**Polaromonas vacuolata** gen. nov., sp. nov., a Psychrophilic, Marine, Gas Vaculate Bacterium from Antarctica

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Several strains of a novel heterotrophic gas vaculate bacterium were isolated from antarctic marine waters. The results of phylogenetic analyses in which 16S ribosomal DNA sequencing was used, coupled with phenotypic tests, indicated that strain 34-P*T* (**T*** = type strain) belongs to a new genus and species of the beta subgroup of the *Proteobacteria*, for which the name *Polaromonas vacuolata* is proposed. Although the other four strains studied probably belong to this new species, DNA-DNA hybridization tests were not conducted. The closest phylogenetic relatives of *P. vacuolata* are the photosynthetic nonsulfur purple bacterium *Rhodobacter capsulatus* and the hydrogen autotroph *Variovorax paradoxus*.

Although gas vaculate heterotrophic bacteria are well-known inhabitants of aquatic ecosystems, until recently none of these organisms had been observed in or isolated from marine habitats. In 1989 several types of gas vaculate bacteria were found in Antarctica growing in association with the sea ice microflora, and in association with the sea ice microflora, and in association with the sea ice microflora. In 1989 several types of gas vaculate bacteria were isolated from the Palmer Peninsula near the zone of clearing that was more than 40 mm wide. The fatty acid compositions of all of the strains and the 16s rDNA nucleotide sequence of 34-PT were determined independently in the laboratory of C. R. Woese (6).

*P. vacuolata* was positive for urease and deaminase activities; susceptible to novobiocin (30 μg), tetracycline (30 μg), and neomycin (30 μg); and resistant to streptomycin (10 μg) and gentamicin (10 μg).

Strain 34-PT grew when the tryptone, yeast extract, beef extract, and vitamins of SWC-m were replaced with vitamin-free Casamino Acids (Difco Laboratories, Detroit, Mich.), indicating that vitamins are not required for growth. Good

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FIG. 1. Phase-contrast photomicrograph showing several cells of strain 34-PT. Note the bright, irregular gas vacuoles in the cells. Bar = 5 μm.

FIG. 2. Electron micrograph of 34-PT showing a cell containing several gas vesicles. The flagella are not attached. Bar = 0.5 μm.

growth occurred in nutrient broth containing only 4.0 g/liter, but there was no growth in nutrient broth containing 8.0 g/liter.

In addition to the carbon sources indicated previously (9), the following carbon sources were utilized by strain 34-PT: fumarate, citrate, succinate, 2-oxoglutarate, d-glucose, oxaloacetate, butyrate, DL-alanine, pyruvate, DL-glutamate, glycerol, DL-proline, propionate, DL-aspartate, DL-asparagine, acetate, and sorbitol.

The following carbon sources were not utilized: maltose, d-fructose, xylose, d-ribose, L-fucose, formate, glycine, DL-serine, malonate, DL-isoleucine, DL-lysine, DL-histidine, DL-methionine, DL-valine, cellobiose, mannose, melibiose, melezitose, rhamnose, sorbose, trehalose, methanol, propanol, benzoate, erythritol, DL-threonine, and DL-tryptophan.

Whole-cell fatty acid analyses were performed on all strains. All strains contained large amounts of 16:1 ω7c (74 to 79%) and smaller amounts of 16:0 (14 to 17%). In addition, a third fatty acid was present in smaller amounts (7 to 9%). This fatty acid was identified as 18:1 ω7c, 18:1 ω9t, or 18:1 ω12t or possibly a combination of more than one of these compounds; its actual identity could not be determined by the procedure and instruments used. Such predominance of a single fatty acid is unusual in bacteria, and this is the highest level of 16:1 ω7c that we are aware of in any bacterial species.

The 16S rDNA sequence of strain 34-PT was compared with the sequences of other bacteria included in the RDP database (Table 1). On the basis of simple sequence homology, the most closely related previously described organisms are *Rhodoferax femzentans*, a nonsulfur purple bacterium (8), and *V. paradoxus*, a chemoorganotroph and facultative lithoautotroph (1, 16). As determined by the same method, however, strain 34-PT is most closely related to the environmental 16S rDNA sequences Str. Stripa and Str. PAD44. The Stripa-derived 16S rDNA sequence was obtained from deep groundwater in the Stripa mine in Sweden (2). The Str. PAD44 or env. PAD44 sequence is a sequence that was obtained from a paddy field (15). However, both of these sequences are environmental sequences without organisms available for comparison, and so it is impossible to determine how similar the actual organisms are to one another in other respects. In addition, these sequences are only partial 16S rDNA sequences, and so the actual levels of relatedness might be different if the complete sequences were available.

