Planctomyces stranskae (ex Wawrik 1952) sp. nov., nom. rev. and Planctomyces guttaeformis (ex Hortobágyi 1965) sp. nov., nom. rev.

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Two related but recognizable different forms belonging to morphotype V of the Blastocaulis-Planctomyces group of budding bacteria were observed during the summer of 1983 in two eutrophic man-made ponds in Tempe, Ariz. Each of these forms can be assigned to previously described species of the genus Planctomyces; because these forms were not included in the 1980 Approved Lists of Bacterial Names (Skerman et al., ed., Int. J. Syst. Bacteriol. 30:225-420), they need to be formally revived. One of these forms (morphotype Vb) is assigned to Planctomyces stranskae (ex Wawrik 1952) sp. nov., nom. rev.; the other form (morphotype Va) is assigned to Planctomyces guttaeformis (ex Hortobágyi 1965) sp. nov., nom. rev. Neither species has been cultivated axenically; hence, both are based on type descriptive material.

In 1924, Gimesi (4) established the genus Planctomyces to accomodate the peculiar aquatic "fungus" Planctomyces bekefi he had observed in a pond (Lake Lágymányos) near the Technical University in Budapest, Hungary. Later, additional species were assigned to this "fungal" genus (11, 24). "Blastocaulis sphaerica" was independently established in 1935 by Henrici and Johnson (6) for unusual (budding and appendaged) bacteria, which later were found (9) to be identical to P. bekefi. Thus, Blastocaulis (a bacterial genus) is an exact later synonym of Planctomyces. Confusion exists about the relationships of these two organisms to each other. At one extreme, P. stranskae" is presumed to be a later synonym of "P. guttateformis" (F. Wawrik, personal communication). Others suggest that "P. stranskae" is (i) within the limits of variability of P. bekefi (3, 12), (ii) unlike P. bekefi (24), (iii) probably identical to "P. guttaeformis" but with the latter epithet (a junior synonym) retained (5, 8), or (iv) a member of the bacterial genus Gallionella (10).

In 1965, Hortobágyi (11) established the genus Planctomyces to accommodate "fungus" Planctomyces bekefi he had observed in a pond (Lake Lágymányos) near the Technical University in Budapest, Hungary. Later, additional species were assigned to this "fungal" genus (11, 24). "Blastocaulis sphaerica" was independently established in 1935 by Henrici and Johnson (6) for unusual (budding and appendaged) bacteria, which later were found (9) to be identical to P. bekefi. Thus, Blastocaulis (a bacterial genus) is an exact later synonym of Planctomyces. Confusion exists about the relationships of these two organisms to each other. At one extreme, P. stranskae" is presumed to be a later synonym of "P. guttateformis" (F. Wawrik, personal communication). Others suggest that "P. stranskae" is (i) within the limits of variability of P. bekefi (3, 12), (ii) unlike P. bekefi (24), (iii) probably identical to "P. guttaeformis" but with the latter epithet (a junior synonym) retained (5, 8), or (iv) a member of the bacterial genus Gallionella (10).

The fortuitous occurrence, during the summer of 1983, of these two organisms side-by-side in two Arizona ponds we regularly study (15, 17, 19) provided an opportunity for us to clarify some of the pertinent taxonomic issues. On the basis of an examination of this Arizona material, samples collected in Austria and Hungary (18), and chemically preserved type material from a Hungarian repository, we believe that "P. stranskae" and "P. gutteformis" are related but recognizable different organisms and that both are budding bacteria rather than fungi.

MATERIALS AND METHODS

Sources of water samples. Water samples containing the budding bacteria described in this report were collected in June and July 1983 from the top 15 cm of water at the edge of a eutrophic, shallow man-made pond in The Lakes, a residential development in Tempe, Ariz. Observations similar to those reported here were made (but are not presented here) on samples from another eutrophic, shallow man-made pond (in Kiwanis Park, Tempe, Ariz.). Both ponds are fed via reservoirs and canals with water from the Salt River Project. Some of the illustrative material for morphotype Va is based on laboratory enrichments prepared from water samples collected in August 1981 from a pond located in the Buda section of Budapest, Hungary, just north of the railroad bridge spanning the Danube River and southwest of the Technical University; this pond is the last remnant of the type locality of the genus Planctomyces (16, 19).

