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Dasania marina gen. nov., sp. nov., of the Order *Pseudomonadales*, Isolated from Arctic Marine Sediment

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An obligately aerobic bacterium, strain KOPRI 20902^T, was isolated from a marine sediment in Ny-Ålesund, Spitsbergen Islands, Norway. Cells were irregular rods and motile with polar monotrichous flagellum. The optimum growth temperature was 17-22°C. Cells grew best in pH 7.0-10.0 and 3-4% sea salts (corresponding to 2.3-3.1% NaCl). The novel strain required Ca^{2+} or Mg^{2+} in addition to NaCl for growth. Sequence analysis of 16S rRNA gene revealed that the Arctic isolate is distantly related with established species (<92.4% sequence similarity) and formed a monophyletic group with *Cellvibrio*, which formed a distinct phylogenetic lineage in the order *Pseudomonadales*. Predominant cellular fatty acids [$C_{16:1}$ ω7c/15:0 iso 2OH (45.3%), $C_{16:0}$ (18.4%), ECL 11.799 (11.2%), $C_{10:0}$ 3OH (10.4%)]; DNA G+C content (37.0 mol%); nitrate reduction to nitrogen; absence of aesculin hydrolysis, *N*-acetyl-β-glucosaminidase and esterase; no assimilation of arabinose, galactose, glucose, lactose, maltose, and trehalose differentiated the strain from the genus *Cellvibrio*. Based on the phylogenetic and phenotypic characteristics, *Dasania marina* gen. nov., sp. nov. is proposed in the order *Pseudomonadales*. Strain KOPRI 20902^T (=KCTC 12566^T=JCM 13441^T) is the type strain of *Dasania marina*.

Keywords: Arctic, *Cellvibrio*, *Dasania marina*, marine bacterium, 16S rRNA gene

The order *Pseudomonadales* was circumscribed on the basis of phylogenetic analysis of 16S rRNA gene sequences and contains two families, *Pseudomonadaceae* and *Moraxellaceae*. Bacteria included in *Pseudomonadales* are usually aerobic chemoorganotrophs with respiratory metabolism and motile by means of flagella (Garrity *et al.*, 2005). They have been isolated from various environments including water, soil, mucosa of animals and men, and have diverse metabolic activities. The family *Pseudomanadaceae* contains the genera *Pseudomonas, Azomonas, Azotobacter, Cellvibrio, Mesophilobacter, Rhizobacter, Rugamonas,* and *Serpens*. The family *Moraxellaceae* includes *Moraxella*, *Acinetobacter*, *Alkanindiges*, *Enhydrobacter*, and *Psychrobacter* (Bogan *et al.*, 2003; Juni and Bövre, 2005).

In this study, we report a strain from Arctic marine sediment to represent a novel genus, *Dasania*, in the order *Pseudomonadales*. Taxonomic position in *Pseudomonadales* was not specified.

Materials and Methods

Strain isolation

A marine sediment sample was collected from the coastal area in Ny-Ålesund, Spitsbergen Islands, Norway in July 2003. One hundred microliter of the sediment sample diluted 1:1000 (w/v) in sea water was spread on marine agar 2216 (Difco) and incubated aerobically at 10°C. Strain KOPRI 20902^T was further purified by sub-culturing on the same condition, routinely maintained on the same media at 20°C after determining optimal growth temperature, and preserved as a glycerol suspension [10% (v/v)] at -80°C.