A phylogenetic analysis of the sequences revealed that the relationship of the organisms was uncertain. A preliminary
phylogenetic set of the four most parsimonious trees obtained with PAUP, version 3.0s (14), was analyzed by using MacClade, version 3.05 (11), to produce a substitution matrix to correct for the different rates obtained for the 12 different types of nucleotide substitutions (e.g., A → C or G → U, etc.) (data not shown). The overall rate of transitions to transversions was determined to be 1.3. A rescaled consistency index weighting mask was also constructed from these trees by using MacClade, version 3.05 (11).

The substitution matrix was reapplied to the aligned data set in PAUP, version 3.0s (14), and the most parsimonious trees were determined by using the branch and bound option with both the original data set and 100 bootstrap-resampled data sets. The original data set (with the applied substitution matrix) yielded four equally parsimonious trees whose length was 638. These trees differed in the relationships among env. PAD44, Rhodoferax fermentans, V. paradoxus, str. Stripa, and strain 34-P".

<table>
<thead>
<tr>
<th>Sequence</th>
<th>mean distance or no. of base differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>34-P&quot;</td>
<td></td>
</tr>
<tr>
<td>str. Stripa</td>
<td>0.05</td>
</tr>
<tr>
<td>Variorovax paradoxus</td>
<td>0.05</td>
</tr>
<tr>
<td>env. PAD44</td>
<td>0.05</td>
</tr>
<tr>
<td>Rhodoferax fermentans</td>
<td>0.05</td>
</tr>
<tr>
<td>Sphaerotilus natans</td>
<td>0.05</td>
</tr>
<tr>
<td>Brachymonas denitrificans</td>
<td>0.05</td>
</tr>
<tr>
<td>Rubrivivax gelatinosus</td>
<td>0.05</td>
</tr>
<tr>
<td>Comamonas testosteroni</td>
<td>0.05</td>
</tr>
<tr>
<td>Thioacillus perometabolis</td>
<td>0.05</td>
</tr>
<tr>
<td>Bordetella parapertussis</td>
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<tr>
<td>Alcaligenes faecalis</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*(The numbers on the upper right are the mean distances between sequence pairs adjusted for missing data, and the numbers on the lower left are total numbers of base differences for the pairs of sequences.)*

Finally, the aligned data set and 100 bootstrap replicates were analyzed by using fastDNAm1 (3, 12), with the base frequencies determined empirically and the ratio of transition to transversion set at 1.3. Again, the more distantly related taxa showed a branching topology similar to that determined with

FIG. 3. Phylogenetic relatedness of P. vacuoluta 34-P" and the most closely related species. This maximum-parsimony tree was determined by an exact (branch-and-bound) search method, using a substitution matrix to correct for the various rates of nucleotide substitutions. This is one of four equally parsimonious trees. The numbers in parentheses near the branch points indicate how many of the four equally most parsimonious trees shared that branch structure. The numbers not in parentheses near the clades indicate the percentages of bootstrap support for the clades based on 100 bootstrap resamplings. Only bootstrap values of 50% or more are shown.
the parsimony and distance trees. As in the neighbor-joining analysis, strain 34-P was between the Stripa mine clone and a clade containing *Rhodoferax fermentans* and *V. paradoxus*. This is in contrast to the results of the parsimony analysis, in which strain 34-P was identified (with weak support) as a sister taxon of the Stripa mine clone. Also, like the parsimony and distance methods, the maximum-likelihood bootstrap analysis produced no significant support for any particular branching order near strain 34-P and only 59% support for a clade that included only strain 34-P, *V. paradoxus*, *Rhodoferax fermentans*, env. PAD44, and stri. Stripa.