Planctomyces guttaeformis" type material. Type material of "P. guttaeformis" Hortobágyi 1965 (11), in the form of pond water microflora preserved in Formalin (as is customary with "mycological" specimens), collected in 1964 by T. Hortobágyi at Buzáí, Hungary, was obtained from L. J. Hajdu, Curator of the Algal Herbarium, Museum of Natural History, Budapest, Hungary.

Microscopic methods. Samples were prepared for phase-contrast light microscopy and for negative-contrast transmission electron microscopy by previously described methods (15, 17, 20). Stained preparations (13), examined by light microscopy, were sometimes used to emphasize spikes, bristles, and other features.

For preparation of thin sections for transmission electron microscopy, 500-ml samples of pond water containing ca. 2 × 106 rosettes of the relevant bacteria per ml were concentrated by centrifugation (8,000 × g; 15 min; refrigerated centrifuge). The pellets (containing rosettes of the budding bacteria together with many algae, cyanobacteria, and other bacteria) were fixed by the procedure of Burdett and Murray (2) with 5.0% acrolein (Eastman Kodak Co., Rochester, N.Y.) plus 0.25% glutaraldehyde (prepared from ampoules

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of 8.0% glutaraldehyde stock solution obtained from Ted Pella, Inc., Tustin, Calif.). A 5-min prefixation in a 1:10 dilution of this fixative was followed by a 3-h treatment in the full-strength fixative buffered at pH 7.4 with 0.05 M sodium cacodylate. A 1-h secondary fixation with 1.0% osmic acid (also buffered at pH 7.4 with 0.05 M sodium cacodylate) followed. The fixed material was enrobed in agar (2.0% Special Agar; Difco Laboratories, Detroit, Mich.), dehydrated in an ethanol series followed by propylene oxide, and embedded in Maraglass (Ted Pella, Inc.). After polymerization, thin sections were cut with a diamond knife (Biomedical Division, Du Pont Co., Wilmington, Del.). Electron-microscopic observations were made with a Philips model EM-300 instrument operated at 80 kV.

RESULTS AND DISCUSSION

More than one form of morphotype V. The various budding and nonprosthecately appendaged bacteria have been sorted into morphotypes of the Blastocaulis-Planctomyces group (17, 20). In our initial categorization (18), morphotype V was represented only by those bacteria assigned to "Planctomyces guttaeformis" Hortobagyi 1965, that is, the bulbiform, budding bacteria that bear spikes and form rosettes (see figures). Up to now, the existence of two similar, yet distinguishable, bulbiform, budding bacteria has not generally been realized. The aforementioned form, with a prominent spike at its globose end, is widely distributed and quite well known (5, 7, 8, 11, 18). The other form, which lacks a spike, was probably sighted by us earlier (see, for example, Fig. 1B and C of reference 18) but not recognized as a separate entity until its fortuitous occurrence during the summer of 1983, while we were seeking "Planctomyces gracilis" (23). Two distinguishable populations of bulbiform, budding bacteria were then seen repeatedly in both Arizona ponds.

Both forms are shown in the same microscopic fields in Fig. 1A, a transmission electron micrograph, and in Fig. 1B, a stained (13) preparation examined by light microscopy. One form (morphotype Va) possesses an apical spike and thin pili on the globose hemisphere. The other form (morphotype Vb) lacks the spike, but its globose hemisphere is covered by numerous rigid, multifibrillar, and tapered appendages, thicker and more substantial than pili; we call them bristles. In stained preparations, the bristles of morphotype Vb appear as a halo not more than 1 to 2 μm in length or diameter, whereas the individual spikes of morphotype Va are thicker and 5 to 10 μm long. Each rosette is homogeneous with regard to the appendages on the cells that make up the rosette; i.e., either every cell of a given rosette has a spike or every cell of a given rosette lacks a spike but has a beard of bristles at its globose end. An examination of over 100 rosettes revealed none with a mixture of morphotype Va and Vb cells, although both forms were abundant in the samples. Assuming rosettes are formed by attachments of individual cells by their holdfasts, this homogeneity speaks for some kind of uniqueness recognized by the

