Assignment of ATCC 27377 to *Planctomyces staleyi* sp. nov. and Conservation of *Pasteuria ramosa* Metchnikoff 1888 on the Basis of Type Descriptive Material Request for an Opinion

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The name *Pasteuria ramosa* Metchnikoff 1888 is used for two quite different kinds of bacteria. We request that the Judicial Commission issue an Opinion to the effect that the name be used only for the sort of bacterial parasite or endosymbiont described by Metchnikoff from the body cavities of cladoceran invertebrates belonging to the family *Daphnidae*. The name should not be used for an organism quite different from that described by Metchnikoff, ATCC 27377^T, a member of morphotype IV of the *Blastocaulis-Planctomyces* group of budding and non-prosthecately appendaged bacteria, for which we propose the name *Planctomyces staleyi* sp. nov. We further recommend that relevant alterations be made in connection with the Approved Lists of Bacterial Names and culture collection catalogs.

Background information. In 1888, Metchnikoff (7) illustrated the developmental stages of a bacterial parasite he had discovered in the body cavities of the cladoceran invertebrates *Daphnia pulex* and *D. magna*. Although he did not succeed in isolating the bacterium, he described it quite adequately by the standards common a century ago and named it *Pasteuria ramosa* (gen. nov., sp. nov.). Some subsequent efforts to find an organism identical to that described by Metchnikoff have led to confusion with a rather different bacterium, and both are now called *Pasteuria ramosa* Metchnikoff 1888 (7, 15). One is the bacterium parasitic or endosymbiotic in cladocerans, seemingly identical to the *Daphnia* organism of Metchnikoff, which has in recent years been rediscovered and described in detail (8–10). Efforts to isolate this cladoceran parasite in axenic culture are under way (R. L. Gherna and R. M. Sayre, in progress).

The other kind of organism to which the name was later applied homonymously is a group of nonparasitic budding bacteria, either free-living or attached to the external surfaces of *Daphnia* and other small invertebrates as well as to other plankton. Certain of these creatures share, perhaps superficially, some (but by no means all) of the properties described by Metchnikoff; some of these look-alikes have also been called *Pasteuria ramosa* Metchnikoff 1888. Among pertinent reports—which, it must be stressed, did not include attempts to search directly for Metchni-

koff's organism in the body cavities of cladocerans—we might mention the findings of Henrici and Johnson (2) concerning a free-living aquatic bacterium which reproduced by budding. Suggesting that the "spore bodies" illustrated by Metchnikoff were not spores but buds, Henrici and Johnson ignored Metchnikoff's *Daphnia* parasite but used its name (*Pasteuria ramosa* Metchnikoff 1888) for certain of the free-living budding and appendaged bacteria they did find. (Zavarzin [19] took exception to this interpretation of Metchnikoff's paper by Henrici and Johnson [i.e., that the "spore bodies" were buds], pointing out it was unlikely that a scientist of Metchnikoff's capability would have confused spore formation with the budding process; Zavarzin's view has been challenged [15], but recent work [9] has supported it.)

More recently, Hirsch (3, 4) equated with Metchnikoff's organism a budding bacterium he had found attached externally to the antennae of *Daphnia*. Hirsch's organism (3) had some properties reminiscent of those described by Metchnikoff for certain developmental stages of the *Daphnia* parasite; however, as later events demonstrated, it was an organism rather different from that of Metchnikoff, being a budding bacterium resembling those that Henrici and Johnson (2) had studied but living externally on *Daphnia*. Hirsch (3, 4) followed Henrici and Johnson's infelicitous nomenclatural action of retaining the name (*Pasteuria ramosa*) Metchnikoff had used

for the *Daphnia* parasite but restricting this name only to these quite different budding and nonparasitic forms. Budding bacteria, probably similar to those studied by Henrici and Johnson (2) and by Hirsch (3), were observed by Staley (15) attached to other aquatic microorganisms, including the carapaces of *Daphnia*. Staley isolated such organisms from aquatic habitats in Michigan and California and referred them to the *Pasteuria-Blastobacter* group (14, 15). A culture of one of these budding bacteria was deposited in the American Type Culture Collection (ATCC) as the type strain of *Pasteuria ramosa* Metchnikoff 1888 under accession number ATCC 27377 (1, 15).