Each of these methods produced slightly different trees. From all of the trees, however, it is clear that strain 34-P is most closely related to *Rhodoferax fermentans*, *V. paradoxus*, stri. Stripa, and env. PAD44. On the other hand, strain 34-P is not photosynthetic and does not grow as a nonsulfur purple bacterium under conditions used for the growth of *Rhodoferax fermentans*. Also, strain 34-P differs by 5 and 7% in 16S rDNA base homology from *V. paradoxus* and *Rhodoferax fermentans*, respectively. Furthermore, other genotypic and phenotypic data indicate that *P. vacuolata*, *V. paradoxus*, and *Rhodoferax fermentans* differ markedly (Table 2); for example, the G+C contents of these organisms are 52 to 57, 67 to 69, and 60 mol%, respectively. In addition, *V. paradoxus* and *Rhodoferax fermentans* are pigmented, are not gas vacuolate, and differ from *P. vacuolata* in cell shape and motility (Table 2).

This appears to be the first report of a gas vacuolate member of the beta subgroup of the Proteobacteria. This is not surprising, however, because some members of both the alpha and gamma subgroups of the Proteobacteria are known to be gas vacuolate. A logical conclusion is that this feature is widespread among this phylogenetic group, many members of which are found in aquatic habitats, where gas vacuolate bacteria most commonly reside.

On the basis of its phenotypic features and the results of an analysis of its levels of 16S rDNA base homology, as discussed above, 34-P is sufficiently different from other bacteria to warrant creation of a new genus. We therefore propose that the new genus *Polaromonas* should be described as follows.

**Description of Polaromonas gen. nov.** *Polaromonas* (Pol.ar.o.mo'na.s. M. L. adj. *polaris*, pertaining to the geographic poles; Gr. fem. *n. monas*, unit; M. L. fem. *n. Polaromonas*, polar bacterium). Cigar-shaped, gram negative rods that are 0.8 by 2.0 to 3.0 \( \mu \)m. Encapsulated. Aerobic. Chemoorganotrophic and catalase and oxidase positive. Requires amino acids, but not vitamins, for growth. Motile by means of a polar flagellum. Cells may contain gas vesicles. Psychrophilic. The maximum growth temperature of known strains is 15°C. The G+C contents are 52 to 57 mol% (as determined by the thermal denaturation method) (9).

The only species is the type species, *Polaromonas vacuolata*.

**Description of Polaromonas vacuolata sp. nov.** *Polaromonas vacuolata* (va.cuo.la'ta. L. adj. *vacuus*, empty; N. L. part. adj. *vacuolata*, equipped with gas vacuoles). Cells contain gas vesicles. The optimum temperature for growth is 4°C, and the growth temperature range is 0 to 12°C. Colonies are white, circular, and convex with smooth surfaces and entire edges. The more gas vesicles within the cells, the whiter the colony. Good growth occurs in media containing NaCl at concentrations ranging from 0 to 6%, but no growth occurs in the presence of 7% NaCl.

Tests for catalase, oxidase, urease, deaminase, and lipase are positive. Amylase, protease (gelatin), trypsinophanase (indole), nitrate reductase, cysteine desulfurase, and agarase tests are negative.

The following carbon sources are utilized: acetate, lactate, malate, fumarate, pyruvate, propionate, citrate, succinate, oxaloacetate, butyrate, 2-oxoglutarate, glucose, glycerol, sorbitol, DL-alanine, DL-glutamate, DL-proline, DL-aspartate, and DL-asparagine.


The fatty acid composition is 75% 16:1 \( \omega 7c \), 17% 16:0, and 8% 18:1 \( \omega 7c \), 18:1 \( \omega 9t \), or 18:1 \( \omega 12t \).

Susceptible to novobiocin, tetracycline, neomycin, and kanamycin. Resistant to bacitracin, streptomycin, and gentamicin.

The G+C content is 52.0 mol% (as determined by the thermal denaturation method).

The type strain is *P. vacuolata* 34-P (= ATCC 51984).

It is likely that the other strains included in this study are members of the same species on the basis of the results of the phenotypic tests that were performed, including the whole-cell fatty acid analysis. However, DNA-DNA hybridization tests were not conducted, and so this has not been verified.

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**REFERENCES**


17. Woese, C. R. Personal communication.