16S rRNA gene sequencing and phylogenetic analysis

The 16S rRNA gene was amplified from a single colony with universal primers, 27F; AGA GTT TGA TCM TGG CTC AG and 1492R; GGT TAC CTT GTT ACG ACT T, as described by Lane (1991) and Lee *et al.* (2006). PCR products were purified using AccuPrep PCR Purification Kit (Bioneer, Korea) and directly sequenced using primers described by Lane (1991), 27F, 337F; GAC TCC TAC GGG AGG CWG CAG, 518R; GTA TTA CCG CGG CTG CTG G, 785F; GGA TTA GAT ACC CCG GTA, and 1225F; TCA CGC ATG MTA CAA TGG. Initially, an almost full-length sequence of the 16S rRNA gene was compared with the sequences of all type strains available in EzTaxon database (Chun *et al.*, 2007) and sequences available in GenBank database, to determine phylogenetic affiliation. For the phylogenetic analyses, sequences were aligned using CLUSTAL X (Thomson *et al.*, 1997) with those of representative species of each family and order in *Gammaproteobacteria*. Ambiguously, aligned sites were excluded from phylogenetic analyses. Phylogenetic trees were constructed by the neighbor-joining (NJ; Saitou and Nei, 1987), maximum parsimony (MP; Fitch, 1971), and maximum-likelihood (ML; Felsenstein, 1981) methods using PAUP program (Swofford, 2002). Evolutionary distance matrix for the neighbor-joining method was generated according to the Kimura's two-parameter model (Kimura, 1980). Maximum parsimony and maximum like-

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lihood trees were constructed by heuristic search option. The confidence level of the tree topology was evaluated by bootstrap analysis (Felsenstein, 1985) using 1,000 replications.

Phenotypic characterization

Temperature range for growth was determined in a temperature-gradient incubator (TVS126MA, Advantec) using marine broth in the range of 4-50°C. Growth at different pH (between pH 5.0 and 10.0 at intervals of 1.0 pH unit) was determined using marine broth at 20°C. Growth at different salt concentrations [between 0 and 10% (w/v) sea salt (Sigma, S9883) at intervals of 1%] was determined using D.W. substituted ZoBell basal medium (Bacto peptone 0.5%, yeast extract 0.1%, ferric citrate 0.01% in distilled water) at 20°C. Requirement for salts was determined by growing for 20 days in modified marine broth (0.5% peptone, 0.1% yeast extract, 0.001% $FePO_4$, and 3% NaCl) supplemented with 0.18% $CaCl_2$, 0.055% KCl, or 0.59% $MgCl_2$. Growth in anaerobic condition was examined in the anaerobic chamber with air of 80% nitrogen, 10% carbon dioxide, and 10% hydrogen at 20°C for up to 5 days using thioglycollate medium (Sigma). Enzyme activities, assimilation and fermentation of sole carbon sources, nitrate reduction, indole production were determined using API 20E, API 20NE, API 50CHB (bioMérieux), and Biolog GN2. Morphological examinations of strain KOPRI 20902^T were performed by DIC microscopy (model ECLIPSE TE2000-U; Nikon) and transmission electron microscopy (model CM-20; Philips) on cells grown on marine agar at 20°C for 3 days. Cellular motility was tested in motility medium (0.3% beef extract, 1% peptone, 0.5% NaCl, 0.4% agar).

Chemotaxonomic characterization

Chemotaxonomic characteristics were determined in cells grown at 20°C on marine agar. Isoprenoid quinones were extracted from 7-day-old cells according to the method of Minnikin *et al.* (1984) and analyzed using HPLC (Collins, 1985). Fatty acid methyl ester analysis was performed by gas chromatography (model 6890A, Hewlet-Packard) according to the Microbial Identification (MIDI) System using 3-day-old cells. The G+C content was determined by thermal melting method (Marmur and Doty, 1962; Mandel *et al.*, 1970; Johnson, 1985) using UV/Vis spectrophotometer with temperature control unit (Pharmacia, Cambridge).

Results and Discussion

Phylogeny

An almost complete 16S rRNA gene sequence of strain KOPRI 20902^T was obtained (1,486 bp) and deposited in GenBank database under the accession number AY771747. Preliminary sequence comparison with 16S rRNA gene se-

