Assignment of ATCC 27377 to *Pirella* gen. nov. as *Pirella staley* comb. nov.

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The necessity to rename strain ATCC 27377^T (T = type strain), *Pasteuria ramosa* sensu Staley, led Starr and his associates to propose placing this organism in the genus *Planctomyces*. Morphological and physiological observations, as well as deoxyribonucleic acid base composition and 16S ribosomal ribonucleic acid oligomer analyses, clearly showed that strain ATCC 27377^T differs significantly from species belonging to the genus *Planctomyces*. Therefore, we propose that strain ATCC 27377^T be renamed *Pirella staley* and afforded the position of the type strain of the type species of the newly created genus *Pirella*. The generic and species descriptions are emended. The request of Starr et al. (Int. J. Syst. Bacteriol. 33:666–671, 1983) for the conservation of *Pasteuria ramosa* is supported.

The recent findings concerning cladoceran bacterial parasites (7–9) have made it obvious that in nature there are organisms that are morphologically identical to *Pasteuria ramosa* Metchnikoff 1888. Fine-structure observations indicate relationships between this bacterium and gram-positive actinomycetes (8). Unfortunately, Hirsch (3) and Staley (13) were unaware of these observations; these authors applied the name *Pasteuria ramosa* to superficially similar budding and rosette-forming bacteria represented by a new isolate labeled strain ATCC 27377^T (T = type strain). Moreover, *Pasteuria ramosa* and its neotype strain (strain ATCC 27377^T) were in the Approved Lists of Bacterial Names (12). Although at present a pure culture of the cladoceran parasite does not exist, the descriptions given by Metchnikoff (6) and Sayre et al. (7–9) clearly indicate that there are substantial differences between the neotype strain and the cladoceran parasite. Thus, the necessity to rename strain ATCC 27377^T arose.

In a recent Request for an Opinion from the Judicial Commission (16), strain ATCC 27377^T was provisionally placed in the genus *Planctomyces*. At the same time, it was the stated aim of Starr et al. to rename this strain at a later date (i.e., when axenic cultures of *Planctomyces bekefii*, the type species, became available and could be studied to define the genus in a modern way). We take exception to this placement and suggest that strain ATCC 27377^T should not be assigned to the genus *Planctomyces*. Our observations and data clearly show major differences between strain ATCC 27377^T and either the unculturable type species, *Planctomyces bekefii* Gimesi 1924, or *Planctomyces maris* strain ATCC 29201^T. The latter organism is the only culturable, named organism in the genus *Planctomyces*.

For several years we have been working with budding bacteria, including a number of recent isolates obtained from freshwater (M. Müller, Ph. D. thesis, Universität Kiel, Kiel, Federal Republic of Germany, 1977), brackish water (H. Schlesner, Ph.D. thesis Universität Kiel, Kiel, Federal Republic of Germany, 1983) or a hypersaline lake near Rio de Janeiro, Brazil. We isolated in pure culture 9 strains of stalked budding bacteria that are morphologically similar to *Planctomyces maris* and 34 strains of budding bacteria (without stalks) which resemble strain ATCC 27377^T. These strains came from various aquatic habitats (Table 1) and they

were compared morphologically and physiologically with strains ATCC 27377^T and ATCC 29201^T (4; Schlesner, Ph.D. thesis). Our results can be summarized as follows: the properties of the *Planctomyces* strains are significantly different from those of strain ATCC 27377^T or of the 34 recently isolated strains with characteristics similar to those of strain ATCC 27377^T. Some of the specific differences observed are described below.

Morphology. The most important morphological variations are shown in Table 2. We specifically emphasize differences in cell shape; strain ATCC 27377^T cells are normally pear or teardrop shaped with a clearly visible pointed attachment pole. *Planctomyces bekefii* cells are spherical, and *Planctomyces maris* cells are spherical to ovoid; both of these organisms have true stalks with holdfasts at the distal ends. Schmidt and Starr (10) stated that morphotype IV cells of the *Planctomyces* group are tapered at the pole from which the stalk arises, although the micrographs of strain ATCC 27377^T published by Starr et al. (16) (Fig. 2 and 3) show nearly spherical cells for this organism. Strain ATCC 27377^T cells do not produce a stalk composed of a flat band or tube, as has been noted for *Planctomyces bekefii* (11). Likewise, the "stalk" of strain ATCC 27377^T cells does not consist of a rigid bundle of long fibrils (Fig. 1), as observed in cultures of *Planctomyces maris*; (1; Schlesner, Ph.D. thesis) (Fig. 2). In a previous report (16) strain ATCC 27377^T cells were said to form "a fragile fascicle" with individual fibrils about 3 nm in diameter. This fascicle was said to be more readily observable in natural material or laboratory enrichments than in axenic cultures. Our own observations suggest that strain ATCC 27377^T and other strains resembling it normally produce a fibrillar holdfast of irregular shape (Fig. 1 and 3) and that these strains lack the rigid, thicker stalks that can be observed in other *Planctomyces* spp. (Fig. 2). The morphological characteristics of members of the genus *Planctomyces* have been described as including multifibrillar, acellular stalks (width, 0.3 to 0.9 µm) and polarly excreted holdfasts (5, 15). The relative positions of flagella and crateriform structures demonstrate additional differences. We believe that the site of flagellar insertion is an important and reliable characteristic of these organisms. Contrary to the judgements expressed in the previous report (16), we do not feel that strain ATCC 27377^T fits the present morphological definition of the genus *Planctomyces*.

