Roseomonas wooponensis sp. nov., isolated from wetland freshwater

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A non-motive, cocobacilli-shaped and pink-pigmented bacterium, designated strain WW53T, was isolated from wetland freshwater (Woopo wetland, Republic of Korea). Cells were Gram-stain-negative, catalase- and oxidase-positive. The major fatty acids were C18:1ω7c, C18:1ω6c and C18:ω6. The predominant quinone and polyamine were ubiquinone 10 (Q-10) and spermidine, respectively. The DNA G+C content was 71 mol%. The major polar lipids were phosphatidylethanolamine, phosphatidylcholine and an unknown aminolipid. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain WW53T belongs to the family Acetobacteraceae, and is related to the genus Roseomonas. Strain WW53T was most closely related to Roseomonas stagni HS-69T (95.3% 16S rRNA gene sequence similarity). Results of a polyphasic taxonomy study suggested that the isolate represents a novel species in the genus Roseomonas, for which the name Roseomonas wooponensis sp. nov. is proposed. The type strain is WW53T (= KCTC 32534T = JCM 19527T).

The genus Roseomonas, a member of the family Acetobacteraceae in the class Alphaproteobacteria, was first proposed by Rihs et al. (1993). Since the description of three novel species in 1993, the number of novel species with validly published names has been increasing. Strains representing the genus Roseomonas have been isolated from clinical specimens and environmental samples such as freshwater, a water-cooling system, drinking water, air and soil samples. Cells of the members in the genus Roseomonas are coccoid or coccobacilli. Colonies of most strains in the genus are pink-pigmented, but some are white- or orange-coloured. Most strains have catalase and oxidase activity. At the time of writing, the genus comprises 19 species and two subspecies with validly published names: Roseomonas gilardii (the type species, with the subspecies R. gilardii subsp. gilardii and R. gilardii subsp. rosea) (Rihs et al., 1993, 1998; Han et al., 2003), R. aerilata (Yoo et al., 2008), R. aerophila (Kim et al., 2013), R. aestuarii (Venkata Ramana and et al., 2010), R. alkaliiterrae (Dong et al., 2014), R. aquatica (Gallego et al., 2006), R. cervicalis (Rihs et al., 1993), R. frigidaqueae (Kim et al., 2009), R. lacus (Jiang et al., 2006), R. ludipueritiae (Sánchez-Porro et al., 2009), R. mucosa (Han et al., 2003), R. pecuniae (Lopes et al., 2011), R. rhizospherae (Chen et al., 2014), R. riguiloci (Baik et al., 2012), R. rosea (Sánchez-Porro et al., 2009), R. soli (Kim & Ka, 2014), R. stagni (Furuhata et al., 2008), R. terrae (Yoon et al., 2007) and R. vinacea (Zhang et al., 2008). In the course of our study on microbial diversity of a wetland, a pink-pigmented bacterium, designated WW53T, was isolated and subjected to investigation.

Strain WW53T was isolated from a freshwater sample obtained from Woopo wetland in Changyeong, Gyeongnam Province, Republic of Korea, by using the standard dilution plating technique. Isolation was achieved from R2A agar (Becton Dickinson) incubated at 25 °C for 7 days. The isolate was routinely cultured on R2A agar and maintained at −80 °C as a suspension in distilled water containing glycerol (20%, w/v). R. stagni KACC 14009T (purchased from culture collection), R. riguiloci 03SU10-PT and R. frigidaqueae CW67T [isolated in the previous studies by Baik et al. (2012) and Kim et al. (2009), respectively] were used as reference strains.

Bacterial DNA preparation, PCR amplification and 16S rRNA gene sequencing were carried out as described by Chun & Goodfellow (1995). Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarities were achieved by using the EzTaxon-
Preliminary sequence comparison with 16S rRNA gene sequences held in GenBank indicated that our isolate was related to the genus *Roseomonas*. A total of 1336 nt in unambiguously aligned positions were used for tree reconstruction. Strain WW53\(^T\) showed the highest 16S rRNA gene sequence similarity with *R. stagni* HS-69\(^T\) (95.3 %), *R. mucosa* ATCC BAA-692\(^T\) (94.9 %) and *R. gilardii* subsp. *rosea* ATCC BAA-691\(^T\) (94.9 %). The neighbour-joining tree (Fig. 1) showed that strain WW53\(^T\) was closely related to the type strains of the genus *Roseomonas*, occupying a distinct position. The trees based on maximum-likelihood and maximum-parsimony methods showed similar topology.

