Proposal To Reclassify Zoogloea ramigera IAM 12670 (P. R. Dugan 115) as Duganella zooglooides gen. nov., sp. nov.

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The taxonomic position of a misclassified strain, Zoogloea ramigera IAM 12670T (= ATCC 25925T = P. R. Dugan 115T), was reevaluated. A phylogenetic analysis based on 16S ribosomal rDNA sequences revealed that this organism was located in the beta subclass of the class Proteobacteria with members of the genus Telluria as its closest relatives. On the basis of phenotypic and phylogenetic information, we propose that this organism should be reclassified in a new taxon with the name Duganella zooglooides gen. nov., sp. nov.

Zoogloea ramigera, which is at this time the only species of the genus Zoogloea Itzigsohn 1868, is an aerobic, chemooorganotrophic, gram-negative, rod-shaped bacterium that forms characteristic cell aggregates surrounded by gelatinous matrices, the so-called zoogloal matrices. This organism has been isolated from wastewater environments, such as activated sludge and trickling filters, and it has been suggested that Z. ramigera plays an important role in these environments. The two strains that were repurified differed in floc formation in addition to colony appearance. Strain 12670A exhibited dispersed growth with no or little formation of visible flocs when it was cultured with shaking in complex liquid media containing peptone. This strain formed visible flocs only when it was grown in chemically defined media supplemented with organic acids, such as tartrate, as the sole carbon source. The flocs formed were amorphous but, as is the case in "typical" Zoogloea strains, were fingerlike occasionally. Strains 12670A and 12670B were indistinguishable from each other in all other characteristics investigated, including cell morphology and physiological, biochemical, and chemotaxonomic characteristics (for details, see the descriptions of the genus and species below). The phenotypic studies indicated that the two strains were variants that originated from a single strain. Variations in colony appearance of the original strain, Z. ramigera P. R. Dugan 115T, on agar media have been reported previously (4).
Also, studies with typical Z. ramigera strains have shown that nonflocculating variants appear spontaneously upon subculture (20).

We confirmed the genetic homogeneity of strains 12670A and 12670B by DNA-DNA hybridization studies, as they showed 93 to 102% hybridization to each other in two different assays. The guanine-plus-cytosine contents of the genomic DNAs of the two strains varied from 63.4 to 63.8 mol%, but the variations appeared to be within the range of analytical error. We also found that the small-subunit rRNA structures of the two strains were identical. Moreover, there was no difference between the two strains in their RAPD patterns with the six arbitrary PCR primers (Fig. 1), suggesting that they were derived from the same strain.

The 16S rDNA sequence analysis of strain IAM 12670T performed in this study revealed that there were some errors in the sequence for the strain previously reported by us (DDBJ, EMBL, and GenBank accession no. D14256) (19). Our revised sequence for strain IAM 12670T differed at only one position from the sequence of strain ATCC 25935T published by Rossello-Mora et al. (accession no. X74914) (17). Previous phylogenetic studies have indicated that strain IAM 12670T (= ATCC 25935T), as well as Z. ramigera ATCC 19544T, belong to the beta subclass of the class Proteobacteria, but that within this subclass, the two strains form different clusters at the generic level (17, 19). We reconstructed a phylogenetic tree based on the updated 16S rDNA sequence information for these Z. ramigera strains and their phylogenetic relatives available from The Ribosomal Database Project database (14) and the DDBJ, EMBL, and GenBank databases. As shown in Fig. 2, the type strain of Z. ramigera formed a cluster with the phototrophic bacterium Rhodococcus purpuratus, whereas strain IAM 12670T was located in a different cluster with members of the genus Tellurita (1) as its closest relatives. The IAM 12670T Tellurita cluster also formed a lineage with the poly-β-hydroxybutyrate-degrading organism Pseudomonas lemoignei (3, 15) as a sister group. The levels of corrected distance (13) were 0.0577 to 0.0650 between strain IAM 12670T and the Tellurita species and 0.0696 between strain IAM 12670T and P. lemoignei. These values may be low enough to separate strain IAM 12670T from its recognized phylogenetic neighbors at the generic level.

On the basis of phenotypic, chemotaxonomic, and phylogenetic evidence noted above and elsewhere (8, 17, 19, 23), we propose that Z. ramigera IAM 12670T should be reclassified as a member of a new genus and new species with the name Duganella zooglooides. Differential characteristics of D. zooglooides and phylogenetically and phenotypically related organisms are summarized in Table 1. Although our proposal allows the existence of only one strain in the new genus at this time, this is reasonable considering the necessity for avoiding further confusion in Zoogloea taxonomy and also the importance of the strain in the field of wastewater microbiology and biotechnology (4).

