The Growth and Metabolic Versatility of the Gram-negative Bacterium NCIB 8250 ('Vibrio 01')

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SUMMARY

The nutritional requirements of the Gram-negative Bacterium NCIB 8250 (formerly referred to as 'Vibrio 01') have been determined. Growth was tested on almost 450 compounds, of which over 100 served as carbon+energy sources and almost 50 as nitrogen sources. The effects of various factors such as pH value, temperature and aeration on the growth rate and cell yield of organism have also been measured.

INTRODUCTION

Until recently the Gram-negative bacterium now in the National Collection of Industrial Bacteria (NCIB, Torry Research Station, Aberdeen, Scotland) as *Achromobacter* sp., NCIB 8250, was generally referred to as 'Vibrio 01', but Sebald & Véron (1963) and Fewson (1967) have shown that it belongs to the *Acinetobacter*–*Moraxella* group of bacteria. This organism has been used in experiments on the metabolism of aromatic compounds (e.g. Cain, 1961; Ali, Callely & Hayes, 1962; Chapman & Dagley, 1962; Griffiths, Rodriques, Davies & Evans, 1964; Callely & Jones, 1965), monocarboxylic and dicarboxylic acids (Dagley & Patel, 1955; Dagley, 1956; Callely, Dagley & Hodgson, 1958) and liquid paraffin (Linday & Donald, 1961). There appears, however, to have been no detailed study of its nutritional requirements or metabolic versatility. This paper records experiments on the growth and nutrition of the Bacterium NCIB 8250. The results obtained in testing growth on a large number of compounds have served to outline the metabolic capabilities of the organism and have indicated which catabolic pathways could be used in experiments on metabolic control.

METHODS

Organism. Stock cultures of Bacterium NCIB 8250 were maintained in Oxoid cooked-meat medium stored at 4°. Subcultures were made into Oxoid nutrient broth at intervals of approximately 2 months and were also kept at 4°. Inocula used in the growth experiments were produced by a further subculture into nutrient broth and incubated for 17–24 hr at 30° immediately before use.

Measurement of growth rates. Cultures were grown under conditions of vigorous aeration in an apparatus similar to that described by Schlegel, Kaltwasser & Gottschalk (1961). Medium (800 ml.) was contained in 1 l. flasks fitted with side-arms to facilitate sampling. Magnetic stirring drive assemblies, constructed by Mr N. Harvey in this Department, were arranged to accommodate 5 or 10 flasks in water baths equipped

with Circotherm II a constant temperature units (Shandon Scientific Co. Ltd., London). Aeration was effected by means of 45 mm. magnetic stirring bars encased in polypropylene. In all experiments the vortex produced by stirring extended to the bottom of the flask and was there disturbed by the stirring bar so that bubbles were introduced into the body of the medium (Schlegel *et al.* 1961). This arrangement allowed a large number of cultures to be grown in the laboratory under constant and identical conditions of aeration, agitation and temperature. Growth was followed by taking 4 ml. samples at 10 to 20 min. intervals and measuring the extinction at 350 m μ in a Spectronic 20 colorimeter.

When testing the effect of various concentrations of nutrients, the inoculum was grown in a chemically defined medium deficient in the appropriate compound. In some cases, e.g. potassium or iron, several subcultures through the defined deficient media were necessary to show any response to the nutrient under test. In all cases the concentration of only one ion was varied at a time and the accompanying ions were added in other forms.