Growth on various standard bacteriological media was tested by using nutrient agar (NA; Becton Dickinson), plate count agar (PCA; Becton Dickinson), R2A agar (Becton Dickinson), tryptic soy agar (TSA; Becton Dickinson), MacConkey agar (Becton Dickinson), MacConkey agar (Becton Dickinson), MacConkey agar (Becton Dickinson), and 5 % sheep

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain WW53\(^T\).
Evolutionary distances, generated using the Jukes & Cantor model, are based on 1336 unambiguously aligned nucleotides. Bootstrap values greater than 70 % (1000 resamplings) for nodes conserved among neighbour-joining analyses are shown. *Endobacter medicaginis* M1MS02\(^T\) (JQ436923) was used as an outgroup. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-parsimony and maximum-likelihood algorithms. Bar, 0.01 substitutions per nucleotide position.
blood agar (Asan Pharmaceutical) according to the manufacturers’ instructions. Cells of strain WW53T grown on R2A agar at 37 °C for 3 days were used for the physiological and biochemical tests. Cell morphology was observed with phase-contrast (ICC50, Leica), transmission (CM-20, Philips) and scanning electron (S-4800, Hitachi) microscopy. Motility was determined by examining wet mounts with a phase-contrast microscope. Growth temperature (4 °C, 10–50 °C at 5 °C intervals and 37 °C) and NaCl tolerance (0.5 % and 1–5 % at 1 % intervals) were determined as described by Lányi (1987). DNase (0.1 %, w/v), gelatin (1 %, w/v) and urea (2 %, w/v) were tested on R2A agar. The pH range for growth was determined in R2A broth adjusted to pH 4–11 (at 1 pH intervals) by using 100 mM acetate buffer (pH 4–5), 100 mM NaH2PO4/Na2HPO4 buffer (pH 6–8) and 100 mM NaHCO3/Na2CO3 buffer (pH 9–11) (Yumoto et al., 2004). Anaerobic growth was tested on R2A agar in a jar containing the AnaeroPack-Anaero (Mitsubishi Gas Chemical), which works as an oxygen absorber and CO2 generator, for up to 10 days. The Gram reaction was determined by using the KOH method (Powers, 1995). Catalase and oxidase activities were determined using 3 % (v/v) hydrogen peroxide and Kovacs’ reagent (Kovacs, 1956), respectively. Acid production from carbohydrates was tested using phenol red broth base (Becton Dickinson). Nitrate reduction was tested on nitrate broth containing 0.1 % KNO3 (Tindall et al., 2007). H2S production was determined on Kligler iron agar (Becton Dickinson) according to the method of Smibert & Krieg (1994). Hydrolysis of casein (2 %, w/v, skimmed milk), CM-cellulose (0.5 %, w/v), starch (0.2 %, w/v), Tween 20 (1 %, v/v), Tween 80 (1 %, v/v) and xylan (1 %, w/v) was tested using R2A agar according to the method of Smibert & Krieg (1994). Decomposition of hypoxanthine (0.5 %, w/v), L-tyrosine (0.5 %, w/v) and xanthine (0.4 %, w/v) was tested using R2A agar according to the method of Gordon et al. (1974). Hydrolysis of aesculin (0.1 %, w/v), gelatin (1 %, w/v) and urea (2 %, w/v) were determined as described by Lányi (1987). DNase activity was determined with DNase test agar (Becton Dickinson). Other biochemical tests and enzyme activities were performed using the API 20NE and API ZYM kits (bioMérieux). Antibiotic sensitivity was determined with the disc diffusion method using commercial discs (BBL, Becton Dickson) impregnated with the following antibiotics (µg per disc unless otherwise stated): amikacin (30), ampicillin (10), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), nalidixic acid (30), penicillin G (10 IU), streptomycin (10) and vancomycin (30). After 7 days of incubation at 37 °C on R2A, the results were interpreted according to the guidelines set forth by CLSI (2009).

Cells were Gram-stain-negative, non-motile and cocco-bacilli-shaped (0.5–1.0 × 0.6–1.6 µm in size; Fig. S1, available in the online Supplementary Material). On R2A agar, strain WW53T was able to grow with 0–0.5 % (w/v) NaCl, at pH 6–8 (optimally at pH 7) and at 15–40 ºC (optimally at 35–37 ºC). The detailed results of physiological and biochemical analyses are given in Table 1. There were several phenotypic characteristics such as optimum growth temperature, acid production from mannitol and hydrolysis of Tween 20 that separated strain WW53T from phylogenetically related species. For cellular fatty acid analysis, strain WW53T and three reference strains were grown on R2A agar under the following conditions: strain WW53T, for 3 days at 37 ºC; R. stagni KACC 14009T, for 3 days at 30 ºC; R. rigui loci 03SU10-PT and R. frigidaquae CW67T, for 5 days at 30 ºC. Extraction of fatty acid methyl esters (FAMEs) and separation by GC were performed by using the Instant FAME method of the Microbial Identification System (MIDI) version 6.1 and the TSBA6 database. Cells grown in R2A broth for 5 days at 37 ºC were harvested and freeze-dried for the extraction of polar lipids and isoprenoid quinones. Polar lipids were analysed by using standard procedures (Minnikin et al., 1984). Extracted lipids were separated by two-dimensional TLC and identified by spraying with appropriate detection reagents (Minnikin et al., 1977; Embley & Wait, 1994). Isoprenoid quinones were extracted and purified according to the method of Minnikin et al. (1984) and analysed by HPLC as described by Collins (1994). Polyamines were extracted according to the method of Scherer & Knefel (1983). The extracted samples were loaded on TLC plates and separated by using ethylacetate/cyclohexane (2 : 3, v/v) as the running solvent. For DNA G+C content calculations, the DNA sample was prepared in triplicate and examined by the thermal denaturation method of Marmur & Doty (1962).

