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

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The taxonomic position of a misclassified strain, *Zoogloea ramigera* IAM 12670^T (= ATCC 25925^T = P. R. Dugan 115^T), 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 zoogloeoides* gen. nov., sp. nov.

Zoogloea ramigera, which is at this time the only species of the genus *Zoogloea* Itzigsohn 1868, is an aerobic, chemoorganotrophic, gram-negative, rod-shaped bacterium that forms characteristic cell aggregates surrounded by gelatinous matrices, the so-called zoogloeal 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 following three strains of *Z. ramigera* have historically been well-known through a number of experimental studies: type strain ATCC19544 (= N. C. Dondero 106) (22), ATCC 19623 (= K. T. Crabtree I-16-M) (2), and ATCC 25935^T (= P. R. Dugan 115^T) (4, 6, 12). During studies to taxonomically revise *Z. ramigera*, however, Unz (22, 23) mentioned that there were major phenotypic differences among these three strains of *Z. ramigera* and that strains ATCC 19623 and ATCC 25935^T should be removed from the genus *Zoogloea*. Recent research on polyamine patterns (8), fatty acid and quinone profiles (9), and 16S ribosomal DNA (rDNA) sequences of *Zoogloea* strains (17, 19) provided evidence that there has been chemotaxonomic and phylogenetic divergence between *Z. ramigera* ATCC 19544^T and the other two strains. The chemotaxonomic and phylogenetic data, together with the earlier phenotypic information, led to a reevaluation of the taxonomic criteria for identification of *Z. ramigera* and emendation of the genus *Zoogloea* (19). The data require that each of the misnamed *Z. ramigera* strains be reclassified as a member of an appropriate known genus or a new genus. Here we report the results of a reexamination of one of the misnamed strains, strain IAM 12670^T (= ATCC 25935^T), and propose that this bacterium should be reclassified as a strain of *Duganella zoogloeoides* gen. nov., sp. nov.

Strain IAM 12670^T was obtained from the Culture Collection Center of the Institute of Applied Microbiology (now IAM Culture Collection, Institute of Molecular and Cellular Biosciences), The University of Tokyo, Tokyo, Japan. After several transfers of the strain on agar media, two morphological groups of colonies appeared and were designated strains 12670A and 12670B. The colonies of strain 12670A, which accounted for more than 70% of the colonies recovered, were glistening, convex with entire margins, viscous, and cream to straw colored. The viscous appearance of this strain became more pronounced with time of incubation. The colonies of strain 12670B were tough, leathery, dry, wrinkled, and pale yellow. We examined both of the newly isolated clones in this study. All phenotypic tests, DNA-DNA hybridization assays, and 16S rDNA sequencing were performed as previously described (9–11, 19). A distance matrix tree based on 16S rDNA sequences was constructed by the neighbor-joining method (18) with the CLUSTAL W program (21). Randomly amplified polymorphic DNA (RAPD) analysis (24, 27) was performed with a Ready-To-Go RAPD analysis kit (Pharmacia, Uppsala, Sweden) and with crude cell lysates as sources of template DNA; these crude cell lysates were prepared by a previously described protocol (11). The PCR primers used were the following six RAPD primers offered as a set by the manufacturer: primer 1, 5′-GGTGCGGGAA-3′; primer 2, 5′-GTTTCGCTCC-3′; primer 3, 5′-GTAGACCCGT-3′; primer 4, 5′-AAGAGCCCGT-3′; primer 5, 5′-AACGCGCAAC-3′; and primer 6, 5′-CCCGTCAGCA-3′. The RAPD reaction and detection of RAPD patterns by gel electrophoresis were performed according to the manufacturer's instructions.

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 medium supplemented with organic acids, such as tartrate, as the sole carbon source. The flocs formed were amorphous. On the other hand, strain 12670B constantly produced cell aggregates both in complex media and in chemically defined media. The flocs formed by this strain 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 115^T, on agar media have been reported previously (4).

