Isosphaera pallida, gen. and comb. nov., a gliding, budding eubacterium from hot springs

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Abstract. An unusual filamentous, budding bacterium was isolated from several North American hot springs and named Isosphaera pallida. Filaments are composed of spherical cells 2.5–3.0 μm in diameter, with cell growth and division occurring by formation of intercalary buds. These obligately aerobic, heterotrophic isolates closely resemble Isocystis pallida Woronichin, which has been previously described as a cyanobacterium, and later as a yeast, based on collected specimens.

Isolates were salmon-colored due to the presence of carotenoids and contained gas vesicles. Growth occurred at temperatures up to 55 °C in defined media using 0.025% glucose or lactate as carbon sources. Glucose concentrations of 0.05% or higher inhibited growth of the culture.

Ultrathin sections observed by TEM revealed an unusual tri-laminar wall structure. Pit-like ultrastructural features were found in the cell wall. Growth of cultures was not inhibited by penicillin G, and the Gram reaction gave variable results.

Cells formed motile, macroscopic aggregates ("comets") when harvested from liquid cultures and plated on media containing Gelrite (Kelco Co.) as a solidifying agent. Aggregation and motility were observed in both the light and the dark. However, comets were strongly phototactic. Negative stains revealed numerous pili, but not flagella.

We propose that this highly unusual prokaryote be placed in a new genus.

Key words: Isosphaera pallida - Thermophile - Hot spring - Phototaxis - Gliding motility - Gas vesicles - Budding - Cell wall

In addition to its unusual morphology, Isosphaera pallida possesses a combination of unusual phenotypic traits which has not been seen in other prokaryotes. It is the only budding microorganism known to move by "gliding", and it is the only heterotrophic prokaryote known to be phototactic. Muramic acid and dianaminopimelic acid, essential components of peptidoglycan, are absent from the cell wall of Isosphaera pallida (Giovannoni, unpublished work).

Phylogenetic analyses based on sequence comparisons of SS rRNAs (Bomar, unpublished work) and 16S rRNAs (Giovannoni, unpublished work) have demonstrated that Isosphaera pallida is related to members of the Planctomyces group of budding bacteria, which also lack peptidoglycan walls (König et al. 1984).

Here we present the results of initial studies on the ultrastructure and physiology of this organism.

Materials and methods

Isolation and culture conditions. Unless otherwise stated, strain IS1B, isolated from Kah-nee-tah Hot Springs in Oregon, was used in all experiments. Strains were isolated by streaking plates of medium IM containing 1.5% Bacto-Agar. Routine cultivation used medium IMC. Large batch cultures were raised in 100 ml "bubbler" vessels (Nelson and Castenholz 1982, for a description). Bubbler cultures were sparged with 5% CO2/95% air. Small batch cultures (50 ml) were grown in 125 ml Erlenmeyer flasks equipped with a Hungate-type septum screw cap, and purged with 5% CO2/95% air. Cultures on plates or in tubes (shakes or slants) were incubated in Baltimore Biological Laboratories "Gas-Paks". A single "Alka-Seltzer Gold" tablet was added to a flask of water in the "Gas-Paks" before closure. One "Alka-Seltzer Gold" tablet evolves 109 ml of gaseous CO2, producing a final concentration of 5.3% CO2 in the "Gas-Pak". Unless otherwise specified, cultures were incubated at 42 °C and cells were harvested in late exponential growth phase (0.5–0.6 OD650 nm).

Medium IM was prepared as follows: 250 ml of solution A was added to 650 ml distilled water (15 g/l agar was added for plates), autoclaved, and cooled to 50 °C. 100 ml of solution B was then added to the sterile medium. Solution A contained (per liter H2O): CaCl2·2H2O 320 mg, MgSO4·7H2O 400 mg, KCl 500 mg, NaCl 1 g, (NH4)2SO4 500 mg, KH2PO4 300 mg, FeCl3 0.292 mg, micronutrient solution SL7 (Pfennig and Trüper 1981): 10 ml, vitamin B12: 0.005 mg. The solution was brought to pH 7.6 with 2 M NaOH, and filtered through Whatman no 1 filter paper to
Carbon source utilization. All carbon sources used in nutritional experiments were filter sterilized and added to medium IM following autoclaving. Either 0.5% agarose (Sigma, type I), or “Gelrite” (Kelco Co.) was used as a solidifying agent. 20 x 50 mm dishes containing 50 ml of medium were used. Carbon sources were added to a concentration of 0.025%. Control plates contained either no carbon source or 0.025% of the carbon source being tested in addition to 0.025% D-glucose and 0.025% casamino acids. One loopful of cells from a fresh plate of medium IM was resuspended in 1 ml of medium IM as an inoculum. 10 gl of source or 0.025% of the carbon source being tested in addition to 0.025% D-glucose and 0.025% casamino acids. One loopful of cells from a fresh plate of medium IM was resuspended in 1 ml of medium IM as an inoculum. 10 μl of this suspension was spotted onto plates and incubated for 2 weeks, followed by scoring.