FIG. 1. Demonstrations in the same fields of the two different kinds of bulbiform, rosette-forming, budding bacteria in material from the pond in The Lakes, Tempe, Ariz. Pg, Planctomyces guttaeformis (ex Hortobagyi 1965) sp. nov., nom. rev.; Ps, Planctomyces stranskae (ex Wawrik 1952) sp. nov., nom. rev. (A) Negative-contrast transmission electron micrograph (1.0% uranyl acetate stain). sp, Spike; br, bristles. ×8,000. (B) Light micrograph of a stained (13) preparation showing a P. guttaeformis rosette, with a long spike (sp) on each of several cells, and a P. stranskae rosette, with no spikes at all but with a prominent halo caused by staining of the bristles (br). ×4,400.
Archival illustrations and preserved type material. Much confusion between the two forms (morphotypes Va and Vb) is evident in the literature (5, 7, 8, 11, 12), especially with respect to the spikes. Relevant archival illustrations are reproduced in Fig. 2. Wawrik's drawings (Fig. 2A) accompanied her original description of "P. stranskae" (24). Other drawings of both forms (Fig. 2B-E) by Heynig (7, 8) and Hortobágyi (11, 12) showed either no spikes or mixtures of spiked and spikeless cells. The only bulbiform, rosette-forming, budding bacterium seen by phase-contrast microscopy in Hortobágyi's preserved type material of "P. guttaeformis" (Fig. 3) was the spiked form.

Hortobágyi's description of "P. guttaeformis" and clarification of its cellular shape. Hortobágyi (11) described "P. guttaeformis" as a new species of truly planktonic fungi (" euplanktonische Pilze"), in the following terms, as translated by us (18) from his Latin diagnosis (the mycological terminology is retained): three to seven drop-shaped [guttaeform], spore-bearing arms [i.e., conidiophores]; one pole [of each arm] is gradually tapered to a point, [several] points [of the three to seven arms] come together at the center of the thallus; the other pole [of each arm] is broadly rounded; the arms bear spores, also drop shaped and contacting the arms by their rounded poles; mature cells are 2.8 to 3.7 μm long, 1 to 1.6 μm wide, and straight or slightly bent. Hortobágyi's sketches (11, 12), reproduced here as Fig. 2C, were based on conventional light microscopy; they depict a drop-shaped (i.e., guttaeform) organism arranged in rosettes.

Whether Hortobágyi's "P. guttaeformis" is bulbiform or drop shaped has been discussed (5, 18). On the basis of transmission electron microscopy and phase-contrast microscopy of material from the same Hungarian sources as Hortobágyi's, Hajdu (5) described this bacterium as "pear shaped" (during an interview in Budapest, Hungary, L. J. Hajdu explained that he used the term "pear shaped" for the shape we call "bulbiform"). We (18) and Hajdu (5) agree that Hortobágyi was understandably unable to resolve the junction between the globose and cylindrical portions of the bulbiform cell by conventional light microscopy and, hence, interpreted what he saw as the smoothly tapered transition of a globose end to a pointed end (i.e., drop shaped). This assumption is supported by our phase-contrast (light) micrographs of Hortobágyi's preserved type material of "P. guttaeformis" (Fig. 3), which unmistakably show rosettes of bulbiform cells. It is, of course, possible that Hortobágyi actually saw drop-shaped cells in his material in 1964 but that they were no longer visible in the preserved type material when examined 2 decades later. However, until evidence of a drop-shaped organism comes to light, we must believe that Hortobágyi's "P. guttaeformis" is bulbiform.