One could argue that the actions up to this point (in the early 1970s) might have been weakly justified by the superficial resemblance of the free-living (or, at least, nonparasitic) organisms described by Henrici and Johnson (2), Hirsch (3, 4), and Staley (15) to some of the developmental stages of the *Daphnia* parasite as described by Metchnikoff (7). However, by 1980, when this particular strain (ATCC 27377) was designated in the Approved Lists of Bacterial Names (14) as the type strain of *Pasteuria ramosa* Metchnikoff 1888, the nonequivalence should have been evident because modern papers on the cladoceran parasite (Metchnikoff's *Pasteuria ramosa*) had appeared during the 1970s (8, 10), before the Approved Lists went to press. These papers, and an even more recent one (9), demonstrate unequivocally that an organism identical to that described by Metchnikoff (7), but quite different from the free-living budding forms (2, 3, 15), really exists.

Moreover, from a series of studies (11–13, 18) on organisms of the sort represented by ATCC 27377^T, as well as that very strain, it is now quite clear that ATCC 27377^T is a typical member of morphotype IV of the *Blastocaulis-Planctomyces* group of budding and nonprosthecately appendaged bacteria (18). ATCC 27377^T bears little relationship to Metchnikoff's organism (7); the unrelatedness becomes even more evident from scrutiny of the modern characterizations of the cladoceran parasite (8–10). ATCC 27377 is an inappropriate type strain for *Pasteuria ramosa* Metchnikoff 1888. The problem of homonymy does not formally disappear by calling ATCC 27377^T "*Pasteuria ramosa* sensu Staley" (17); however, when it is viewed solely as a trivial name, this designation does at least have the advantage of distinguishing between two different organisms both named *Pasteuria ramosa*.

The overall nomenclatural confusion—including homonymy and designation of improper type material—requires the assignment of ATCC 27377^T to a more appropriate taxon as well as

the judicial intervention and other actions proposed herein.

Assignment of ATCC 27377^T to the genus *Planctomyces*. To propose new generic taxa before completion of the relevant portions of our extensive comparative studies on some 40 strains belonging to the *Blastocaulis-Planctomyces* group (M. P. Starr and J. M. Schmidt, manuscript in preparation) would be premature (11, 12). Moreover, basing new taxa in this bacterial group (or any other, for that matter) on only one or two strains is not a sound taxonomic practice. However, to have the accompanying Request for an Opinion considered on an adequate basis, we were urged (W. E. C. Moore, personal communication) to recommend assignment of ATCC 27377^T to a definite taxon in the present paper. Under these circumstances, the action currently most appropriate in our view was to name ATCC 27377^T as a new species of the genus *Planctomyces*, on the basis that ATCC 27377^T does fit the present definition of the genus *Planctomyces* (5, 12, 17). When our comprehensive examination of the entire group is completed, this organism may be transferred to a different genus (12).

In its present meaning (5, 12, 17), the genus *Planctomyces* is quite heterogeneous. It contains two validly published species, *Planctomyces bekefii* (the type species of the genus) and *Planctomyces maris*, and a number of irregular "species" (5, 12). To the extent determinable in the absence of axenic cultures of *Planctomyces bekefii* (12), these two valid species (as well as many of the irregular ones) are probably sufficiently unrelated (11, 12) to warrant eventual placement in different genera. ATCC 27377^T differs substantially from both of these *Planctomyces* species; albeit our present impression is that ATCC 27377^T is taxonomically closer to *Planctomyces maris* than to *Planctomyces bekefii*. Taking all of these factors into account, we now formally propose assignment of ATCC 27377^T to *Planctomyces staley* sp. nov., with ATCC 27377 as the type strain.

Emended description of *Planctomyces staley* sp. nov. *Planctomyces staley* has been described previously by Staley (15, 16) under the name *Pasteuria ramosa* Metchnikoff 1888, with ATCC 27377 as its type strain. We wish now only to make the following alterations in those published descriptions, based mainly on our findings by the methodologies we usually employ (11–13).

