CHAPTER 18
Characterization and Identification of the Anoxygenic Phototrophic Bacteria
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Characterization and Differentiation of Higher Taxa Down to the Four Families

The anoxygenic phototrophic bacteria (Anoxyphotobacteria Gibbons and Murray 1978) perform photosynthesis with a bacteriochlorophyll (bchl) under anaerobic conditions, using reduced sulfur compounds, molecular hydrogen, or simple organic carbon compounds as electron donors. These bacteria do not use water as a photosynthetic electron donor and do not produce molecular oxygen during photosynthesis. The ability to fix molecular nitrogen occurs in nearly all species tested so far (Siefert, 1976).

The Anoxyphotobacteria comprise the two orders Rhodospirillales (Pfennig and Trüper, 1971b) and Chlorobiales (Gibbons and Murray, 1978). This division is based on clear differences in the fine structure and pigments of the photosynthetic apparatus. In the Rhodospirillales ("purple bacteria"), the entire photosynthetic apparatus is located in the cytoplasmic membrane; this membrane is usually enlarged by several types of infoldings into the cytoplasm (intracytoplasmic membrane system) and carries bchl $a$ or $b$ as the main pigment (i.e., as reaction center as well as antenna bacteriochlorophyll). In the Chlorobiales, the antenna bacteriochlorophyll (i.e., the bulk bacteriochlorophyll, consisting of bchl $c$, $d$, or $e$) is located in distinct organelles (chlorosomes), underlying and attached to the inner side of the cytoplasmic membrane. Only the reaction center bacteriochlorophyll (comparatively small amounts of bchl $a$ only) is located in the cytoplasmic membrane itself. Anoxyphotobacteria are discussed further in this Handbook, Chapter 7, and in Pfennig (1977).

The order Rhodospirillales consists of two families, the Rhodospirillaceae (Pfennig and Trüper, 1971b; "purple nonsulfur bacteria") and the Chromatiaceae (Bavendamm 1924, "purple sulfur bacteria"). The differentiation between these two families is relatively clear-cut, with the exception of the position of the genus Ectothiorhodospira (see this Handbook, Chapter 15), which so far is considered to belong to the Chromatiaceae (Trüper, 1968). All other members of the Chromatiaceae, when growing with sulfide as the electron donor, will form conspicuous globules of elemental sulfur inside the cells. Hansen (1974) clearly showed that some members of the Rhodospirillaceae are able to utilize sulfide. However, when growing with sulfide, they either will not produce elemental sulfur at all (i.e., they oxidize sulfide to higher oxidized sulfur compounds, such as sulfate or tetrathionate) or will deposit elemental sulfur outside the cells in the medium. However, they do not further oxidize elemental sulfur to sulfate in most cases.

The Rhodospirillaceae show the strongest photo-organotrophic tendency of all phototrophic bacteria; simple organic carbon compounds are the preferred phototrophic electron donors and carbon sources, in the presence or absence of carbon dioxide. Many strains will also grow photolithotrophically with molecular hydrogen, and some with hydrogen sulfide or thiosulfate. All species are microaerophilic, and many will grow at full atmospheric oxygen tension in either the dark or the light. The formation of photosynthetic pigments and intracytoplasmic membranes is regulated by the partial pressure of oxygen.

In the Chromatiaceae, facultative photoorganoheterotrophy is limited to the species capable of assimilatory sulfate reduction. The other species depend upon the presence of sulfide in the medium and, as a consequence, grow photolithoautotrophically. The DNA base ratios of the Rhodospirillaceae cover the relatively narrow range between 61 and 73 mol% G+C, while those of the Chromatiaceae cover the wide range between 45 and 71 mol% G+C (Mandel et al., 1971).