Assignment of Hortobágyi's organism to Planctomyces guttaeformis (ex Hortobágyi 1965) sp. nov., nom. rev. Although the foregoing points are historically interesting, they are taxonomically moot because "P. guttaeformis" Hortobágyi 1965 was not included in the 1980 Approved Lists of Bacterial Names (21). We now formally revive the name of Hortobágyi's organism (our morphotype Va), using the same name, Planctomyces guttaeformis (ex Hortobágyi 1965) sp.
holdfasts at the narrower ends of the several cells. Neither is by budding, with a mirror-image bud formed at the globose bulb). A prominent multifibrillar appendage, a spike, extends from the globose (spherical) end of the cell. The spike attachment site. Resistant to beta-lactam antibiotics. Occur in rosettes, consisting of as many as 12 cells attached via holdfasts at the narrower ends of the several cells. Neither motility nor flagella have been observed. Common in eutrophic freshwater habitats, usually ascribed to the rather thin cell envelope, the crateriform surface structures, and the spike and its basal attachment site. Resistant to beta-lactam antibiotics. Occur in rosettes, consisting of as many as 12 cells attached via holdfasts at the narrower ends of the several cells. Neither motility nor flagella have been observed. Common in eutrophic freshwater habitats, usually accompanying phototrophs (algae and cyanobacteria). Has not been cultivated axenically, although blooms occur in laboratory enrichments. The type material consists of the present description and illustrations (P in Fig. 1; Fig. 3 made from Hortobágyi's preserved type material; and Fig. 4; see also Fig. 2 of reference 18 and Fig. 2 of reference 20). The type locality is a eutrophic man-made pond in a residential district (The Lakes) in Tempe, Ariz. Frequently confused with Planctomyces stranskae (ex Wawrik 1952) sp. nov., nom. rev., from which it can be distinguished on the basis of cellular size and shape, and the absence of numerous multifibrillar bristles in P. guttaeformis and its absence in P. stranskae, and the presence of numerous multifibrillar bristles in P. guttaeformis and its absence in P. stranskae.

Wawrik's description of "P. stranskae". In 1952, Wawrik (24) described "P. stranskae" as a new fungal species. Her German text and Latin diagnosis, translated and paraphrased here rather freely (and with the mycological terminology retained), distinguished the new species from the gradually tapering (i.e., club-shaped) conidiophores of "P. stranskae". The daughter spores were reported to arise from the most strongly refractile end of the conidiophore, in a shallow concavity thereof. The diameter of the thallus was given as 8.8 to 12 μm, the diameter of the daughter spore was given as 1.5 μm; and the diameter of the conidiophore was given as 1.0 μm. Iron encrustation was observed. Her description of "P. stranskae" included sketches, which are reproduced here, particularly Figs. 1A and 5A-C, and 6A and of a thin section (Fig. 5D) of morphotype Vb, as well as light micrographs (Fig. 1B and 6B), are presented (for a comparison with morphotype Va, see Fig. 1 through 4). Invoking Rule 28a and Provisional Rule B3 of the International Code of Nomenclature of Bacteria (14), we now apply to morphotype Vb the name Planctomyces stranskae (ex Wawrik 1952) sp. nov., nom. rev. No type cultures are available because P. stranskae has not been cultivated axenically; hence, type descriptive material must suffice.

Description of Planctomyces guttaeformis (ex Hortobágyi 1965) sp. nov., nom. rev. Planctomyces guttaeformis [guttaeformis. L. gen. noun guttate(a) a drop; L. suffix -formis in the form or shape of; M.L. adj. guttaeformis drop shaped]. Cells are relatively large (2.9 to 3.0 μm in length, 1.15 to 1.3 μm in diameter at the globose end, and 0.6 to 0.7 μm in diameter at the cylindrical end) and bulbiform (i.e., bulb shaped; a cylinder blending into a spherical terminal dilatation; shaped like a common screw-in incandescent light bulb). A prominent multifibrillar appendage, a spike, extends from the globose (spherical) end of the cell. The spike is not a stalk, as it lacks the function of connecting the cell to other cells or to a substrate via a terminal holdfast. Division is by budding, with a mirror-image bud formed at the globose end of the cell. The bud is often slightly off-center with respect to the longitudinal axis of the mother cell, whereas the spike is usually exactly polar. Numerous crateriform surface structures and fine pili are present on the globose end of the cell. The fine structure is that of a typical bacterium, except for the rather thin cell envelope, the crateriform surface structures, and the spike and its basal attachment site. Resistant to beta-lactam antibiotics. Occur in rosettes, consisting of as many as 12 cells attached via holdfasts at the narrower ends of the several cells. Neither motility nor flagella have been observed. Common in eutrophic freshwater habitats, usually accompanying phototrophs (algae and cyanobacteria). Has not been cultivated axenically, although blooms occur in laboratory enrichments. The type material consists of the present description and illustrations (Ps in Fig. 1; Fig. 3 made from Hortobágyi's preserved type material; and Fig. 4; see also Fig. 2 of reference 18 and Fig. 2 of reference 20). The type locality is a eutrophic man-made pond in a residential district (The Lakes) in Tempe, Ariz. Frequently confused with Planctomyces stranskae (ex Wawrik 1952) sp. nov., nom. rev., from which it can be distinguished on the basis of cellular size and shape, and the absence of numerous multifibrillar bristles in P. guttaeformis and its absence in P. stranskae, and the presence of numerous multifibrillar bristles in P. guttaeformis and its presence in P. stranskae.