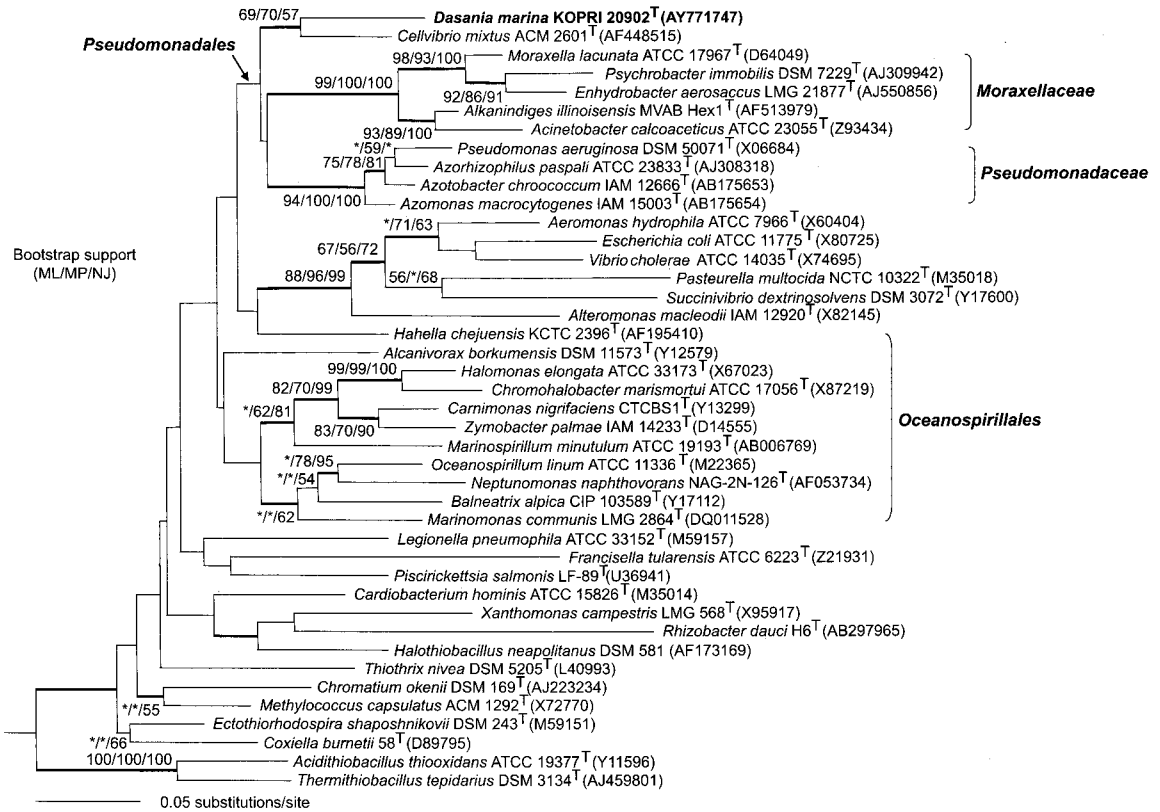

Fig. 1. Phylogenetic position of KOPRI 20902^T in the *Gammaproteobacteria* based on 16S rRNA gene sequences. The tree was reconstructed by the heuristic search with the maximum likelihood criterion. Branches that were conserved in maximum likelihood, maximum parsimony, and neighbor-joining analyses were presented by thick lines. Percent bootstrap supports (>50%) were given at each node (ML/MP/NJ). *Bacillus subtillis* ATCC 43116^T was used as an outgroup.