Planctomyces staley sp. nov. (M. L. adj.; sta'ley.i.), named in honor of James T. Staley, imaginative and industrious isolator of unusual bacteria, including ATCC 27377^T (15).

Synonyms: *Pasteuria ramosa* sensu Staley (15, 16). Morphotype IV (in part, at least) of the

FIG. 1. Reproductive half of a cell of *Planctomyces staleyii* sp. nov., ATCC 27377^T, negatively stained with 1.0% uranyl acetate. cr, Crateriform surface structures; bu, bud; p, pili. Bar, 0.5 μ m.

Blastocaulis-Planctomyces group of budding and nonprosthecately appendaged bacteria (11–13). Not *Pasteuria ramosa* Metchnikoff 1888 sensu stricto (7, 9, 13, 17).

The type strain of *Planctomyces staleyii* sp. nov. is ATCC 27377 (ICPB 2138; Staley "Michigan strain"). A putative reference strain, with which we have had considerable experience (13, 18), is ICPB 4362 (Schmidt strain CLPM, "white" clone derived from ICPB 4232; ATCC 35122).

ATCC 27377^T was isolated from Lake Lansing, Michigan (15), and ICPB 4362 was isolated from Campus Lake, Louisiana State University, Baton Rouge (13, 18). Other populations of *Planctomyces staleyii* and close relatives also belonging to morphotype IV have been found by us in aquatic habitats the world over. These organisms are usually attached to filamentous algae and cyanobacteria by a holdfast located at the distal end of the fascicle (the multifibrillar major appendage, which is further considered below) or at the nonreproductive (nonbudding and nonpiliated; see below) pole of the cell, if a fascicle is not present.

Although occasional cells take the Gram stain, the reaction of *Planctomyces staleyii* is predominantly gram negative, as is to be expected from the gram-negative cell envelope profile of strains belonging to morphotype IV of the *Blastocaulis-Planctomyces* group (13); in those morphotype IV strains thus examined, no discrete peptidoglycan layer can be visualized in thin sections by transmission electron microscopy (13).

ATCC 27377^T, ICPB 4362, and other strains belonging to morphotype IV (as well as strains

belonging to other morphotypes) of the *Blastocaulis-Planctomyces* group are highly resistant to beta-lactam antibiotics (e.g., methicillin, penicillin G, ampicillin, cephalosporin C) and other inhibitors of peptidoglycan synthesis (e.g., vancomycin, bacitracin). Although ATCC 27377^T has not yet been thus examined, the cell envelopes of other strains belonging to morphotype IV (and morphotype III) contained little or no muramic acid or diaminopimelic acid (O. Kandler, personal communication).

Planctomyces staleyii has numerous crateriform surface structures (11–13), a defining characteristic of the entire *Blastocaulis-Planctomyces* group. There are two kinds of crateriform structures in ATCC 27377^T; the larger ones (12-nm diameter) are located on the reproductive (budding) pole of the cell, from which short and brittle pili (5- to 6-nm diameter) also extend (Fig. 1). Smaller crateriform or porelike structures (5- to 7-nm diameter), which occur on the nonreproductive and nonpiliated pole of the cell opposite the budding site, are not as common in ATCC 27377^T as in ICPB 4362 and other strains of *Planctomyces staleyii* (see Fig. 3E of reference 18).

Planctomyces staleyii forms a fragile fascicle (11–13), a bundled or braided multifibrillar appendage, originating from the nonreproductive and nonpiliated pole of the cell. The individual fibrils of the fascicle are about 3 nm in diameter and several micrometers in length. This fascicle, a third kind of nonprosthecate appendage (i.e., in addition to the sheathed flagellum of the swimmers and the short pili), is the least obvious appendage in axenic cultures of *Planctomyces staleyii*. Typical fascicles of ATCC 27377^T are shown here in Fig. 2 and 3; the fascicle of another *Planctomyces staleyii* strain is illustrated elsewhere (see Fig. 4A of reference 18). Fascicles are very easy to overlook in transmission electron microscopic preparations of axenic cultures. They are seen more readily in natural material or laboratory enrichments of *Planctomyces staleyii* and other morphotype IV strains, in which they frequently terminate in a definite holdfast (as shown here in Fig. 4). The fascicles of ATCC 27377^T in axenic culture are not only inconspicuous (Fig. 2), but also quite fragile (Fig. 3).