Distinctions between P. stranskae and P. guttaeformis. F. Wawrik (personal communication) has maintained quite firmly that the two organisms are identical and, hence, that Planctomyces guttaeformis (first described in 1965) is a later synonym of Planctomyces stranskae (first described in 1952). Before our recognition of morphotype Vb in the Arizona material, we would have accepted her opinion. However, these two budding bacteria are discernibly different in the following respects. (i) P. guttaeformis (morphotype Va) invariably has a prominent...
FIG. 4. Morphology and fine structure of morphotype Va, Planctomyces guttaeformis (ex Hortobágyi 1965) sp. nov., nom. rev. (A) Five-membered rosette in material from a pond near the Budapest railroad bridge, with one cell bearing a smaller, mirror-image daughter cell, a bud (Bu). Each of the five mature cells bears a prominent multifibrillar spike (sp) extending outward from the globose end of the cell. Negative contrast; 1.0% uranyl acetate stain. ×8,580. (B) Budding cell of morphotype Va in material from a pond near the Budapest railroad bridge, showing the crateriform surface structures (arrowheads) and fine pili (p) on the globose end of the cell. The bud (Bu) is slightly off-center with respect to the longitudinal axis of the parent cell, whereas the spike is centrally located. Negative contrast; 1.0% uranyl acetate stain. ×26,000. (C) Thin section of a morphotype Va cell in material from the pond in The Lakes, Tempe, Ariz., showing typical bacterial organization, the thin cell envelope, and the basal attachment site (arrowhead) of the spike protruding from the globose end of the cell (cf. with Fig. 5D). The fixation method used was that described by Burdett and Murray (2). ×48,600.
FIG. 5. Morphology and fine structure of morphotype Vb, *Planctomyces stranskii* (ex Wawrik 1952) sp. nov., nom. rev., in material from the pond in The Lakes, Tempe, Ariz. (A) Mature cell, showing a young bud (Bu) and numerous multifibrillar bristles (br). Negative contrast; 1.0% uranyl acetate stain. ×27,000. (B) Budding cell of morphotype Vb with mirror-image bud (Bu) at a later stage of development than in (A); note the prominent bristles (br). Negative contrast; 1.0% uranyl acetate stain. ×19,375. (C) Six-membered rosette of morphotype Vb. No cell has a spike, but all have prominent bristles (br). Negative contrast; 1.0% uranyl acetate stain. ×11,480. (D) Thin section of a morphotype Vb cell showing its bacterial ultrastructure; no spike can be seen (cf. with Fig. 4C). The fixation method used was that described by Burdett and Murray (2). ×51,300.
The cellular shape of \textit{P. stranskae}; in contrast, the cellular shape of \textit{P. guttaeformis} usually is a sphere merging into a cylinder fairly uniform in diameter. (iii) In natural material, \textit{P. stranskae} cells are larger than \textit{P. guttaeformis} cells. Cell size is dependent upon nutrient conditions and the age of samples or enrichments, but the dimensions given above are typical for organisms observed in natural samples. (iv) \textit{P. guttaeformis} has prominent pili; in \textit{P. stranskae}, the presence of pili is suspected (although the situation is confused by the plethora of bristles and fibrils shed from them); in any case, piliation of \textit{P. stranskae} is not the prominent feature it is in \textit{P. guttaeformis}.

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LITERATURE CITED


FIG. 6. Some details of morphotype Vb, \textit{Planctomyces stranskae} (ex Wawrik 1952) sp. nov., nom. rev., in material from the pond in the Lakes, Tempe, Ariz. (A) Numerous multifibrillar bristles (br) extend from the globose end of the cell. Multiple crateriform surface structures (arrowheads) are discernible over the globose end of the cell. Negative contrast; 1.0% uranyl acetate stain. ×68,000. (B) Phase-contrast (light) micrograph of a rosette, showing the association of numerous bacteria with the bristles of \textit{P. stranskae}; such associations are particularly evident in preparations made directly from natural samples.