The order Chlorobiales consists of the two families Chlorobiaceae (Copeland, 1956; "green sulfur bacteria") and Chloroflexaceae (Trüper, 1976; "flexible, filamentous, gliding, green bacteria"); see this Handbook, Chapter 17). The differentiation between these two families is absolutely sharp. The Chlorobiaceae are all obligately phototrophic,
<table>
<thead>
<tr>
<th>Species</th>
<th>Cell shape, width, length (μm)</th>
<th>Intracytoplasmic membrane system</th>
<th>DNA base ratio (mol% G+C)</th>
<th>Predominant carotenoids</th>
<th>Color of cell suspension under anaerobic growth conditions</th>
<th>Ability to grow aerobically or microaerobically in the dark</th>
<th>Growth factors required</th>
<th>Type or neotype strains</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rhodocyclus purpureus</em></td>
<td>Half circle/circle 0.6–0.7 x 2.7–5</td>
<td>T</td>
<td>65.3</td>
<td>rl/rh</td>
<td>Purple-violet</td>
<td>m</td>
<td>B&lt;sub&gt;17&lt;/sub&gt;</td>
<td>DSM 168</td>
</tr>
<tr>
<td><em>Rhodomicrobium vannielli</em></td>
<td>Ovoid and stalk 1.0–1.2 x 2–2.8</td>
<td>L</td>
<td>61.8–63.8</td>
<td>sp, β&lt;sub&gt;c&lt;/sub&gt;</td>
<td>Orange-brown</td>
<td>m</td>
<td>None</td>
<td>DSM 162 ( = ATCC 17100)</td>
</tr>
<tr>
<td><em>Rhodopseudomonas acidophila</em></td>
<td>Rod 1.0–1.3 x 2–5</td>
<td>L</td>
<td>62.2–66.8</td>
<td>rh, rg, rlg</td>
<td>Purple-red or orange-brown</td>
<td>m, ae</td>
<td>None</td>
<td>DSM 137 ( = ATCC 25092)</td>
</tr>
<tr>
<td><em>capulata</em></td>
<td>Rod/sphere 0.5–1.2 x 2–2.5</td>
<td>V</td>
<td>65.5–66.8</td>
<td>sn, se</td>
<td>Yellow to brown</td>
<td>ae</td>
<td>Thiamine ± biotin ± niacin</td>
<td>ATCC 11166</td>
</tr>
<tr>
<td><em>gelatinosa</em></td>
<td>Rod 0.4–0.5 x 1–2</td>
<td>T</td>
<td>70.5–72.4</td>
<td>sn, se</td>
<td>Yellow-brown to pinkish</td>
<td>ae</td>
<td>Biotin + thiamine</td>
<td>ATCC 17011</td>
</tr>
<tr>
<td><em>globiformis</em></td>
<td>Sphere 1.6–1.8</td>
<td>V</td>
<td>66.3</td>
<td>kts</td>
<td>Purple-red</td>
<td>m</td>
<td>Biotin + pAB</td>
<td>DSM 161</td>
</tr>
<tr>
<td><em>palastris</em></td>
<td>Rod 0.6–0.9 x 1.2–2</td>
<td>L</td>
<td>64.8–66.3</td>
<td>sp, ly, rh</td>
<td>Red-brown</td>
<td>ae</td>
<td>pAB ± biotin</td>
<td>DSM 126 ( = ATCC 17001)</td>
</tr>
<tr>
<td><em>sphaeroidea</em></td>
<td>Sphere/ovoid 0.7 x 2–2.5</td>
<td>V</td>
<td>68.4–69.9</td>
<td>sn, se</td>
<td>Green-brown to brown</td>
<td>ae</td>
<td>Biotin + thiamine + niacin</td>
<td>ATCC 17023</td>
</tr>
<tr>
<td><em>sulfidophila</em></td>
<td>Rod/sphere 0.6–0.9 x 0.9–20</td>
<td>V</td>
<td>67.0–71.0</td>
<td>sn, se</td>
<td>Yellow-brown to red</td>
<td>ae</td>
<td>pAB + biotin + thiamine + niacin</td>
<td>DSM 1374</td>
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<tr>
<td><em>sulfoviridis</em></td>
<td>Rod 0.6–0.9 x 1.2–2</td>
<td>L</td>
<td>67.8–68.4</td>
<td>2H-neu (?)</td>
<td>Green</td>
<td>m</td>
<td>Yeast extract</td>
<td>DSM 729</td>
</tr>
<tr>
<td><em>viridis</em></td>
<td>Rod 0.6–0.9 x 1.2–2</td>
<td>L</td>
<td>66.3–71.4</td>
<td>2H-neu 2H-ly (?</td>
<td>Green</td>
<td>m</td>
<td>pAB + biotin</td>
<td>DSM 133 ( = ATCC 19567)</td>
</tr>
<tr>
<td><em>Rhodospirillum fulvum</em></td>
<td>Spiral 0.5–0.7 x 3.5</td>
<td>S</td>
<td>64.3–65.3</td>
<td>ly, rh</td>
<td>Brown</td>
<td>m</td>
<td>pAB</td>
<td>DSM 113 ( = ATCC 157980)</td>
</tr>
<tr>
<td><em>molischianum</em></td>
<td>Spiral 0.7–1.0 x 5–8</td>
<td>S</td>
<td>61.7–64.8</td>
<td>ly, rh</td>
<td>Brown</td>
<td>m</td>
<td>Amino acids</td>
<td>DSM 120 ( = ATCC 14031)</td>
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<tr>
<td><em>photometricum</em></td>
<td>Spiral 1.2–1.5 x 7–10</td>
<td>S</td>
<td>65.8</td>
<td>ly, rh</td>
<td>Brown</td>
<td>m</td>
<td>Yeast extract</td>
<td>DSM 122</td>
</tr>
<tr>
<td><em>rubrum</em></td>
<td>Spiral 0.8–1.0 x 7–10</td>
<td>V</td>
<td>63.8–65.8</td>
<td>sp</td>
<td>Red</td>
<td>ae</td>
<td>Biotin</td>
<td>DSM 467 ( = ATCC 11170)</td>
</tr>
<tr>
<td><em>tenuis</em></td>
<td>Spiral 0.3–0.5 x 3–6</td>
<td>T</td>
<td>64.8</td>
<td>ly, rh, rl</td>
<td>Purple-violet or brown-orange</td>
<td>ae</td>
<td>None</td>
<td>DSM 109 ( = ATCC 25093)</td>
</tr>
</tbody>
</table>

*aL, Lamellae; S, lammelar stacks; T, tubes; V, vesicles. £βc, β–Carotene; 2H–ly, 1,2 dihydrolycopene; 2H–neu, 1,2-dihydro-neurosporene; kts, diketo–tetrahydrospirilloxanthin; ly, lycopene; rg, rhodopin glucoside; rh, rhodopin; rl, rhodopinal; rlg, rhodopinal glucoside; se, spheroidene; sn, spheroidenone; sp, spirilloxanthin. cae, Aerobic; m, microaerophilic.