Poly-β-hydroxybutyrate (PHB), a polymer discovered by Lemoigne in *Bacillus megaterium* (Lemoigne, 1926), occurs in a variety of bacteria, among them many species of aerobic pseudomonads (Stanier et al., 1966). Granules of the polymer accumulate preferably during growth in media of high C/N ratio and can be observed best under phase-contrast optics after staining with Sudan Black.

PHB was detected in aerobic pseudomonads of the genera *Burkholderia* and *Ralstonia* (group II), the family Comamonadaceae (group III) and genus *Brevundimonas* (group IV) (Palleroni et al., 1973; Palleroni, 1993; Kersters et al., 1996). Most strains of *Pseudomonas* and those of group V (*Xanthomonas* and *Stenotrophomonas*) do not synthesize PHB, which confirms the close positions of these organisms in the γ-Proteobacteria. Among the exceptions are some weak accumulator strains of *Pseudomonas pseudoalcaligenes* (Stanier et al., 1966), strain DSM 50338 of *Pseudomonas viridiflava* (Timm & Steinhübel, 1990), *Pseudomonas corrugata* (Scarlett et al., 1978) and *Pseudomonas ficiuserectae* (Goto, 1983).

In the 1960s and 1970s, synthesis of medium chain length poly-β-hydroxyalkanoates (mcl-PHAs) by bacteria was reported, but the evidence presented failed to elicit much interest (Steinhübel & Valentin, 1995). The situation changed in the following decade and PHAs composed of a large variety of monomeric units were described. At least 92 different monomers are now known to be present in natural PHAs (Steinhübel & Valentin, 1995; He et al., 1998).

As with PHB, accumulation of mcl-PHAs can be elicited in media of high C/N ratio and the polymer also can be clearly seen under phase-contrast after staining with Sudan Black. Granules of PHB and PHA cannot be differentiated by simple observation, and the identification may involve either GC analysis (Timm & Steinhübel, 1990) or a solubility test [mcl-PHAs are soluble in acetone, whereas PHB is not (Abe et al., 1994)].

The first report of mcl-PHA formation by a *Pseudomonas* species, *Pseudomonas oleovorans*, described the production of a polymer containing 3-OH-octanoate by assimilation of medium and long chain length fatty acids (de Smet et al., 1983). This capacity is also expressed by growth on alkanes (Lageveen et al., 1988), but not on carbohydrates (Timm & Steinhübel, 1990).

Further studies established that synthesis of PHAs is a common feature of fluorescent pseudomonads (Huisman et al., 1989; Timm & Steinhübel, 1990), which suggested the possibility of using this characteristic for taxonomic purposes (Huisman et al., 1989). In contrast to *P. oleovorans* and another fluorescent species, *Pseudomonas resinovorans*, *Pseudomonas aeruginosa* PAO1 and *Pseudomonas putida* KT2442 and *Pseudomonas* sp. NCIMB 40135 can synthesize PHAs from carbohydrates (Timm & Steinhübel, 1990; Haywood et al., 1990; Ramsay et al., 1992).

**Keywords:** poly-β-hydroxybutyrate, poly-β-hydroxyalkanoates, *Pseudomonas* taxonomy
Table 1. PHAs synthesized by Pseudomonas corrugata and Pseudomonas ficuserectae at the expense of two substrates

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate</th>
<th>CDW (% PHA)</th>
<th>C6</th>
<th>C8</th>
<th>C10</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. corrugata</td>
<td>Octanoate</td>
<td>0.9</td>
<td>61</td>
<td>15</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>0.5</td>
<td>14</td>
<td>4</td>
<td>29</td>
</tr>
<tr>
<td>P. ficuserectae</td>
<td>Octanoate</td>
<td>0.17</td>
<td>3</td>
<td>25</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>0.31</td>
<td>0.4</td>
<td>ND</td>
<td>14</td>
</tr>
</tbody>
</table>

Abbreviations: CDW, cell dry weight (g l⁻¹) determined by the absorbance at 450 nm (Witholt, 1972); % PHA, percentage weight per cell dry weight (Lageveen et al., 1988); C6, 3-OH-hexanoate; C8, 3-OH-octanoate; C10, 3-OH-decanoate; ND, not detectable.

Whereas the formation of endocellular PHB granules by P. pseudoalcaligenes has been confirmed by identification of the monomer 3-OH-butyrate (Timm & Steinbüchel, 1990), such confirmation is still lacking for the reserve material of P. corrugata and P. ficuserectae. Consequently, the reported capacity for PHB biosynthesis by strains of the two species was re-examined in this study.

P. pseudoalcaligenes strain LMG 1225ᵀ (included as control), P. corrugata strain LMG 2172ᵀ and P. ficuserectae strain LMG 5694ᵀ were grown in shake flasks in nitrogen-limited minimal medium 0.1N E2 (Huisman et al., 1989). For P. corrugata and P. ficuserectae, the medium was supplemented with either 14 mM octanoate or 0.8% glucose (final concentrations). P. pseudoalcaligenes was grown on 14 mM octanoate. The cells of P. corrugata and P. pseudoalcaligenes were harvested after 52 h incubation at 28 °C on a rotary shaker; those of P. ficuserectae were harvested after 92 h under the same conditions. Cell preparations were stained with Sudan Black (Schaad, 1988) and observed under phase-contrast microscopy for the detection of polymer granules.

The cellular PHA content and composition were determined using a GC 8000 (Fisons) equipped with a...
PHB and PHA in *Pseudomonas* taxonomy


