sequence information may be obtained by using mass spectrometry.

We thank Dr. K. Brew for advice on α -lactalbumin, Drs. D. H. Williams and H. R. Morris for assistance and mass-spectrometric facilities at the University of Cambridge and the Science Research Council and Muscular Dystrophy Association of America for financial support.

Biemann, K., Gapp, F. & Seibl, J. (1959) *J. Amer. Chem. Soc.* **81**, 2274

Brew, K., Castellino, F. J., Vanaman, T. C. & Hill, R. L. (1970) *J. Biol. Chem.* **245**, 4570

Morris, H. R. (1970) Ph.D. Thesis, University of Leeds

Patterns of Microbial Threonine Catabolism: a Survey

By S. C. BELL, J. M. TURNER, JENNIFER COLLINS and T. R. G. GRAY (*Departments of Biochemistry and Botany, University of Liverpool, Liverpool L69 3BX, U.K.*)

A total of 75 micro-organisms capable of growth on L-threonine as the major source of carbon and nitrogen has been isolated from soils and other materials. A survey of catabolic routes has included growth, micro-respirometric and enzymic measurements.

The occurrence of L-threonine aldolase in extracts of threonine-grown isolates (Morris, 1969) was rare (six cases). L-Threonine dehydrogenase (Neuberger & Tait, 1960), the synthesis of which was induced (specific activities up to 2000 nmol/min per mg of protein), predominated in most cases (46). Sixteen isolates possessed enzymes acting on threonine that were either of low activity (less than 50 nmol/min per mg of protein) or undetectable when assayed under standard conditions. A fourth group of four isolates possessed only low threonine dehydrogenase activity but relatively high L-threonine dehydratase (deaminating) activity (Umbarger & Brown, 1957), which was constitutive. Another group (three isolates) possessed both threonine dehydrogenase and threonine dehydratase activities.

Classification of isolates was carried out by using numerical taxonomic procedures (Sokal & Sneath, 1963), and they were identified by standard diagnostic testing methods. To date, genus names have been assigned to the one-third of the isolates examined in detail. The largest group of strains was made up mostly of *Brevibacterium* spp., which possessed high threonine dehydrogenase activity after growth on L-threonine. Named strains, e.g. *Brevibacterium divaricatum* N.C.I.B. 9379 and *Brevibacterium linens* N.C.I.B. 8546, also grew well on threonine medium supplemented with 0.05% of yeast extract, and possessed high threonine dehydrogenase activity. Other

isolates in the same group included *Achromobacter* and *Flavobacterium* species.

Isolates with apparently low enzyme activities towards L-threonine, suspected to utilize novel routes of threonine catabolism or possess enzymes with atypical properties, included *Pseudomonas*, *Alcaligenes* and perhaps *Corynebacterium* species.

The further metabolism of the C₂ fragments of threonine catabolism catalysed by threonine aldolase or by threonine dehydrogenase plus 2-amino-3-oxobutyrate-CoA ligase (McGilvray & Morris, 1969; Blackmore & Turner, 1971) was also studied. Measurements of glyoxylate carboligase and serine dehydratase activities, diagnostic of routes for glycine catabolism via the glycerate pathway and the serine pathway respectively, permitted further subdivision of the isolates. One isolate, metabolizing threonine via 2-oxobutyrate and propionate, appeared to employ a constitutive 'biosynthetic' threonine dehydratase (Umbarger & Brown, 1957) to catalyse the initial step. Preliminary results of the survey show strong correlation between classifications according to classical taxonomic procedures on the one hand and enzymic analysis of catabolic pathways on the other.

Blackmore, M. A. & Turner, J. M. (1971) *J. Gen. Microbiol.* **67**, 243

McGilvray, D. & Morris, J. G. (1969) *Biochem. J.* **112**, 657

Morris, J. G. (1969) *Biochem. J.* **115**, 603

Neuberger, A. & Tait, G. H. (1960) *Biochim. Biophys. Acta* **41**, 164

Sokal, R. R. & Sneath, P. H. A. (1963) *Principles of Numerical Taxonomy*, Freeman, San Francisco and London

Umbarger, H. E. & Brown, B. (1957) *J. Bacteriol.* **73**, 105

Utilization of Pairs of Substrates by Bacterium N.C.I.B. 8250 Growing in Batch Culture

By A. M. COOK and C. A. FEWSON (*Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, U.K.*)

The induction and co-ordinacy of the enzymes involved in mandelate oxidation by bacterium N.C.I.B. 8250 have been the subject of previous investigations (Kennedy & Fewson, 1968a,b; Livingstone & Fewson, 1971). We have now used the patterns of growth on pairs of substrates as indications of other aspects of control such as repression and permeation.