A number of strains that show high levels of 16S rDNA sequence similarity to D. zooglooides have recently been isolated from soil (16). The partial 16S rDNA sequences (ca. 500 bases) of these new strains (DDBJ, EMBL, and GenBank accession no. D84564, D84572, D84574, D84576, and D84577) have similarity levels of 96.7 to 98.1% with the sequence of D. zooglooides IAM 12670T, suggesting that all of these organisms may form a phylogenetically coherent group at the generic level. Further study of the new soil strains noted above should provide more criteria to circumscribe the new genus Duganella. Also, the D. zooglooides description should be
come much more valuable when the characteristics of these isolates are included.

**Description of Duganella gen. nov.** Duganella (Du.ga.nel’la. M.L. dim. ending -ella; M.L. fem. n. Duganella, named after P. R. Dugan, an American microbiologist who isolated the organism). The description of the genus is based on information from references 4, 8, 9, 19 and 23 and this study. Cells are gram-negative, non-spore-forming, motile rods. Aerobic chemorganotrophs having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. No chemolithotrophic growth occurs with molecular hydrogen. The characteristics are the same as those described above for the genus.* Zoogloea.*

**Description of Duganella zoogloeoides** sp. nov. Duganella zoogloeoides (zo.o.gloe.o’i.des. M.L. bacterial genus name Zoogloea; Gr. suf. -oides, similar to; M.L. adj. zoogloeoides, similar to Zoogloea). The characteristics are the same as those described above for the genus. Other properties, based on information from references 4, 12, 23 and this study, are as follows. Cells are straight or slightly curved rods that are 0.6 to 0.8 µm wide and 1.8 to 3.0 µm long. Motile by means of single flagella. The G+C content of the genomic DNA is 63 to 64 mol%. The phylogenetic position is in the beta subclass of the class Proteobacteria, with members of the genus *Telluria* as phylogenetic neighbors. The type species is *D. zoogloeoides*.

**TABLE 1. Differential characteristics of Duganella gen. nov. and related genera or species of the beta subclass of the class Proteobacteria**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Duganella gen. nov.</th>
<th>Acidovarax</th>
<th>Burkholderia</th>
<th>Comamonas</th>
<th>Propionibacterium</th>
<th>Ralstonia</th>
<th>Telluria</th>
<th>Zoogloea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell diam &gt; 1.0 µm</td>
<td>-</td>
<td>Polar (m)</td>
<td>Polar (m)</td>
<td>V</td>
<td>Polar (m)</td>
<td>Polar (m)</td>
<td>Mix, polar (m)</td>
<td>Polar (m)</td>
</tr>
<tr>
<td>Flagellation</td>
<td>Polar (m)</td>
<td>Polar (m)</td>
<td>Polar (m,t)</td>
<td>Polar (m)</td>
<td>Polar (m)</td>
<td>Polar (m)</td>
<td>Mix, polar (m)</td>
<td>Polar (m)</td>
</tr>
<tr>
<td>Flocculent growth</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth on nutrient agar</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Nondiffusible yellow pigment</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Diffusible pigment</td>
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<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>+</td>
<td>+</td>
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<tr>
<td>H₂ autotrophy</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>-</td>
<td>-</td>
<td>V</td>
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<td>Oxidative acid produced from glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>Hydrolysis of starch</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Hydrolysis of gelatin</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Major respiratory quinone(s)</td>
<td>Q-8</td>
<td>Q-8</td>
<td>Q-8</td>
<td>Q-8</td>
<td>Q-8</td>
<td>Q-8</td>
<td>Q-8</td>
<td>Q-8, RQ-8</td>
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<tr>
<td>Major 3-OH fatty acid(s)</td>
<td>C₁₀₂₀</td>
<td>C₁₀₂₀</td>
<td>C₁₆₂₀</td>
<td>C₁₆₂₀</td>
<td>C₁₆₂₀</td>
<td>C₁₄₂₀</td>
<td>C₁₆₁₁</td>
<td></td>
</tr>
<tr>
<td>G+C content of DNA (mol%)</td>
<td>63-64</td>
<td>62-66</td>
<td>59-70</td>
<td>60-69</td>
<td>58</td>
<td>64-67</td>
<td>67-72</td>
<td>67-69</td>
</tr>
</tbody>
</table>

* Abbreviations: +, positive; (+), weakly positive; −, negative; v, variable among species or strains; polar (m), polar monotrichous; polar (t), polar tuft; per, peritrichous; mix, mixed flagella; Q-8, ubiquinone with eight isoprene units; RQ-8, rhodoquinone with eight isoprene units. Information from references 1, 3, 7, 15, 19, 25, 26, and 28 and this study.

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**REFERENCES**