Base ratios. A comparison of the base ratio values (gua-

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FIG. 1. Two cells of strain ATCC 27377^T grown in liquid culture for 26 days, showing the irregularly arranged, fibrillar holdfast substance (FS) and polar crateriform structures (CR). Negatively stained. Bar = 1 μm.

FIG. 2. Cell of a 3-day-old liquid culture of *Planctomyces maris* ATCC 29201^T, showing the well-organized, rigid fibrillar stalk (S), a terminal holdfast (H), mother and daughter cell flagella (F), mother cell pili (P), and uniformly distributed crateriform structures (CR). Bar = 1 μm.

FIG. 3. Cell from a 2-day-old liquid culture of strain IFAM 1310, an isolate closely resembling strain ATCC 27377^T. The irregular arrangement of the fibrillar substance (FS) and the polar appearance of crateriform structures (CR) are clearly visible. Bar = 1 μm.

TABLE 1. Origins and numbers of new strains of nonprosthecate budding bacteria currently under study in Kiel, Federal Republic of Germany

Origin	No. of strains related to <i>Planctomyces maris</i> ATCC 29201 ^T	No. of strains related to strain ATCC 27377 ^T
Freshwater	2	2
Brackish water	1	31
Seawater	1	0
Hypersaline lake ^a	5	1

^a Lagoa Vermelha, near Rio de Janeiro, Brazil.

nine-plus-cytosine [G+C] contents) of strains resembling *Planctomyces maris* ATCC 29201^T, strain ATCC 27377^T, and related strains is shown in Table 3. The *Planctomyces* strains cluster around a G+C ratio of 50 to 55 mol%, whereas strain ATCC 27377^T and strains which we believe are related to this organism have G+C ratios of 56 to 59 mol%. Although more cultures in each group will have to be compared, we note that the group of strains resembling strain ATCC 27377^T is distinct from the *Planctomyces* cluster.

Ribonucleic acid oligomer catalogs. Examination of the 16S ribosomal ribonucleic acids isolated from representatives of the strain ATCC 27377^T and *Planctomyces* groups provided the following additional information: (i) the data indicate that these strains belong to a eubacterial group that stands isolated from all other eubacteria investigated so far; and (ii) within the strain ATCC 27377^T-*Planctomyces* group, the similarity factor (S_{AB} value; 1a between a strain related to *Planctomyces maris* and a strain similar to strain ATCC 27377^T was determined to be 0.30. This indicates a very distant relationship, as distant, for example, as the relationship between the genera *Bacillus* and *Clostridium* (12a).

Conclusions. We believe that we have demonstrated a number of important differences between strain ATCC 27377^T and species or cultures of the genus *Planctomyces*, as this genus is presently defined (5, 14). We quote Starr et al. (16): "ATCC 27377 differs substantially from both of the (se) *Planctomyces* species; albeit our present impression is that ATCC 27377 is taxonomically closer to *P. maris* than to *P. bekefi*."

At present, we see insufficient reasons for placing strain ATCC 27377^T with *Planctomyces* spp., based on the obvious differences outlined above. Taxonomy and nomenclature should be clear to avoid confusion, and renaming an organism with the immediate intention to again rename it in the

TABLE 3. Base ratios of strain ATCC 27377^T and related strains compared with those of stalked cultures of *Planctomyces* spp.