Screening carbon and nitrogen sources. The basal media were as follows. (i) For testing carbon + energy sources: 4 g. KH_2PO_4 + 2 g. $(\text{NH}_4)_2\text{SO}_4$ dissolved in 1 l. glass-distilled water and adjusted to pH 7.0. (ii) For testing nitrogen sources: 4 g. KH_2PO_4 + 5.4 g. sodium succinate. $6\text{H}_2\text{O}$ dissolved in 1 l. distilled water and adjusted to pH 7.0. Carbon or nitrogen sources at 10 mM, adjusted to pH 7.0, were sterilized by filtration through Millipore filters (GSWP047 00, 0.22 μ). Suitable volumes of the sterile solutions and sterile water to give final concentrations of 0.1, 0.5 and 5 mM in a total volume of 50 ml. were then added to 25 ml. amounts of autoclaved basal medium in 250 ml. Erlenmeyer flasks plugged with cotton wool. Heat-stable compounds were frequently sterilized by autoclaving at 109° rather than by filtration. Water-insoluble volatile compounds (e.g. benzene, pentane) were placed in test tubes held upright in the growth flasks by the cotton-wool plugs, and thus could distil over into the half-strength basal medium as carbon sources. Other insoluble compounds were added to the half-strength basal medium either before or after autoclaving, depending on their heat stability. These substances, such as stearic acid, were therefore tested as saturated solutions and not at known concentrations. Then were added 1.0 ml. sterile 2% (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and, as inoculum, 0.1 ml. of a 24 hr nutrient broth culture. Growth was followed for 5 days at 30° on a rotary shaker (L. H. Engineering Co., Bells Hill, Stoke Poges, Bucks; Mk V) moving at about 180 oscillations/min. The growth response to different carbon or nitrogen sources was compared with that obtained in the presence of succinate as carbon source and ammonium as nitrogen source. Controls of the basal media alone were also included. Visual estimation of the growth responses to different substrates was made by comparison with standard flasks containing 0.1, 0.3, 1 and 3 mg. wet wt. bacteria/ml. This method gave results which were as satisfactory as turbidity measurements with an absorptiometer or nephelometer: indeed the final results of response to carbon and nitrogen sources reported in this paper are given as 'growth' or 'no growth'. In doubtful cases, such as those where the substrate itself produced turbidity, growth was checked by the colony count technique of Miles & Misra (Mackie & McCartney, 1953).

Materials. Dr W. H. Holms of this Department kindly synthesized the *N*-*o*-carboxy-phenyl-D-glucosylamine (Bayne & Holms, 1952). All other compounds were from commercial sources.

RESULTS

Composition of medium and conditions for growth

The composition of the basal medium was examined by varying the concentration of each nutrient in turn. Extinction measurements gave an adequate estimate of cell mass since even under conditions of nitrogen or phosphate limitation the specific extinction was found to be almost the same as that of the bacteria grown in complete medium ($E_{350} = 1$ was equivalent to 0.28 mg. dry wt. organism/ml.). The effects of different concentrations of phosphate, ammonium, magnesium and sulphate are shown in Fig. 1. The results from this experiment were used to formulate the basal media used subsequently.

Nitrate, nitrite or ammonium were used as sources of inorganic nitrogen. Hydroxylamine gave growth at low concentrations but hydrazine was not utilized nor was there any evidence of nitrogen fixation. The phosphate in the medium could be replaced by pyrophosphate, ribose-5-phosphate, fructose-1,6-diphosphate or glucose-6-phosphate. The usual source of sulphur was sulphate but bacterium NCIB 8250 could also utilize sulphite, thiosulphate, cysteine, cystine, methionine, glutathione or thiourea. Neither biotin nor 2-mercaptobenzoate was able to supply the sulphur requirements.

A significant dependence of bacterial yield on potassium was shown only after five successive subcultures, with 1% (v/v) inocula, through K^+ -deficient media. Neither sodium, caesium nor rubidium replaced potassium although rubidium had a small but reproducible sparing effect on the potassium requirement (Table 1). No requirement for sodium, caesium, rubidium or chloride in the growth of bacterium NCIB 8250 was observed. A decreased yield in the absence of added iron was found only after about seven subcultures (1%, v/v, inocula) through deficient media. Iron ($5 \mu M$ as $FeCl_3$) was added to routine media only when preliminary experiments had shown that the inoculum was such that there would be an increased yield. No increase in either the growth rate or yield was found when yeast extract (1 mg./l.) or a mixture of biotin ($2.5 \mu g./l.$), pyridoxine (0.5 mg./l.), nicotinamide (0.4 mg./l.) and thiamine (0.4 mg./l.) was added to a simple acetate + salts medium. No effect of the addition of any vitamins was observed after 130 subcultures (1%, v/v, inocula) through chemically defined media.