quences from EzTaxon database (Chun *et al.*, 2007) revealed that the isolate showed highest sequence similarity to *Cellvibrio japonicus* NCIMB 10462^T (92.4%), followed by *Saccharophagus degradans* DSM 17024^T (91.7%), *Marinimicrobium agarilyticum* DSM 16975^T (91.4%), and *Cellvibrio ostraviensis* LMG 19434^T (91.2%). Among sequence entries in GenBank database, marine gammaproteobacterium HTCC 2143 (Cho and Giovannoni, 2004) had the highest sequence similarity (95%). In the preliminary phylogenetic analysis with representative species of each family in the *Gammaproteobacteria*, it was revealed that strain KOPRI 20902^T was closely related to *Pseudomanadales* and *Oceanospirillales* (data not shown). To examine the phylogenetic position of strain KOPRI 20902^T, neighbor-joining, maximum parsimony, and maximum likelihood analyses were conducted including sequences of representative species of each family of *Gammaproteobacteria* and representative species of each genus in *Pseudomonadales* and *Oceanospirillales* (Fig. 1). In all of the phylogenetic trees by ML, MP, and NJ methods, it was revealed that strain KOPRI 20902^T was related to *Cellvibrio mixtus*. The bootstrap supports were 69, 70, and 57% by ML, MP, and NJ methods, respectively. Independent phylogenetic lineage of the *Cellvibio*-KOPRI 20902^T group in *Pseudomonadales* were maintained in ML, but not in MP or NJ analyses. The *Cellvibio*-KOPRI 20902^T group formed a monophyletic group with *Moraxellaceae* in the MP tree and with *Pseudomonadaceae* in the NJ tree. The sequence similarity between members of *Cellvibrio*-KOPRI 20902^T group and members of *Moraxellaceae* group was 86.2% in average (84.7-87.3%). The sequence similarity between members of *Cellvibrio*-KOPRI 20902^T group and members of *Pseudomonadaceae* group was 88.7% in average (87.5-90.0%). The very low sequence similarities of strain KOPRI 20902^T with described species (<92.4%) and distant relationship between the *Cellvibrio*-KOPRI 20902^T group and the other families in the order *Pseudomonadales* suggested that the strain represented a novel genus and species in the order *Pseudomonadales*.

Phenotypic characteristics of the Arctic isolate

Strain KOPRI 20902^T was irregular rods (0.3-0.4×0.7-2.2 um), motile with polar monotrichous flagellum approximately 4-5 μm in length (Fig. 2). Colonies of the strain grown on marine agar at 10°C for 2 weeks were 1-2 mm in diameter, yellowish, translucent, glistening with an entire margin. No growth was detected under anoxic conditions. The temperature range for growth was 4-30°C, with optimum temperature 17-22°C. The strain grew in pH 7.0-10.0, with optimum pH 7.0-8.0 and in 1-9% sea salt concentration, optimally in 3-4% sea salt. Other physiological characteristics were given in the species description. Differential characteristics of strain KOPRI 20902^T and *Cellvibrio* were presented in Table 1.

Chemotaxonomic characteristics

The fatty acid profile (each comprising >1%) was summarized in Table 2. Relatively high proportion of $C_{16:1}$ ω7c/$C_{15:0}$ iso 2OH and unknown fatty acid of ECL 11.799 differentiated the strain from *Cellvibrio* species. The major respiratory quinone was ubiquinone-8 (Q-8). The DNA G+C content of strain KOPRI 20902^T was 37.0%, as determined by thermal melting method.

Taxonomic conclusions

Strain KOPRI 20902^T had low sequence similarities (<92.4%) with members of the genus *Cellvibrio* and were differentiated by phenotypic and chemotaxonomic characteristics, including fatty acid composition, G+C content, nitrate reduction to nitrogen, absence of aesculin hydrolysis, *N*-acetyl-β-glucosaminidase, esterase, and utilization of several sole carbon sources as presented in Table 1. From these results, a novel genus *Dasania* is proposed for strain KOPRI 20902^T. Strain KOPRI 20902^T and *Cellvibrio* formed a monophyletic group by three tree reconstruction methods with moderate bootstrap supports. The group formed a distinct lineage from families *Maraxellaceae* and *Pseudomonadaceae* in the order

Table 1. Differential characteristics of strain KOPRI 21702^T and *Cellvibrio*

Characteristics	KOPRI 21702^T	*Cellvibrio* (n=7)
G+C%	37.0	47.4-53.3
Aesculin hydrolysis	−	+
Nitrate reduction to nitrogen	+	−
N-acetyl-β-glucosaminidase	−	+
Esterase	−	+
Utilization of		
Arabinose	−	+
Galactose	−	+
Glucose	−	+
Lactose	−	+
Maltose	−	+
Trehalose	−	+