The fatty acid profile of strain WW53T (>5.0 % of total fatty acids) included summed feature 8 (C18:1ω7c/C18:1ω9c) (32.5 %), C16:0 (18.5 %), summed feature 3 (C14:0ω6c/C16:1ω7c) (9.6 %), summed feature 4 (anteiso-C17:1B/iso-C17:1ω13c) (7.7 %), C14:0 (6.6 %) and C18:0 (6.2 %) (Table 2). This fatty acid profile was similar to those of species of the genus Roseomonas. But, proportions of summed feature 4 and C18:0 in WW53T were higher and lower, respectively, than those of the most closely related species. The predominant polar lipids were phosphatidylcholine, phosphatidylethanolamine and one unknown amino lipid; smaller amounts of diphosphatidylglycerol and phosphatidylglycerol were also detected. (Fig. S2). The major isoprenoid quinone of strain WW53T was ubiquinone-10 (Q-10), and a small amount of ubiquinone-9 (Q-9) was also found (Q-10 : Q-9, 87 : 13). Spermidine was the major polyamine, whereas putrescine was detected in a small amount (Fig. S3). The DNA G+C content of strain WW53T was 71 mol%.

Therefore, on the basis of the data presented, strain WW53T represents a novel species within the genus Roseomonas, for which the name Roseomonas wooponensis sp. nov. is proposed.
**Description of *Roseomonas wooponensis* sp. nov.**

*Roseomonas wooponensis* (woo.po.nen sis. N.L. fem. adj. *wooponensis* of or belonging to Woopo wetland, Republic of Korea, the geographical origin of the type strain of the species).

Cells are coccobacilli (0.5–1.0 × 0.6–1.6 μm in size), occurring singly and in pairs. Gram-stain-negative, non-motile, non-spore-forming, aerobic and pink-pigmented bacterium. Cells grow best on media such as R2A agar and NA; slowly on TSA and PCA; not on MacConkey agar or 5 % sheep blood agar. Colonies on R2A agar are convex, smooth, circular to slightly irregular and 1.0–2.0 mm in diameter after 5 days at 37 °C. Growth occurs with 0–0.5 % (w/v) NaCl, at pH 6–8 (optimally at pH 7) and at 15–40 °C (optimally at 35–37 °C). Catalase and oxidase activities are present. Reduces nitrate. Does not produce indole or H2S. Tween 80 is hydrolysed, but aesculin, casein, CM-cellulose, DNA, gelatin, hypoxanthine, starch, xanthine and xylan, activity of *N*-acyetyl-β-glucosaminidase, α-chymotrypsin, cystine arylamidase, ᾱ-fucosidase, ᾱ-galactosidase, β-galactosidase, β-glucuronidase, leucine arylamidase, lipase (C14), ᾱ-mannosidase, trypsin and valine arylamidase, and acid production from lactose, maltose, ᾱ-mannose, ᾱ-rhamnose and sucrose and assimilation of adipate, arabinose, caprate, citrate, gluconate, glucose, malate, maltose, mannitol, mannosone, *N*-acytylglycerolamine and phenylacetate. All strains were sensitive to ampicillin, chloramphenicol, erythromycin, kanamycin, nalidixic acid, penicillin and streptomycin and resistant to vancomycin.

**Table 1. Phenotypic characteristics that differentiate strain WW53T from phylogenetically related species**

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<td>Habitat</td>
<td>Fresh water</td>
<td>Fresh water</td>
<td>Fresh water</td>
<td>Cooled water</td>
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<td>Motility</td>
<td></td>
<td>+</td>
<td>−</td>
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<td>Optimum growth temperature</td>
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<td>25–30</td>
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<td>Growth on:</td>
<td>MacConkey agar</td>
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<td>Nitrate reduction</td>
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<td>Hydrolysis of:</td>
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<td>Tween 80</td>
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<td>Tyrosine</td>
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<td>Esterase lipase (C8)</td>
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<td>ᾱ-Glucosidase</td>
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<td>Naphthol-AS-Bl-phosphohydrolase</td>
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<td>Acid production from:</td>
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<td>ᾱ-Xylose</td>
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<td>Antibiotic susceptibility (µg)</td>
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<td>Amikacin (30)</td>
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<td>Gentamicin (10)</td>
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<td>R</td>
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<td>DNA G+C content (mol%)</td>
<td>71</td>
<td>72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>a</sup>Data from other studies as indicated: a, Furuhata et al. (2008); b, Baik et al. (2012); c, Kim et al. (2009).
The type strain, WW53<sup>T</sup> (= KCTC 32534<sup>T</sup> = ICM 19527<sup>T</sup>), was isolated from the freshwater of Woopo wetland in Gyeongnam Province, Republic of Korea. The DNA G+C content of the type strain is 71 mol%.

**Acknowledgements**

This research was supported by the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR) under the Ministry of Environment, Republic of Korea. This work was also supported by Sunchon National University Research Fund in 2015.

**References**