Bacterium NCIB 8250 grows only under aerobic conditions. The degree of aeration under the conditions used was adequate to give maximum growth rates with acetate as carbon source (mean generation time, 45 min.). There was no increase in growth rate or yield when more air or oxygen was passed through the medium. No growth occurred under anaerobic conditions, even with 0.1% (w/v) potassium nitrate present to act as a possible alternative electron acceptor.

The influence of temperature on the growth rate is shown in Fig. 2. The optimal temperature was from 29° to 32°; most subsequent experiments were made at 30°. The Arrhenius plot (Fig. 2, inset) gave a straight line up to 29° and the apparent activation energy was calculated to be 14,700 cal. mole⁻¹.

The effect of the initial pH value on the mean generation time of bacterium NCIB 8250 is illustrated in Fig. 3. The response to the pH value was independent of the carbon and nitrogen sources tested; the optimum was pH 7.

The variations of growth rate and yield with changing concentrations of the carbon + energy sources are illustrated in Fig. 4. Increasing amounts of acetate, succinate

Fig. 1

Fig. 1. Effect of concentration of nutrients on final yield of *Bacterium* NCIB 8250. The carbon + energy source was 5 mM-succinate which gave a maximum yield (E_{350}) of about 1.0. O, NH_4^+ ; Δ , PO_4^{3-} ; $\bullet$, SO_4^{2-} ; $\square$, Mg^{2+} .

Fig. 2

Fig. 2. Effect of temperature on growth rate of *Bacterium* NCIB 8250. The carbon + energy source was 5 mM-succinate. Inset: Arrhenius plot of growth rate.

Table 1. *Effect of potassium on growth of Bacterium NCIB 8250*

The basal medium contained 2.6 g. $(\text{NH}_4)_2\text{SO}_4$ + 1.0 ml. glacial acetic acid + 2.0 ml. 98 % (v/v) phosphoric acid + 0.4 g. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in 1 l. distilled water adjusted to pH 7.0. The inoculum was prepared by making 4 successive subcultures (1 %, v/v, inocula) in this medium in order to show a significant dependence on K^+ . Erlenmeyer flasks (250 ml.) containing basal medium (50 ml.) + appropriate amounts of K_2SO_4 , Na_2SO_4 , Cs_2SO_4 or Rb_2SO_4 were inoculated with 0.1 ml. of the K^+ -deficient culture and shaken at 30° for 48 hr. before measuring the extinction with a Spectronic 20 colorimeter.

Concentration of K^+ and other ions (μM) in medium	Final yield (E_{350})
0	0.29
10 K^+	0.50
100 K^+	0.73
100 Na^+	0.31
100 Cs^+	0.29
100 Rb^+	0.36
10 K^+ + 100 Na^+	0.50
10 K^+ + 100 Cs^+	0.52
10 K^+ + 100 Rb^+	0.57
100 K^+ + 100 Rb^+	0.72

and benzoate gave proportionate increases in yield. The results approximate to molar growth yields, although more sophisticated techniques would be required for a more accurate determination of this parameter. It is of interest that the yield from benzoate ($E_{350} = 0.30/\text{mm}$) was almost exactly equal to the sum of the yields from succinate ($E_{350} = 0.19/\text{mm}$) and acetate ($E_{350} = 0.12/\text{mm}$): benzoate is oxidized by conversion to succinate and acetyl-CoA (Ornston & Stanier, 1964). The change in mean generation time with concentration of substrate is shown in the upper half of Fig. 4. The effects of substrate concentration on growth rate would be better determined in a continuous

flow apparatus, but the primary purpose of the present experiments was to provide data for the growth of batch cultures for enzyme studies. Succinate and acetate tended to provide a constant maximum growth rate (mean generation time 45 min.); these two compounds gave the fastest rate of growth of *Bacterium* NCIB 8250 in chemically defined medium of any tested. Higher benzoate concentrations depressed the growth rate; this effect was noted with several substrates, e.g. 2-hydroxybenzyl alcohol, phenol and 2-aminobenzaldehyde.

