

We therefore propose the photometabolism of benzoate by *Rhodospseudomonas palustris* occurs by the following pathway:

Benzoate → hydrogenated benzoate derivatives → cyclohexanol-2-carboxylate → [cyclohexanone-2-carboxylate] → pimelate.

Further metabolism may be accomplished by β -oxidation.

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The Extracellular Agarase from a *Cytophaga* Species

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A *Cytophaga* species (N.C.M.B. 1327), previously shown to produce intracellular enzymes that cause breakdown of the polysaccharides agarose and porphyran (Turvey & Christison, 1967*a,b*), was used as the source of an extracellular enzyme with similar activity. When the bacterial cells were removed from cultures at the exponential phase of growth, the cell-free supernatant solution had enzyme activity. The enzyme was isolated by precipitation with ammonium sulphate followed by chromatography on DEAE-Sephadex A-50 (OH⁻ form). The latter step was necessary to free the enzyme preparation from associated polysaccharides.

The purified enzyme differed from that obtained by ultrasonic disintegration of the bacterial cells in that it appeared to be a single enzyme. The ratio of its activity on porphyran measured viscometrically to that measured by production of reducing power (Turvey & Christison, 1967*a,b*) was 25:1 compared with the corresponding ratio 1:9 found for the intracellular enzyme preparation. The extracellular enzyme is thus seen to be an endo-enzyme, producing rapid depolymerization of the substrate without production of low-molecular-weight sugars. This type of activity was confirmed by studying the products of its action on porphyran. The major products were oligosaccharides with only small amounts of the disaccharide, neoagarobiose, and no monosaccharides, compared with the action of the intracellular enzyme, which produced some monosaccharides.

The enzyme displays greatest activity to (a) agarose, with decreasing activity to (b) alkali-treated porphyran (Rees, 1961), (c) porphyran, (d) the

alkali-treated galactan sulphate of *Laurencia pinnatifida*, and (e) the native galactan sulphate of *L. pinnatifida* (Bowker & Turvey, 1968*a,b*), in that order. This is consistent with a specificity for substrates containing an alternating sequence of 3-linked D-galactose and 4-linked 3,6-anhydro-L-galactose units, as in agarose. Increasing departure from this type of structure results in lowering of the extent of enzyme action.

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Metabolism of Monofluorobenzoates by Bacterium N.C.I.B. 8250

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Ali, Cally & Hayes (1962) showed that bacterium N.C.I.B. 8250 oxidized *m*- and *p*-fluorobenzoate after growth on benzoate. Cally & Jones (1965) subsequently demonstrated that *o*- and *p*-fluorobenzoate, and possibly *m*-fluorobenzoate, were inducers for the benzoate-oxidizing system in this organism. We have confirmed these observations in the course of investigations into the metabolism of aromatic compounds by bacterium N.C.I.B. 8250 (Kennedy & Fewson, 1968) and have now examined the metabolic fates of the monofluorobenzoates.

None of the monofluorobenzoates was utilized as sole source of carbon and energy for growth of bacterium N.C.I.B. 8250. Cell yield on limiting benzoate, salicylate or succinate, however, was increased by suitable concentrations of *o*-fluorobenzoate. Yield in the presence of *m*-fluorobenzoate was slightly augmented but the appearance of brown material in the medium made estimates of growth difficult. *p*-Fluorobenzoate generally decreased the yield on other carbon sources but was itself metabolized. Both *o*-fluorobenzoate and, to a smaller extent, *m*-fluorobenzoate appeared to be incorporated into cell material since the specific radioactivity of cells grown on [*ring*-U-¹⁴C]-benzoate was decreased in the presence of these two compounds.

Examination of the medium during growth on benzoate + *o*-fluorobenzoate showed the transitory appearance of a compound reacting positively in the

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