Armatimonas rosea gen. nov., sp. nov., of a novel bacterial phylum, Armatimonadetes phyl. nov., formally called the candidate phylum OP10

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A novel aerobic, chemoheterotrophic bacterium, strain YO-36T, isolated from the rhizoplane of an aquatic plant (a reed, Phragmites australis) inhabiting a freshwater lake in Japan, was morphologically, physiologically and phylogenetically characterized. Strain YO-36T was Gram-negative and ovoid to rod-shaped, and formed pinkish hard colonies on agar plates. Strain YO-36T grew at 20–40 °C with optimum growth at 30–35 °C, whilst no growth was observed at 15 °C or 45 °C. The pH range for growth was 5.5–8.5 with an optimum at pH 6.5. Strain YO-36T utilized a limited range of substrates, such as sucrose, gentiobiose, pectin, gellan gum and xanthan gum. The strain contained C16 : 0, C16 : 1, C14 : 0 and C15 : 0 as the major cellular fatty acids and menaquinone-12 as the respiratory quinone. The G+C content of the genomic DNA was 62.4 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain YO-36T belonged to the candidate phylum OP10 comprised solely of environmental 16S rRNA gene clone sequences except for two strains, P488 and T47 isolated from geothermal soil in New Zealand; strain YO-36T showed less than 80% sequence similarity to strains P488 and T47. Based on the phylogetic and phenotypic findings, a new genus and species, Armatimonas rosea gen. nov., sp. nov., is proposed for the isolate (type strain YO-36T =NBRC 105658T =DSM 23562T). In addition, a new bacterial phylum named Armatimonadetes phyl. nov. is proposed for the candidate phylum OP10 represented by A. rosea gen. nov., sp. nov. and Armatimonadaceae fam. nov., Armatimonadales ord. nov., and Armatimonadia classis nov.

Culture-independent rRNA-based molecular approaches have revealed that prokaryotic microbial communities in natural ecosystems are extremely diverse, and that most prokaryotic microbes have not yet been cultivated (Achtman & Wagner, 2008; Kamagata & Tamaki, 2005; Sekiguchi, 2006). At the time of writing, approximately 100 phylogenetic lineages equivalent to bacterial phyla have been reported (Achtman & Wagner, 2008; Alain & Querellou, 2009) and over 70 lineages are still so-called candidate phyla comprised exclusively of 16S rRNA gene clone sequences derived from various natural environments. According to The Taxonomic Outline of Bacteria and Archaea, Release 7.7, March 6, 2007 (Garrity et al., 2007), the total number of bacterial phyla with validly published names was 25. The list contains two bacterial phyla, Gemmatimonadetes (Zhang et al., 2003) and Lentisphaerae (Cho et al., 2004), formally known as the candidate phyla BD and VadinBE97, respectively. More recently, the new bacterial phylum Caldiserica (formally the candidate phylum OP5) was also proposed (Mori et al., 2009), hence a total of 26 phyla have been recognized in the domain Bacteria.

Candidate phylum OP10 was originally described based on isolates found in Obsidian Pool in Yellowstone National Park (Hugenholtz et al., 1998b) and harbours a diverse array of environmental clones retrieved from various environments, such as hot springs (Kanokratana et al., 2004), geothermal soils (Stott et al., 2008), forest and...
alpine soils (Dunbar et al., 2002; Lipson & Schmidt, 2004), freshwater lakes and rivers (Crump & Hobbie, 2005; Urbach et al., 2001; Wu et al., 2007), water discharged from manures (Simpson et al., 2004), chlorobenzene-contaminated sediments (von Wintzingerode et al., 1999) and activated sludges (Bond et al., 1995; Dalevi et al., 2001). Strains P488 and T49, belonging to the candidate phylum OP10, were isolated from geothermal soils (Stott et al., 2001), though their traits have not been described at the time of writing. In our previous study, a new member in the candidate division OP10, designated strain YO-36, was also successfully isolated from rhizoplanes of an aquatic plant, *Phragmites australis* (reed), inhabiting a mesophilic freshwater lake (Tanaka and others, unpublished). In this study, we characterized strain YO-36T in detail and propose the novel genus and species, *Armatimonas rosea* sp. nov., for this strain and the new phylum *Armatimonadetes* phyl. nov. for the candidate phylum OP10.