Fig. 3

Fig. 4

Fig. 3. Effect of initial pH value on growth rate of *Bacterium* NCIB 8250. The pH values of the media were measured during growth but in no case did a change occur before the last generation when some cultures, initially at relatively low or high pH, shifted towards neutrality by up to 0.5 pH unit. Carbon and nitrogen sources were: ○, acetate + glutamate; △, mandelate + glutamate; ●, acetate + ammonium; □, acetate + nitrate.

Fig. 4. Effect of substrate concentration on growth rate and final yield of *Bacterium* NCIB 8250. ○, acetate; △, succinate; □, benzoate.

Screening carbon and nitrogen sources

The metabolic versatility of *Bacterium* NCIB 8250 was tested by screening a large number of compounds as carbon and nitrogen sources for growth. The yield of organism was recorded at three concentrations of substrate as indicated in Methods. Some compounds, e.g. 2-hydroxybenzoate, gave growth at 0.1 and 0.5 mM but not at 5 mM: toxic effects of this type were not uncommon. In contrast to this, a few compounds such as D,L-tropate showed growth only at the higher concentrations; this may have reflected the inefficiency of these compounds as inducers. With still other compounds,

such as vanillin (mean generation time about 200 min.), growth was extremely slow even at the optimum concentration. Most compounds which supported growth gave approximately the same yield of bacteria as when an equal amount of carbon or nitrogen was provided in the form of succinate or ammonium respectively. In a few cases, e.g. with some amino acids as nitrogen sources, the amount of growth was not as great as that given by the same molar concentration of ammonium or glutamic acid. When compounds did not support growth under the ordinary conditions they were occasionally further tested by growing the inoculum in nutrient broth or glutamate + salts medium + the compound under test, in an attempt to pre-induce enzymes necessary for their utilization. In no case, however, did this method show growth where it had not already occurred in the routine test system. Despite complications such as toxicity, slow growth rates and low yields, it was possible to divide the compounds tested into those which supported growth and those which did not, and thus to delineate the metabolic versatility of *Bacterium* NCIB 8250.

Table 2. *Sugars and related compounds which did not serve as carbon sources for growth of Bacterium NCIB 8250*

D-isomers were used unless otherwise stated. The basal medium contained KH_2PO_4 + MgSO_4 + ammonium as nitrogen source. A nutrient broth culture was used as inoculum (0.2 %, v/v) and flasks were shaken at 30° for 5 days.

<i>N</i> -Acetylglucosamine	6-Phosphogluconate	Mannitol
L-Arabinose	Glucosamine	Mucate
Ascorbate	Glucose	Raffinose
Cellobiose	Glucose-6-phosphate	L-Rhamnose
2-Deoxyribose	Glucuronate	Ribose
Dihydroxyacetone	D,L-Glyceraldehyde	Ribose-5-phosphate
<i>penta</i> -Erythritol	Glycerol	Sorbitol
Fructose	α -Glycerophosphate	Starch
Fructose-1,6-diphosphate	<i>meso</i> Inositol	Sucrose
Galactose	Lactose	Xylose
Gluconate	Maltose	

Bacterium NCIB 8250 showed no growth with any of the sugars or related compounds which were tested (Table 2). Experiments in which the organism was grown on benzoate, succinate or glutamate in the presence of glucose or ribose showed no increase in yield over controls in the absence of sugars. The pattern of utilization of amino acids and related compounds is shown in Table 3. There was a much greater lag in the utilization of L-serine than of D- or D,L-serine, but this effect was not noted with the isomers of alanine or glutamate. Those amino acids which did not serve as carbon sources were further tested in the presence of nitrate rather than ammonium as nitrogen source, but in no case was growth obtained. Table 4 records the growth on normal alkanes, normal alcohols and monocarboxylic and dicarboxylic acids. The failure to grow on alkanes containing less than eight carbon atoms may have been due to their toxicity but they did not depress growth in nutrient broth. All the normal alcohols except methanol gave growth, as did isobutanol but not isopropanol or *sec*-butanol (Table 5). Monocarboxylic and dicarboxylic straight-chain acids, with the exception of formate, oxalate and malonate, supported growth. Growth also occurred with a number of other aliphatic and related compounds (Table 5) including several members

of common metabolic sequences. Most purines, pyrimidines and their derivatives served as nitrogen but not carbon sources (Table 6). Adenosine supported an extremely small amount of growth; confirmation of this result must await further work. A large