Strain KOPRI 21702^T and *Cellvibrio* species were positive for catalase, oxidase, alkaline phosphatase, leucine arylamidase, motility, but negative for indole production, gelatin hydrolysis, arginine dihydrolase, urease, α-chymotrypsin, α-fucosidase, β-galactosidase, β-glucuronidase, lipase, α-mannosidase, trypsin, utilization of acetate, adipate, caprate, citrate, cluconate, inositol, lactate, malate, mannitol, phenyl-acetate, succinate. Data for *Cellvibrio* species (*C. fibrivorans, C. fulvus, C. gandavensis, C. japonicus, C. mixtus, C. ostraviensis*, and *C. vulgaris*) came from Humphry *et al.* (2003) and Mergaert *et al.* (2003).

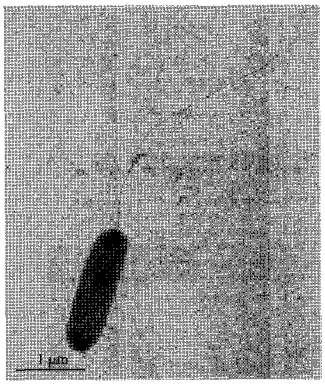

Fig. 2. Transmission electron micrograph of strain KOPRI 20902^T showing polar flagella.

Table 2. Fatty acid compositions of KOPRI 20902^T and *Cellvibrio* species

Fatty acid	1	2	3	4	5	6	7
10:0	1.4	4.1-8.2	4.8-7.1	4.2	4.3-6.9	2.6-5.8	1.6-3.3
10:0 3-OH	10.4	5.5-13.4	7.7-10.7	6.5	6.1-10.9	13.0	3.0-6.5
ECL 11.799	11.2	–	–	–	–	–	–
12:0	–	4.0-7.2	5.4-7.7	4.4	4.4-5.8	tr	5.0-8.6
11:0 3-OH	–	tr	0.0-1.1	–	–	tr	–
12:0 2-OH	–	2.8-6.0	3.3-5.4	3.6	2.6-3.9	7.8-11.0	–
12:1 3-OH	–	–	–	1.3	–	–	3.7-7.4
12:0 3-OH	–	tr	1.0-1.5	–	–	tr	4.0-7.4
14:0	5.9	1.2-2.4	1.6-2.5	tr	0.9-1.2	1.3-1.7	–
15:0	–	tr	0.0-1.3	2.2	tr	0.9-1.9	0.0-1.8
16:0	18.4	18.5-27.8	15.7-20.0	19.7	17.6-20.9	14.3-18.0	17.4-25.5
17:1ω8c	–	–	tr	tr	tr	tr	0.0-1.1
17:0	–	0.0-1.9	1.6-4.5	5.7	1.3-2.0	2.2-3.7	0.0-1.7
18:1ω7c	4.1	7.3-15.3	11.4-19.0	16.8	12.9-19.8	7.5-11.5	15.5
18:1ω6c	–	0.0-2.8	–	–	0.0-4.3	0.0-3.3	–
18:0	1.8	0.0-1.6	0.0-2.8	3.1	tr	tr	0.0-1.4
ECL 18.814	–	–	tr	–	–	–	0.0-2.9
Summed feature 3	45.3	34.4-38.1	25.6-34.0	30.9	34.1-38.2	30.4-34.9	34.6-47.6
Summed feature 5	–	–	–	–	–	–	0.0-1.1

Values are percentages of total fatty acids. Strains designations are as follows: 1, *Dasania marina* sp. nov. KOPRI 20902^T; 2, *Cellvibrio ostraviensis*, eight strains; 3, *Cellvibrio fibrivorans*, six strains; 4, *Cellvibrio fulvus* LMG 2847^T; 5, *Cellvibrio fulgaris*, four strains; 6, *Cellvibrio mixtus*, two strains; 7, *Cellvibrio gandavensis*, 12 strains. –, Not detected; tr, trace (≤1.0% of total). Unknown fatty acids are designated by their equivalent chain-length (ECL), relative to the chain lengths of known saturated fatty acids. Summed feature 3 comprises $C_{15:0}$ iso 2-OH, $C_{16:1}$ω7c or both. Summed feature 5 comprises $C_{18:2}$ω6,9c, $C_{18:0}$ anteiso or both. Data for *Cellvibrio* species were obtained from Mergaert *et al.* (2003)

Pseudomonadales and had low sequence similarity values (<90.0%). However, taxonomic position of the group was not specified in this study because the placement of the group in the order *Pseudomonadales* was not conserved in three tree reconstruction methods or supported by high bootstrap values.