Diauxic growth was observed when cells previously grown on benzoate were inoculated into benzoate+mandelate-salts medium. Benzoate was utilized first, and mandelate oxidation started only after the disappearance of benzoate. Inoculation of cells

grown on mandelate into benzoate+mandelate-salts medium gave rapid growth in two distinct phases. In this case the utilization of mandelate was immediate but arithmetic, and accelerated only after exhaustion of the benzoate. When benzoate was added to a culture growing in mandelate-salts medium, the utilization of mandelate became arithmetic but was not inhibited. Utilization of mandelate during growth was also suppressed by succinate or catechol. No substantial feedback inhibition was observed of mandelate oxidation in washed-cell suspensions or of enzyme activities in cell-free preparations. Benzoate repressed the synthesis of L-mandelate dehydrogenase in a mutant strain devoid of benzoate oxidase activity. It seems likely therefore that the mandelate pathway of bacterium N.C.I.B. 8250 is subject to multi-sensitive end-product repression of the type observed in *Pseudomonas putida* (Mandelstam & Jacoby, 1965; Stevenson & Mandelstam, 1965).

Growth in benzyl alcohol+benzoate-salts medium gave a different pattern of results. If the inoculum was grown on benzoate then diauxie was observed, with benzoate utilized before the benzyl alcohol. If, however, the inoculum was grown on benzyl alcohol there was no diauxie and utilization of both substrates was exponential. By analogy with a similar situation in *Pseudomonas aeruginosa* growing on glucose+citrate (Hamilton & Dawes, 1961; Hamlin *et al.*, 1967), the different behaviour in benzoate+benzyl alcohol-salts medium depending on the nature of the inoculum suggests the possibility of a specific and inducible transport mechanism for benzyl alcohol in this organism

This work was supported in part by a grant from the Medical Research Council, and A. M. C. acknowledges the award of a Science Research Council Studentship.

- Hamilton, W. A. & Dawes, E. A. (1961) *Biochem. J.* **79**, 25 P
 Hamlin, B. T., Ng, F. M.-W. & Dawes, E. A. (1967) in *Microbial Physiology and Continuous Culture* (Powell, E. O., Evans, C. G. T., Strange, R. E. & Tempest, D. W., eds.), p. 211, H.M.S.O., London
 Kennedy, S. I. T. & Fewson, C. A. (1968a) *J. Gen. Microbiol.* **53**, 259
 Kennedy, S. I. T. & Fewson, C. A. (1968b) *Biochem. J.* **107**, 497
 Livingstone, A. & Fewson, C. A. (1971) *Biochem. J.* **121**, 8 P
 Mandelstam, J. & Jacoby, G. A. (1965) *Biochem. J.* **94**, 569
 Stevenson, I. L. & Mandelstam, J. (1965) *Biochem. J.* **96**, 354

Possible Transport Mechanisms for Mandelate and Benzoate in Bacterium N.C.I.B. 8250

By A. M. COOK and C. A. FEWSON (*Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, U.K.*)

Relatively little work has been done on the mechanism of entry of non-amino aromatic compounds into bacterial cells. Hegeman (1966) found no evidence for a permease for mandelate in *Pseudomonas putida*. We have developed an assay for measuring pools of certain aromatic compounds in cells of bacterium N.C.I.B. 8250, and have obtained evidence to indicate that benzoate and mandelate enter this organism by facilitated diffusion.

Cell suspensions containing carboxy-¹⁴C-labelled substrates were incubated at 30°C with aeration. Samples were taken at intervals, washed on filters under rigorously defined conditions and their radioactivities counted by liquid-scintillation spectrometry. Corrections were made for interstitial and bound material, and the concentrations of intracellular material were calculated.

Various degrees of permeability barrier to mandelate were observed in cells of bacterium N.C.I.B. 8250 that had been grown under conditions that did not induce the enzymes for mandelate oxidation. For instance, mandelate entered cells grown in nutrient broth at a low but detectable rate and entered cells grown on benzyl alcohol even more slowly. No pool of mandelate could be detected in cells of bacterium N.C.I.B. 8250 induced to, and incubated in the presence of, mandelate. With a mutant strain lacking L-mandelate dehydrogenase and grown in the presence of phenylglyoxylate (Livingstone & Fewson, 1971), however, the intracellular concentration of mandelate rapidly reached the extracellular concentration.

No permeability barrier to benzoate could be detected in non-induced cells, but evidence for facilitated diffusion was obtained from exit counterflow experiments (see, e.g., Wong & Wilson, 1970). Salicylate was not decarboxylated by cells that had been grown on benzoate, but did rapidly enter these cells to attain and maintain the extracellular concentration. The addition of benzoate to cells in suspension and containing salicylate caused an exit of salicylate against a concentration gradient. Further, the decarboxylation of 4-fluorobenzoate was prevented by benzoate. Presumably this interaction occurred at the cell membrane, since no 4-fluorobenzoate could be detected inside the cells.

This work was supported in part by a grant from the Medical Research Council, and A. M. C. acknowledges the award of a Science Research Council Studentship.