reductase activity would be greater in β -hydroxybutyrate-grown cells than in ethanol-grown cells. Because hydroxypyruvate reductase and serine-glyoxylate aminotransferase are co-ordinately regulated (Dunstan *et al.*, 1972b) the activities of both enzymes should be higher, although only the first is likely to be essential for growth on β -hydroxybutyrate. Measurement of the two enzyme activities has confirmed this prediction; the specific activities ($\mu\text{mol/h}$ per mg of protein) of hydroxypyruvate reductase and serine-glyoxylate aminotransferase were 39 and 1.8 respectively in β -hydroxybutyrate-grown organisms.

The present results indicate that malonate and β -hydroxybutyrate are assimilated in *Pseudomonas* AM1 by the novel pathway previously shown to operate during growth on ethanol. This route presumably also provides a means of generating precursors for biosynthesis from the endogenous reserves of poly- β -hydroxybutyrate, which constitute up to 8% of the dry weight of these organisms.

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Dunstan, P. M., Anthony, C. & Drabble, W. T. (1972a) *Biochem. J.* **128**, 99–106

Dunstan, P. M., Anthony, C. & Drabble, W. T. (1972b) *Biochem. J.* **128**, 107–115

Microbial Growth on C_1 and C_2 Compounds: the Metabolism of Acetate to Glycine in *Pseudomonas* AM1

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Pseudomonas AM1 is a pink bacterium capable of growth on C_1 compounds. These substrates are assimilated by condensation of 5,10-methylenetetrahydrofolate with glycine to give serine, which is converted into glycolytic intermediates (Large & Quayle, 1963). To maintain the operation of this pathway glyoxylate must be synthesized from C_1 units and then transaminated to glycine (Harder & Quayle, 1971). Evidence has been presented that acetate and glycollate are the precursors of glyoxylate (Dunstan *et al.*, 1972b). The enzymes converting acetate into glyoxylate also operate during growth on C_2 compounds. In this case glyoxylate is not transaminated to glycine but condenses with acetyl-CoA to give malate (Dunstan *et al.*, 1972b).

Consideration of the observations presented above indicates that glycine should be an early product of [^{14}C]acetate metabolism by methanol-grown cells containing induced activities of the specific serine-glyoxylate aminotransferase. This prediction was

tested by incubating methanol-grown organisms with [^{14}C]acetate; samples were removed at various time-intervals and radioactive compounds were separated and identified as described by Dunstan *et al.* (1972a). From these results the proportions of radioactivity in non-volatile metabolic intermediates at each sampling time were determined. The proportion of radioactivity in glutamate was 4.5% at the first sample time (7s) and increased during the course of the experiment. The proportion of radioactivity in malate increased from 15 to 23% (at 30s), then decreased. A similar pattern of incorporation was measured for succinate+fumarate (which were not distinguished), but the proportion of radioactivity in these compounds was about one-quarter of that detected in malate. The only compounds that appeared to be early intermediates (i.e. those for which the proportion of radioactivity was constant or decreased from the earliest times) were glycine (6% in the first sample) and an unknown substance (compound 'B') (15% in the first sample). This compound 'B' has been previously implicated in the metabolism of acetate by *Pseudomonas* AM1. These labelling patterns contrast with those obtained with ethanol-grown organisms, where the early intermediates in the metabolism of acetate were glycollate, malate and compound 'B'.

In summary, the results presented here confirm that enzymes capable of rapid metabolism of acetate to glycine are present during growth of *Pseudomonas* AM1 on methanol.

We thank the Science Research Council for financial assistance.

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The Microbial Metabolism of Nitrilotriacetate

By R. E. CRIPPS and A. S. NOBLE (*Borden Microbiological Laboratory, Shell Research Ltd., Sittingbourne, Kent, U.K.*)

An organism that is capable of utilizing nitrilotriacetate as sole source of carbon and nitrogen for growth was isolated by elective culture and tentatively identified as a pseudomonad. The generation time on this substrate was 6h. Unlike a previously described pseudomonad (Focht & Joseph, 1971), the present organism grew more rapidly on nitrilotriacetate than on its possible metabolites iminodiacetate and glycine, and washed suspensions of nitrilotriacetate-grown cells showed very little ability

to oxidize iminodiacetate and glycine. The ability to oxidize nitrilotriacetate was inducible; cells grown on acetate showing tenfold less activity.