Description of Dasania gen. nov.

Dasania (Da.sa'ni.a. N.L. fem. n. honoring Dasan, a Korean scientist in 18th and 19th century and after the name of Korean Arctic research station, Dasan Station, in Ny-Ålesund, Spitsbergen Islands, Norway where the strain was isolated) Cells are aerobic, rods that are motile with polar monotrichous flagellum. Positive for catalase, oxidase, acetoin production, nitrate reduction to nitrite and nitrogen, cytochrome oxidase. Negative for indole production, gelatin hydrolysis, aesculin hydrolysis, N-acetyl-β-glucosaminidase, esterase, and utilization of arabinose, galactose, glucose, lactose, maltose and trehalose. The major fatty acids are $C_{16:1}$ ω7c/15:0 iso 2OH, $C_{16:0}$, ECL 11.799, $C_{10:0}$ 3OH, $C_{14:0}$. The only respiratory quinone detected is Q-8. The G+C content of the type species is 37.0 mol%. Phylogenetically, the genus forms a monophyletic group with *Cellvibrio,* which forms a distinct phylogenetic lineage from *Moraxellaceae* and *Pseudomonadaceae* in the order *Pseudomonadales*. The type species of the genus is *Dasania marina*.

Description of Dasania marina sp. nov.

Dasania marina (ma.ri'.na. L. fem. adj. marina, of the sea, marine referring the environment where the type strain was isolated)

In addition to properties given in the genus description, the species is characterized as follows. Cells are irregular rods (0.3-4×0.7-2.2 μm). Colonies are circular, convex with entire edges and yellow colored. Diffusible pigments are not produced. Growth occurs at pH 7.0-10.0 (optimum pH 7.0-8.0) and with 1-9% sea salts (optimum 3-4%). Grows at 4-30°C (optimum 17-22°C). Acid is not produced from *N*-acetyl-glucosamine, adonitol, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, arbutin, cellobiose, dulcitol, erythritol, esculin, fructose, D-fucose, L-fucose, galactose, gentiobiose, gluconate, glycerol, glycogen, inositol, inulin, 2-keto-gluconate, 5-keto-gluconate, lactose, lucose, D-lyxose, maltose, mannitol, mannose, melibiose, melezitose, α-methyl-D-glucoside, α-methyl-D-mannoside, β-methyl-D-xyloside, raffinose, rhamnose, ribose, salicin, sorbitol, sorbose, starch, sucrose, D-tagatose, trehalose, D-turanose, xylitol, D-xylose, and L-xylose (API 50CHB). Cytochrome oxidase is present, but citrate is not utilized. Arginine dihydrolase, β-galactosidase, gelatinase, H_2S production, indole production, lysine decarboxylase, ornithine decarboxylase, typtophane deaminase, and urease are absent. Glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amgdalin, and arabinose are not oxidized (API 20E). Esculin and gelatin are not hydrolyzed. Glucose, arabinose, mannose, mannitol, *N*-acetyl-

glucosamine, maltose, gluconate, caprate, adipate, malate, citrate, phenyl-acetate are not assimilated (API 20NE). Predominant cellular fatty acids are 16:1 ω7c/15:0 iso 2OH (45.3%), 16:0 (18.4%), ECL 11.799 (11.2%), 10:0 3OH (10.4%), 14:0 (5.9%), 18:1 ω7c (4.1%), 18:0 (1.8%), 10:0 (1.4%).

The type strain KOPRI 20902^T (=KCTC 12566^T =JCM 13441^T) was isolated from a marine sediment sample from Ny-Ålesund, Spitsbergen Islands, Norway.

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