Strain YO-36T was isolated from the root of reed (*P. australis*) collected from a mesophilic freshwater lake in Yamanashi prefecture in Japan. Cultivation and isolation were performed using a low nutrient medium, DTS plate medium (pH 7.0) containing 0.17 g Bacto tryptone l⁻¹ (Difco), 0.03 g Bacto soyton 1⁻¹ (Difco), 0.025 g glucose 1⁻¹, 0.05 g NaCl 1⁻¹ and 0.025 g K₂HPO₄ 1⁻¹, at 25 °C under dark conditions. The isolate was also able to form colonies on R2A (Difco) plates, generally known as a relatively low-nutrient medium commonly used for cultivation of environmental micro-organisms. Colonies of strain YO-36T were circular, smooth, pink, significantly hard and 1–2 mm in diameter on R2A agar plates after one week of incubation at 30 °C. The morphology of cells was examined by phase-contrast microscopy and transmission electron microscopy (Tamaki et al., 2003). Cells were non-motile and ovoid to rod-shaped, 1.4–1.8 μm in width and 2.4–3.2 μm in length (mean size 1.6 × 2.8 μm) (Fig. 1a).

Neither flagella nor spores were detected in ultrathin sections (Fig. 1b) and negative staining observations with an electron microscope. Invagination of cytoplasmic membranes was not observed. Cells were negatively stained by Gram-staining (Tamaki et al., 2003).

The following physiological properties of strain YO-36T were investigated in R2A liquid cultures and on 1.5 % R2A agar plates. The strain grew at 20–40 °C with an optimum temperature of 30–35 °C; no growth was observed at 15 or 45 °C. The pH range for growth was 5.5–8.5, with an optimum at pH 6.5. The strain did not require NaCl for growth and tolerated up to 0.5 % NaCl. No growth was observed with 1.0 % NaCl.

Strain YO-36T was an aerobic, chemoheterotrophic bacterium. There was no evidence of growth under anaerobic conditions: the isolate showed neither nitrate respiration nor fermentative growth. Catalase and cytochrome oxidase tests were negative. The strain did not grow on common nutrient rich media such as Luria-

![Fig. 1. Photomicrographs of strain YO-36T grown in R2A medium under aerobic conditions at 30 °C. (a) Phase-contrast photomicrograph (bar, 10 μm); (b) transmission electron micrograph of ultrathin section (bar, 1.0 μm).](http://ijs.sgmjournals.org)
Phylogenetic analysis based on 16S rRNA gene sequences demonstrated that strain YO-36\textsuperscript{T} belongs to candidate phylum OP10 together with the other cultured strains, P488 and T49, although strain YO-36\textsuperscript{T} is only distantly related to strains P488 and T49. The morphological, physiological and nutritional characteristics of strains P488 and T49 were investigated by Stott et al. (2008) to some extent. Interestingly, they shared the following features: non-motile Gram-negative cells, pinkish pigment production, oligotrophy, and capability of degrading polysaccharides, i.e. gellan gum and xanthan gum. These traits may be common characteristics typical of the phylum.

While strain YO-36\textsuperscript{T} and strains P488 and T49 possess features in common, there was a notable physiological difference between them; strain YO-36\textsuperscript{T}, isolated from the rhizoplane of an aquatic plant inhabiting a mesophilic freshwater lake, is a mesophilic bacterium, while strains P488 and T49, isolated from geothermal soils, are thermophilic bacteria showing growth at 65 °C. As mentioned above, subgroup 1 of candidate phylum OP10, including strain YO-36\textsuperscript{T}, comprises environmental clones derived from mesophilic ecosystems such as freshwater rivers, lakes, terrestrial soils and water discharged from manures, whilst subgroup 3, containing strains P488 and T49, mainly comprises clones obtained from thermophilic environments such as hot springs. The two phylogenetic lineages, taxonomically regarded as classes (subgroups 1 and 3), could be easily differentiated by the temperatures of their habitats. These findings strongly suggested that a new class should be created within this phylum for mesophilic strain YO-36\textsuperscript{T}. Likewise, another class could possibly be created for the thermophilic isolates P488 and T49, once more detailed biochemical and physiological features such as the respiratory quinone, cellular fatty acids and carbon source utilization are characterized. In addition to their growth temperature, the following phenotypic differences were also noted: 1) the pH range for the growth of strain YO-36\textsuperscript{T} was pH 5.5–8.5, whereas that for strain P488 was pH 3.8–5.5; 2) strain YO-36\textsuperscript{T} was able to utilize pectin but not CM-cellulose, xylan or alginate, while strain P488 did not utilize pectin but could utilize CM-cellulose, xylan and alginate; 3) strain YO-36\textsuperscript{T} grew on R2A but strain P488 did not; 4) alkaline phosphatase and catalase were positive and negative, respectively, in strain YO-36\textsuperscript{T}, but negative and positive, respectively, in strain P488. These characteristics were also important features to differentiate strain YO-36\textsuperscript{T} from the thermophilic strains.

