microbiomes in Agriculture and Food, Ministry of Agriculture, Food and Rural Affairs (as part of the multi-ministerial)

Genome Technology to Business Translation Program, Republic of Korea.

Acknowledgements
The authors would like to thank the nomenclature reviewer for the support regarding the nomenclature of the micro-organism.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Kämpfer P, Rosselló-Mora R, Falsen E, Busse HJ, Tindall BJ. *Cohnella thermotolerans* gen. nov., sp. nov., and classification of *'Paenibacillus hongkongensis'* as *Cohnella hongkongensis* sp. nov. *Int J Syst Evol Microbiol* 2006;56:781–786.
2. Khianngam S, Tanasupawat S, Akaracharanya A, Kim KK, Lee KC *et al.* *Cohnella thailandensis* sp. nov., a xylanolytic bacterium from Thai soil. *Int J Syst Evol Microbiol* 2010;60:2284–2287.
3. Cho EA, Lee JS, Lee KC, Jung HC, Pan JG *et al.* *Cohnella laeviribosi* sp. nov., isolated from a volcanic pond. *Int J Syst Evol Microbiol* 2007;57:2902–2907.
4. García-Fraile P, Velázquez E, Mateos PF, Martínez-Molina E, Rivas R. *Cohnella phaseoli* sp. nov., isolated from root nodules of *Phaseolus coccineus* in Spain, and emended description of the genus *Cohnella*. *Int J Syst Evol Microbiol* 2008;58:1855–1859.
5. Yoon MH, Ten LN, Im WT. *Cohnella panacarvi* sp. nov., a xylanolytic bacterium isolated from ginseng cultivating soil. *J Microbiol Biotechnol* 2007;17:913–918.
6. Choi JH, Seok JH, Jang HJ, Cha JH, Cha CJ. *Cohnella saccharovorans* sp. nov., isolated from ginseng soil. *Int J Syst Evol Microbiol* 2016;66:1713–1717.
7. Lee KC, Kim KK, Kim JS, Kim DS, Ko SH *et al.* *Cohnella collisoli* sp. nov., isolated from lava forest soil. *Int J Syst Evol Microbiol* 2015; 65:3125–3130.
8. Huang Z, Yu YJ, Bao YY, Xia L, Sheng XF *et al.* *Cohnella nanjingensis* sp. nov., an extracellular polysaccharide-producing bacterium isolated from soil. *Int J Syst Evol Microbiol* 2014;64:3320–3324.
9. Shiratori H, Tagami Y, Beppu T, Ueda K. *Cohnella fontinalis* sp. nov., a xylanolytic bacterium isolated from fresh water. *Int J Syst Evol Microbiol* 2010;60:1344–1348.
10. Khianngam S, Tanasupawat S, Akaracharanya A, Kim KK, Lee KC *et al.* *Cohnella cellulosilytica* sp. nov., isolated from buffalo faeces. *Int J Syst Evol Microbiol* 2012;62:1921–1925.
11. Hameed A, Hung MH, Lin SY, Hsu YH, Liu YC *et al.* *Cohnella formosensis* sp. nov., a xylanolytic bacterium isolated from the rhizosphere of *Medicago sativa* L. *Int J Syst Evol Microbiol* 2013;63: 2806–2812.
12. Kämpfer P, Glaeser SP, Mcinroy JA, Busse HJ. *Cohnella rhizosphaerae* sp. nov., isolated from the rhizosphere environment of *Zea mays*. *Int J Syst Evol Microbiol* 2014;64:1811–1816.
13. Kämpfer P, Glaeser SP, Busse HJ. *Cohnella lubricantis* sp. nov., isolated from a coolant lubricant solution. *Int J Syst Evol Microbiol* 2017;67:466–4471.
14. Kazamia E, Czesnick H, Nguyen TT, Croft MT, Sherwood E *et al.* Mutualistic interactions between vitamin B_{12}-dependent algae and heterotrophic bacteria exhibit regulation. *Environ Microbiol* 2012; 14:1466–1476.
15. Kouzuma A, Watanabe K. Exploring the potential of algae/bacteria interactions. *Curr Opin Biotechnol* 2015;33:125–129.
16. Jeong SE, Jeon SH, Chun BH, Kim DW, Jeon CO. *Marinicauda algicola* sp. nov., isolated from a marine red alga *Rhodosorus marinus*. *Int J Syst Evol Microbiol* 2017;67:3423–3427.
17. Jeong SE, Kim KH, Baek K, Jeon CO. *Parasphingopyxis algicola* sp. nov., isolated from a marine red alga *Asparagopsis taxiformis* and emended description of the genus *Parasphingopyxis* Uchida *et al.* 2012. *Int J Syst Evol Microbiol* 2017;67. doi:10.1099/ijsem.0.002215.
18. Lee HJ, Jeong SE, Cho MS, Kim S, Lee SS *et al.* *Flavihumibacter solisilvae* sp. nov., isolated from forest soil. *Int J Syst Evol Microbiol* 2014;64:2897–2901.
19. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y *et al.* Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017; 67:1613–1617.
20. Nawrocki EP, Eddy SR. Query-dependent banding (QDB) for faster RNA similarity searches. *PLoS Comput Biol* 2007;3:e56.
21. Felsenstein J. *Phylip (Phylogeny Inference Package), Version 3.6a*. Seattle: Department of genetics, University of Washington, Seattle, WA, USA; 2002.
22. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30: 1312–1313.
23. Smibert RM, Krieg NR. Phenotypic characterization. In: Gerhardt P (editor). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology; 1994. pp. 607–654.
24. Lányí B. Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* 1987;19:1–67.
25. Komagata K, Suzuki K. Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* 1987;19:161–208.
26. Sasser M. *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc; 1990.
27. Gonzalez JM, Saiz-Jimenez C. A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* 2002;4:770–773.
28. Minnikin DE, Patel PV, Alshamaony L, Goodfellow M. Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* 1977;27:104–117.