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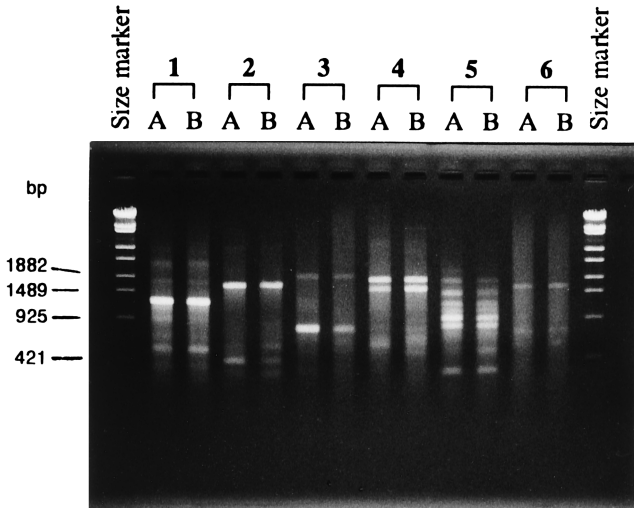

FIG. 1. RAPD patterns of strains 12670A and 12670B. The patterns obtained with arbitrary PCR primers 1 to 6 (indicated at the top) are shown. Lanes A and B contained strains 12670A and 12670B, respectively. PCR products were separated by gel electrophoresis (2% agarose) and were detected by ethidium bromide straining. Size marker, λ-*Eco*T14 I digest.

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 12670^T 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 12670^T differed at only one position from the sequence of strain ATCC 25935^T published by Rossello-Mora et al. (accession no. X74914) (17). Previous phylogenetic studies have indicated that strain IAM 12670^T (= ATCC 25935^T), as well as *Z. ramigera* ATCC 19544^T, 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 *Rhodocyclus purpureus*, whereas strain IAM 12670^T was located in a different cluster with members of the genus *Telluria* (1) as its closest relatives. The IAM 12670^T-*Telluria* cluster also formed a lineage with the poly(3-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 12670^T and the *Telluria* species and 0.0696 between strain IAM 12670^T and *P. lemoignei*. These values may be low enough to separate strain IAM 12670^T 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 12670^T should be reclassified as a member of a new genus and new species with the name *Duganella zoogloeoides*. Differential characteristics of *D. zoogloeoides* 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. zoogloeoides* 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. zoogloeoides* IAM 12670^T, 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. zoogloeoides* description should be-

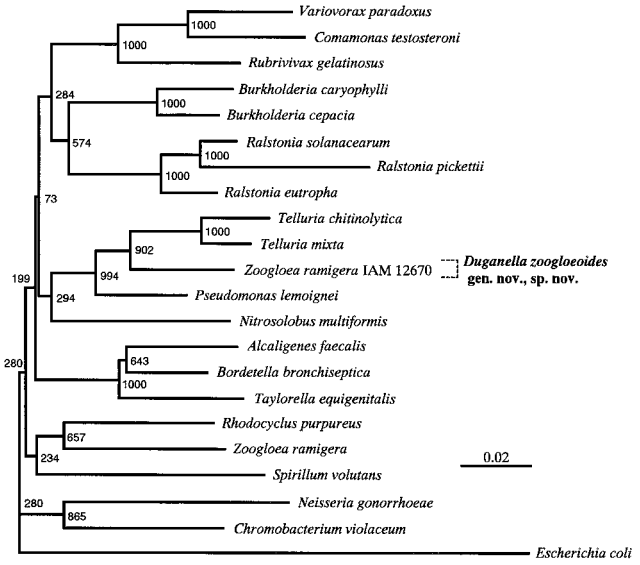

FIG. 2. Phylogenetic tree derived from an analysis of the 16S rDNA sequences of strain IAM 12670^T and related organisms belonging to the beta subclass of the class *Proteobacteria*. The sequence of *Escherichia coli* was used as an outgroup to root the tree. Bootstrap confidence values obtained with 1,000 resamplings (5) are given at branch points. Scale bar indicates 2 nucleotide substitutions per 100 nucleotides. The reference organisms and the accession numbers for their 16S rDNA and rRNA sequences used in the phylogenetic analysis are as follows: *Alcaligenes faecalis* ATCC 8750^T, M22508; *Bordetella bronchiseptica* ATCC 19395^T, U04948; *Burkholderia caryophylli* ATCC 25418^T, X67039; *Burkholderia cepacia* ATCC 25416^T, M22518; *Chromobacterium violaceum* ATCC 12472^T, M22510; *Comamonas testosteroni* ATCC 11996^T, M11224; *Escherichia coli*, J01695; *Neisseria gonorrhoeae* NCTC 8375^T, X07714; *Nitorosolobus multiformis* ATCC 25196^T, L35509; *Pseudomonas lemoignei* LMG 2207^T, X92554; *Ralstonia eutropha* ATCC 17697^T, M32021; *Ralstonia pickettii* ATCC 27512, X67042; *Ralstonia solanacearum* PDDCC17127^T, U28221; *Rhodocyclus purpureus* 6770^T, M34132; *Rubrivivax gelatinosus* ATCC 17011^T, D16213; *Spirillum volutans* ATCC 19554^T, M34131; *Taylorella equigenitalis* NCTC 11184^T, X68645; *Telluria chinolytica* ACM 3522^T, X65590; *Telluria mixta* ACM 1762^T, X65589; *Variovorax paradoxus* IAM 12373^T, D30793; and *Zoogloea ramigera* IAM 12136^T, D14254.