Electron microscopy. Exponential growth phase cells were resuspended in 50 mM sodium cacodylate buffer containing 2% glutaraldehyde and fixed on ice for 1.5 h. Equal volumes of fixed cells were then added to a warm solution of 0.8% agarose in 50 mM cacodylate buffer, and drawn up in the tips of Pasteur pipettes. The agarose containing the cells was then extruded from the pipettes and fixed in 1% OsO4 for 1 h at room temperature. After osmium fixation, the agarose strands were washed in several changes of buffer, dehydrated in an ethanol series, and embedded in Epon/Araldite. Sections were cut using a Reichert OM-U2 ultramicrotome, placed on copper grids, and poststained in 1 or 2% uranyl acetate. Samples were examined and photographed using a Philips 300 transmission electron microscope. Pili were visualized by negative staining with 2% uranyl acetate or by rotary shadowing with platinum.

Oxygen uptake measurements. All O2 uptake measurements were carried out using a polarographic oxygen electrode mounted in a temperature-controlled glass cuvette equipped with a magnetic stirrer. Inhibitors were added through a glass capillary using a 100 μl Hamilton syringe.

Optical absorbance spectra. A Cary 14R spectrophotometer was used for measurement of optical absorbance. Cell-free extracts were prepared by sonicating cell suspensions in 50 mM pH 7.5 potassium phosphate buffer, followed by centrifugation at 12,000 g for 10 min to remove unbroken cells. Samples were oxidized by adding hydrogen peroxide (final concentration ca. 0.03%). “Reduced” samples were stored in a sealed Hungate tube under nitrogen. Protein was measured by the dye binding method of Bradfield (1976).

DNA base composition. DNA was purified by the method of Marmur (1961). The mole percent guanine plus cytosine base ratio was determined by buoyant density centrifugation in a cesium chloride density gradient.

Results
Isolation and cultivation. Isosphaera pallida was isolated from hot springs by streaking samples directly onto agar plates of medium IM and incubating the plates for two weeks in darkness at 45°C. After incubation Isosphaera pallida colonies were usually the most conspicuous on the plate. Since medium IM contains no organic constituents except vitamin B12, growth of Isosphaera pallida under these conditions appeared to be occurring on organic contaminants of agar. Isolation was accomplished by re-streaking, followed by transfer to medium IM for routine cultivation.

A list of strains is shown in Table 1. Unless otherwise noted, the experiments described here were done with strain IS1B, a variant of strain IS1 which forms short (ca. 2-6 cell) chains, rather than the usual filaments of indefinite length.

<table>
<thead>
<tr>
<th>Collection</th>
<th>Locality</th>
<th>Temperature (°C)</th>
</tr>
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<tbody>
<tr>
<td>IS1B</td>
<td>Kah-nee-tah Hot Springs, Oregon, USA</td>
<td>44</td>
</tr>
<tr>
<td>IS2</td>
<td>Painted Pool, Mammoth Hot Springs, Yellowstone Nat. Park, USA</td>
<td>40</td>
</tr>
<tr>
<td>IS4</td>
<td>“Rabbit Creek Spouter”, Midway Geyser Basin, Yellowstone Nat. Park, USA</td>
<td>35-38</td>
</tr>
<tr>
<td>IS5</td>
<td>Bath Lake, Mammoth Hot Springs, Yellowstone Nat. Park, USA</td>
<td>44</td>
</tr>
<tr>
<td>IS6</td>
<td>White Sulfur Spring, Thermopolis, Wyoming, USA</td>
<td>51</td>
</tr>
<tr>
<td>IS9</td>
<td>Big Spring, Thermopolis, Wyoming, USA</td>
<td>36</td>
</tr>
</tbody>
</table>

Colony and cell morphology. Wild-type Isosphaera pallida forms small, pink, convex colonies of firm consistency when grown on Bacto-agar. Colonies may migrate on wet agar plates or Gelrite plates. Migrating colonies are phototactic and often comet-shaped (Fig. 1). The cells are spherical in shape and from 2.5 to 3.0 μm in diameter (Fig. 2). Wild-type cells form chains of indefinite length. Cell growth and division occur by formation of intercalary buds (Fig. 2). Bud formation always occurs on the chains axis; branching was never observed. Budding begins with the formation of a small protuberance connected to the mother cell by a narrow constriction (Fig. 3a). Buds enlarge until they reach the approximate size of the mother cell, at which time “pinching off” occurs (Fig. 3b-d). Cytoplasmic connections are not visible between mature cells of a chain.

Gas vesicles are often present in recent isolates of Isosphaera pallida, but disappear during extended cultivation. When observed by phase-contrast microscopy, Isosphaera often appears to have a capsule. In fact, light