Cell-free extracts were able to catalyse the degradation of nitrilotriacetate. This activity was assayed by the release of $^{14}\text{CO}_2$ from nitrilotri[1- ^{14}C]acetate and was dependent on the presence of thiamin pyrophosphate, Mg^{2+} and NADH. NADPH was not an effective substitute for NADH. The CO_2 produced in this assay was derived solely from C-1 of nitrilotriacetate, as no release of $^{14}\text{CO}_2$ occurred when nitrilotri[2- ^{14}C]acetate was used. In addition, no $^{14}\text{CO}_2$ was produced from nitrilotri[1- ^{14}C]acetate in incubations under anaerobic conditions. The formation of glyoxalate from nitrilotriacetate was demonstrated in incubation mixtures containing phenylhydrazine, when glyoxalate phenylhydrazone accumulated. The formation of this material was dependent on the presence of NADH. The formation of glycine from nitrilotriacetate was shown in incubation mixtures containing nitrilotri[1- ^{14}C]acetate, NADH and cell-free extract to which non-labelled glycine was added. Under these conditions radiolabel appeared in the glycine.

Cell-free extracts were examined for the presence of enzymes capable of transforming glyoxalate and glycine. When supplemented with thiamin pyrophosphate, Mg^{2+} and NADH, extracts of nitrilotriacetate-grown cells catalysed the anaerobic conversion of glyoxalate into glycerate. In this process 0.5 mol of CO_2 was produced/mol of glyoxalate utilized. When NADH was omitted, a product accumulated whose dinitrophenylhydrazone possessed the spectral properties of the tartronic semialdehyde derivative (Kornberg & Gotto, 1961). This product was metabolized rapidly by extracts in the presence of NADH. Hence the presence of glyoxalate carboligase and tartronic semialdehyde reductase was inferred. The former enzyme was induced by growth on nitrilotriacetate. In addition, nitrilotriacetate-grown cells possessed increased activities of serine hydroxymethyltransferase and hydroxypyruvate reductase, indicating the presence of a metabolic sequence for the conversion of glycine into glycerate. Attempts to demonstrate enzymes capable of the interconversion of glycine and glyoxalate were unsuccessful.

These results indicate a degradatory pathway in which nitrilotriacetate is converted into glyoxalate and glycine. The requirements of NADH and O_2 for this process suggest that a mixed-function oxidase may be involved (Large, 1971). The release of CO_2 from C-1 of nitrilotriacetate and the cofactors required are in accordance with the further metabolism of glyoxalate, by the glyoxalate carboligase reaction, to tartronic semialdehyde and then glycerate. Glycine is apparently also metabolized to glycerate by a separate sequence of reactions.

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Metabolism of Piperonylate by *Pseudomonas fluorescens*

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Methylenedioxyphenyl compounds, important commercially as insecticide synergists, are demethylated by NADPH-dependent mono-oxygenases in rat liver microsomal fraction (Kamiński & Casida, 1970), although cleavage of the methylene bridge was not the major metabolic route of piperonylate.

Cell-free extracts of *Pseudomonas fluorescens* strain PM 3 grown on anisate as the sole carbon source were previously shown to metabolize piperonylate (3,4-methylenedioxybenzoate) as well as the methoxylated compound (Buswell & Mahmood, 1972*a,b*). Oxidative attack to yield protocatchuate and formate involved absorption of 1 mol of O_2 /mol of piperonylate and required NADH as cofactor. Mono-oxygenase activity was present in the soluble fraction (164000g for 3h) and was stable for several months when extracts were stored at -20°C . The data indicate that the methylene carbon is hydroxylated to the unstable hydroxymethylenedioxy derivative, which undergoes hydrolysis to formate and the dihydroxy acid.

Protocatchuate, confirmed as an intermediate on the breakdown pathway by chemical isolation from enzyme-piperonylate incubations and identification by m.p. and by i.r. and u.v. spectrophotometry, was further metabolized via intradiol ring fission to 3-oxoadipate.

Piperonylate mono-oxygenase and 4-O-demethylase activity in extracts of anisate-grown cells was probably catalysed by the same enzyme because: (i) thermal deactivation at 45°C led to a correlated decrease in activity on each substrate; (ii) the presence of inhibitors resulted in equal inhibition of both activities; (iii) addition of either 4-methoxybenzoate or piperonylate to the assay mixture did not increase the rate of reaction when the other substrate was present alone at saturating concentrations; (iv) repression produced by succinate in the growth medium was identical for both activities.

This oxidative attack appears similar to the mono-oxygenase activity towards safrole and dihydro-safrole found by Harrison & Ribbons (1971) in several *Pseudomonas* strains grown on these substrates, although soluble enzyme systems from piperonylate-grown cells were reported not to catalyse the oxygenation of the methylene bridge.