Planctomyces staleyii goes through a developmental cycle, which has been described elsewhere (18). The mature bud develops a sheathed flagellum attached near the piliated pole (opposite the fascicle origin) and becomes a swimmer; the swimmer loses its flagellum and becomes a sessile mother cell (with a distal holdfast and eventually a fascicle at the pole opposite the piliated and budding pole); the mother cell develops a bud; etc.

FIG. 2. Cell of *Planctomyces staley* sp. nov., ATCC 27377^T, negatively stained with 1.0% uranyl acetate. fa, Fascicle; F, sheathed flagellum. Bar, 0.5 μ m.

The guanine plus cytosine (G+C) content of the DNA of ATCC 27377^T (M. Mandel, personal communication) is 59 mol% by buoyant density (variance in four independent determinations was zero); the value reported elsewhere (15) for the same strain as analyzed by the same method and by the same analyst was 57 mol% G+C. The corresponding value for ICPB 4362 is 58 mol% G+C (M. Mandel, personal communication). Other strains belonging to morphotype IV (though not necessarily to *Planctomyces staley*) give values ranging from 56 to 63 mol% G+C (M. Mandel, personal communication).

Request for an Opinion. After the present assignment of ATCC 27377 as the type strain of *Planctomyces staley* sp. nov., we request that

the Judicial Commission issue an Opinion to conserve the name *Pasteuria ramosa* Metchnikoff 1888 on the basis of the original description by Metchnikoff (7) as emended by others in recent years (8–10).

There are several bases for this request. The name used by Metchnikoff for the cladoceran parasite is the senior homonym. The expedient assignment of a member of morphotype IV of the *Blastocaulis-Planctomyces* group as the type strain (of *Pasteuria ramosa*) in the 1980 Approved Lists of Bacterial Names (14) was counter to type material available in 1979 and

FIG. 3. Cell of *Planctomyces staley* sp. nov., ATCC 27377^T, negatively stained with 1.0% uranyl acetate, showing its fragile fascicle, the attachment of which to the cell is disrupted. fa, Fascicle; p, pili; cr, crateriform surface structures. Bar, 0.5 μ m.

FIG. 4. Morphotype IV cell (either *Planctomyces staleyi* or a close relative) observed directly from an enrichment culture (11) prepared from a water sample collected in September 1982 from a lily pond in Burgers Park, Pretoria, South Africa. The cell was negatively stained with 1.0% uranyl acetate. The fascicle in this cell from a crude enrichment culture is much more conspicuous (and possibly also wider and composed of more fibrils) compared with the corresponding structures in cells from axenic cultures. fa, Fascicle; h, holdfast; p, pili; cr, crateriform surface structures. Bar, 0.5 μ m.

has led to nomenclatural confusion that, in view of extensive historical interest in the species, would have continued as long as the name also was associated with the junior homonym. Perusal of provisions of the *International Code of Nomenclature of Bacteria* (6) regarding noncultivable bacteria leads to the conclusion that the type material for *Pasteuria ramosa* has been,

since 1888 (7) and until the Approved Lists (14) was published in 1980, Metchnikoff's description and illustrations (7), enhanced by the modern descriptions and illustrations, especially the electron micrographs (8–10). We request that this material be retained as the type material pending designation of a type strain if, as we hope, the natural material yields axenic cultures of Metchnikoff's *Pasteuria ramosa* (R. L. Gherna and R. M. Sayre, in progress, personal communication).

The Opinion would designate Metchnikoff's description and illustrations—as enhanced by modern descriptions (8–10) of the cladoceran parasite—as the type material of *Pasteuria ramosa* Metchnikoff 1888, replacing ATCC 27377 as the type strain (14).

Although it is not in the purview of the Judicial Commission, accessioning information in culture collection records should be corrected to show the true nature of ATCC 27377 as the type strain of *Planctomyces staleyi* sp. nov. and as a representative of morphotype IV of the *Blastocaulis-Planctomyces* group of budding and non-prosthecately appendaged bacteria (11–13, 18).

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