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

Characteristic	*Duganella* gen. nov.	*Acidovarax*	*Burkholderia*	*Comamonas*	*P. lemoignei*	*Ralstonia*	*Telluria*	*Zoogloea*
Cell diam > 1.0 μm	−	−	−	V	−	−	−	+
Flagellation	Polar (m)	Polar (m)	Polar (m,t) or none	Polar (t)	Polar (m)	Polar (m), per, or none	Mix, polar (m)	Polar (m)
Flocculent growth	+	−	−	−	−	−	−	+
Growth on nutrient agar	+	+	+	+	−	+	−	(+)
Nondiffusible yellow pigment	+	−	−	−	−	−	V	−
Diffusible pigment	−	−	+	−	−	V	−	−
H_2 autotrophy	−	V	−	−	−	V	−	−
Oxidative acid produced from glucose	+	+	+	−	−	V	+	−
Hydrolysis of starch	+	−	−	−	−	−	+	−
Hydrolysis of gelatin	+	V	V	−	−	V	+	+
Major respiratory quinone(s)	Q-8	Q-8	Q-8	Q-8		Q-8	Q-8	Q-8, RQ-8
Major 3-OH fatty acid(s)	$C_{10:0}$	$C_{10:0}$	$C_{14:0}$, $C_{16:0}$	$C_{10:0}$	$C_{10:0}$	$C_{14:0}$		$C_{10:0}$
G+C content of DNA (mol%)	63-64	62-66	59-70	60-69	58	64-67	67-72	67-69

[a] 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.

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 chemoorganotrophs having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. No chemolithotrophic growth occurs with molecular hydrogen. Amorphous or fingerlike flocculent growth occurs in liquid media. Mesophilic and neutrophilic. Growth is good on ordinary nutrient media and also occurs in mineral media supplemented with simple organic compounds as carbon and energy sources. Catalase and oxidase are present. Acid is produced oxidatively from glucose and other carbohydrates. Amylase, gelatinase, and urease are produced. Major components of the cellular fatty acids are $C_{16:0}$ and $C_{16:1}$, and 3-OH-$C_{10:0}$ is the major hydroxy fatty acid. Ubiquinone 8 is the sole respiratory quinone. Putrescine and hydroxyputrescine are intracellular polyamines. 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.*

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, and 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 polar flagella. Colonies on nutrient agar media are glistening, convex with entire margins, viscous, and pale yellow to straw colored. Tough, leathery, dry, wrinkled colonies appear in some cases. Aerobic chemoorganotrophy is the mode of growth. Nitrate is not used as a terminal electron acceptor for growth; denitrification is negative. No growth factor is required for growth, but yeast extract stimulates growth. Forms an esterlike sweet odor in cultures with organic acids as carbon sources. Produces acid from and grows with the following carbohydrates (as carbon sources): L-arabinose, D-xylose, D-glucose, D-fructose, D-galactose, D-mannose, maltose, sucrose, cellobiose, lactose, and glycogen. Growth and acid production are negative with D-ribose, L-rhamnose, glycerol, D-mannitol, D-sorbitol, and inositol. Carbon sources other than carbohydrates that are utilized are pyruvate, citrate, succinate, fumarate, malate, malonate, tartrate, ethanol, alanine, aspartate, asparagine, glutamate, and proline. No growth or little growth occurs with formate, acetate, propionate, butyrate, caproate, caprylate, methanol, propanol, benzoate, *p*-hydroxybenzoate, glycine, histidine, arginine, lysine, ornithine, or tryptophan. Source: sewage and polluted water. The type strain is IAM 12670 (= ATCC 25935 = P. R. Dugan 115).

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