Organism	Strain	G+C content (mol%)	Reference(s)
<i>Pasteuria ramosa</i>	ATCC 27377 ^T	57.1 ^a	13
<i>Planctomyces staley</i>	ATCC 27377 ^T	59.0 ± 0 ^a	16
<i>Planctomyces staley</i>	ICPB 4362	58 ^a	16
<i>Pirella staley</i>	ATCC 27377 ^T	56.4 ± 0.4	Schlesner ^b
<i>Pirella</i> sp. ^c	IFAM 1310 ^d	56.4 ± 0.2	Schlesner
<i>Pirella</i> sp. ^c	IFAM 1313	57.4 ± 0.3	Schlesner
<i>Pirella</i> sp. ^c	IFAM 1318	56.9 ± 0.3	Schlesner
<i>Pirella</i> sp. ^c	IFAM 1009	57.1	Müller ^f
<i>Planctomyces maris</i>	ATCC 29201 ^T	50.5 ± 0.2	1; Schlesner
<i>Planctomyces maris</i>	IFAM 1317	53.6 ± 0.3	Schlesner
<i>Planctomyces</i> sp. ^c	IFAM 1007	54.4 ± 0.7	Müller
<i>Planctomyces</i> sp.	IFAM 1008	51.3 ± 1.3	Müller

^a Determined by the buoyant density method. The other values were determined by the melting temperature method.

^b Schlesner, H. Schlesner, Ph.D. thesis.

^c New species to be described shortly.

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^e To be described as *Pirella staley*.

^f Müller, M. Müller, Ph.D. thesis.

near future appears to us to be unwarranted. We agree with Starr et al. (16) concerning the need for transfer of this species to a different genus. Therefore, for the reasons given above, we formally propose to name strain ATCC 27377^T *Pirella staley*.

Description of the genus *Pirella* (Schlesner and Hirsch 1984). *Pirella* (Pi.rel' la. L.n.pirum pear; M.L. dim. ending -ella; M.L.fem.n. *Pirella* small pear, referring to the usually pear-shaped cells) cells are motile, budding, and nonstalked and frequently form rosettes by attachment with the pointed cell pole. We determined that the G+C content of the deoxyribonucleic acid ranges from 56.4 ± 0.4 to 57.4 ± 0.3 mol% (Table 3), as determined by the melting point method. Starr et al. (16) gave values of 58 to 59 mol% G+C; the values of these authors are derived from buoyant density data.

The type species is *Pirella staley* Schlesner and Hirsch 1984, and the genus is monotypic.

Emended description of the type species, *Pirella staley* Schlesner and Hirsch 1984 sp. nov. The description of *Pirella staley* (*Pasteuria ramosa* Staley 1973 [13]; *Pasteuria ramosa* sensu Staley, Staley et al. [15]; sta'ley. i. M.L. adj. *staley* named after James T. Staley, who isolated strain

TABLE 2. Morphological characteristics of strain ATCC 27377^T compared with those of the type species *Planctomyces bekefi* Gimesi 1924 and the type culture of *Planctomyces maris*, strain ATCC 29201

Characteristic	Strain ATCC 27377 ^a	<i>Planctomyces bekefi</i>	<i>Planctomyces maris</i>
Mature cell shape	Teardrop to pear shaped; attachment pole slightly pointed	Spherical	Spherical to ovoid
Cell size (µm)	0.5–1.0 by 1.0–5.0	1.4–1.7	0.4–1.5
Stalk shape and structure	Absent	Probably tubular, rigid ^b	Always rigid bundle of twisted fibers, ropelike
Stalk size (µm)		0.16–0.3 by 6	0.05–0.1 by 5
Position of holdfast	Narrow or pointed cell pole	Distal end of stalk	Distal end of stalk
Position of crateriform structures	On reproductive cell pole only	Scattered all over cell surface	Scattered all over cell surface
No. of flagella on bud	Monotrichous	Information lacking	Monotrichous
Position of flagellum	Reproductive cell pole	Information lacking	Attachment cell pole

^a Data from original descriptions (13, 14), as well as from our own observations with the type strain, strain ATCC 27377.

^b See reference 11.

ATCC 27377^T) is the same as the description for the genus. A description of the type strain, strain ATCC 27377^T, under the name *Pasteuria ramosa* has been given by Staley (13, 14). However, contrary to the original description, the cells are gram-negative and do not utilize lyxose, D-ribose, fucose, L-rhamnose, fructose, or inulin as a carbon source. Additional characteristics include the following. Pectin, lactose, maltose, melibiose, raffinose, sucrose, and trehalose are utilized as carbon sources. The maximum salt tolerance is 50% artificial seawater. The cells are weakly inhibited by artificial light (2,400 lx). The following carbon sources are not utilized: adipate, citrate, L-alanine, L-glutamate, gluconate, and urea.

The type strain is strain ATCC 27377.

We support the request of Starr et al. (16) to conserve the name *Pasteuria ramosa*.

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