From these polyphasic characterizations, we concluded that a novel genus and species should be created for strain YO-36\textsuperscript{T} with the name *Armatimonas rosea* gen. nov., sp. nov.
**Fig. 2.** Phylogenetic relationships between candidate phylum OP10 and other recognized phyla in the domain *Bacteria*, inferred from the ARB program (Ludwig et al., 2004) based on the slightly modified dataset of 16S rRNA gene sequences described previously (Hugenholtz, 2002; Zhang et al., 2003). The tree was reconstructed using the neighbour-joining method. Shaded wedges indicate phyla with validly published names and unshaded wedges indicate phyla currently represented by environmental sequences. Bar, 0.10 substitutions per nucleotide position. Nodes with bootstrap values ≥90% and ≥80%, estimated using neighbour-joining, maximum-parsimony and maximum-likelihood methods, are shown as filled circles and open circles, respectively. GenBank/EMBL/DDBJ accession numbers are shown in parentheses.
The isolate is the first thoroughly described species within the candidate phylum OP10 and therefore is considered as a representative of this phylum. We also propose a new phylum with the name Armatimonadetes phyI. nov. for the candidate phylum OP10, and the new class, Armatimonadaceae classis nov., for subgroup I within this phylum.

Description of Armatimonas gen. nov.

Armatimonas (Ar.m.a.ti.mo’nas. L. adj. ar matau armoured or armour-clad; L. fem. n. monas a unit; N.L. fem. n. Armatimonas an armour-clad unit, referring to the hard colonies).

Gram-negative. Cells are non-motile, ovoid to rod-shaped. Spores and flagella are not found. Mesophilic. Aerobic. The major respiratory quinone is MK-12. The main fatty acids are C16:0, C16:1, C14:0 and C15:0. The G+C content of genomic DNA of the type species is 62.4 %. The type species is Armatimonas rosea.

Description of Armatimonas rosea sp. nov.

Armatimonas rosea (ro’se.a. L. fem. adj. rosea rose-coloured or rosy, referring to the pinkish colour of the colonies).

Displays the following characteristics in addition to those given in the genus description. Cells are ovoid to rod-shaped (1.4–1.8 μm wide and 2.4–3.2 μm long). Grows between 20 and 40 °C with optimum growth at 30–35 °C. The pH range for growth is 5.5–8.5; optimum growth at pH 6.5. NaCl is not required for growth. Catalase and oxidase are not produced. Aerobic. The following substrates can be utilized as sole carbon source: D-arabinose, raffinose, maltose, sucrose, yeast extract, pectin and gellan gum. The following enzymes are positive: alkaline phosphatase, esterase, esterase-lipase, leucine arylamidase, valine arylamidase, tryptophan, chymotrypsin, acid phosphatase, naphthol-AS-Bl-phosphohydrolase, β-galactosidase, β-glucosidase and gelatinase. Sensitive to the following antibiotics: ampicillin, vancomycin, chloramphenicol, tetracycline and rifampicin; resistant to kanamycin. Colonies are circular, smooth, pink and hard, and 1–2 mm in diameter on R2A agar plates.

The type strain, YO-36 T (=NBRC 105658 T =DSM 23562 T), was isolated from the rhizoplane of an aquatic plant, Phragmites australis (reed), inhabiting a mesophilic freshwater lake in Japan.

Description of Armatimonadaceae fam. nov.

Armatimonadaceae (Ar.m.a.ti.mo.na.da ce’e.ae. N.L. fem. n. Armatimonas type genus of the family; -aceae ending to donate a family; N.L. fem. pl. n. Armatimonadaceae the family of the genus Armatimonas).

The description is the same as for the genus Armatimonas.

Type genus: Armatimonas.

Description of Armatimonadetes ord. nov.

Armatimonadetes (Ar.m.a.ti.mo.na.de’tes. N.L. fem. pl. n. Armatimonadetes type order of the phylum; N.L. fem. pl. n. Armatimonadetes phylum of the order Armatimonadales).

The phylum Armatimonadetes is defined on a phylogenetic basis by comparative 16S rRNA gene sequence analysis of strain YO-36 T (=NBRC 105658 T =DSM 23562 T; GenBank accession no. AB529679) and other cultures (strains P488 and T49; GenBank accession nos AM749768 and AM749780, respectively) and uncultured representatives from various terrestrial and aquatic habitats including thermophilic environments such as hot springs and geothermal soils. Common phenotypic features of the three cultured strains are non-motile Gram-negative cells, pinkish pigment production, oligotrophy and the ability to degrade polysaccharides.

Type order: Armatimonadetes.

Description of Armatimonadiales phyl. nov.

Armatimonadales (Ar.m.a.ti.mo.na.de’les. N.L. fem. n. Armatimonas type genus of the order; -ales ending to donate an order; N.L. fem. pl. n. Armatimonadales the order of the genus Armatimonas).

The description is the same as for the genus Armatimonas.

Type genus: Armatimonas.

References