Table 3. *Amino acids and related compounds as carbon or nitrogen sources for growth of Bacterium NCIB 8250*

The basal medium contained $\text{KH}_2\text{PO}_4 + \text{MgSO}_4$ + either succinate as carbon source or ammonium as nitrogen source. A nutrient broth culture was used as inoculum (0.2 %, v/v) and flasks were shaken at 30° for 5 days.

	As carbon source	As nitrogen source		As carbon source	As nitrogen source
D-, L- or D,L-Alanine	+	+	δ -Hydroxy-D,L-lysine	—	Not tested
β -Alanine	+	+	L-Hydroxy proline	+	+
D,L-2-Amino- <i>n</i> -butyrate	—	+	L-Leucine	—	+
4-Amino- <i>n</i> -butyrate	—	—	D,L-Isoleucine	—	+
2-Aminoisobutyrate	—	—	D,L-Norleucine	—	—
L-Arginine	—	—	L- or D,L-Lysine	—	—
L-Asparagine	+	+	L- or D,L-Methionine	—	+
L- or D,L-Aspartate	+	+	D,L-Ornithine	—	—
L- or D,L-Citrulline	—	+	L- or D,L-Phenylalanine	—	+
Creatine	—	—	<i>N</i> -Phenylglycine	—	—
L- or D,L-Cysteine	—	+	Phenyl-D,L-serine	—	+
L-Cystine	—	+	L-Proline	+	+
D,L- α - ϵ -Diaminopimelate	—	—	D-, L- or D,L-Serine	+	+
D-, L- or D,L-Glutamate	+	+	D,L-Homoserine	—	+
L-Glutamine	+	+	L- or D,L-Threonine	—	—
Glycine	—	+	L-Tryptophan	—	+
Glycylglycine	—	+	L-Tyrosine	+	+
Glycylglycylglycine	—	+	L- or D,L-Valine	—	+
L- or D,L-Histidine	+	+	D,L-Isovaline	—	—

Table 4. *Normal alkanes, normal alcohols, monocarboxylic and dicarboxylic acids as carbon sources for growth of Bacterium NCIB 8250*

The basal medium contained $\text{KH}_2\text{PO}_4 + \text{MgSO}_4$ + ammonium as nitrogen source. A nutrient broth culture was used as inoculum (0.2 %, v/v) and flasks were shaken at 30° for 5 days.

No. of C-atoms	$\text{CH}_3(\text{CH}_2)_n\text{CH}_3$	$\text{CH}_3(\text{CH}_2)_n\text{CH}_2\text{OH}$	$\text{CH}_3(\text{CH}_2)_n\text{COOH}$	$\text{HOOC}(\text{CH}_2)_n\text{COOH}$				
1	.	.	Methanol	—	Formate	—	.	—
2	.	.	Ethanol	+	Acetate	+	Oxalate	—
3	.	.	Propanol	+	Propionate	+	Malonate	—
4	.	.	Butanol	+	Butyrate	+	Succinate	+
5	Pentane	—	Pentanol	+	Valerate	+	Glutarate	+
6	Hexane	—	Hexanol	+	Caproate	+	Adipate	+
7	Heptane	—	Heptanol	+	Heptoate	+	Pimelate	+
8	Octane	+	Octanol	+	Caprylate	+	Suberate	+
9	Nonane	+	.	.	.	.	Azelate	+
10	Decane	+	Decanol	+	Caprate	+	Sebacate	+
11	Undecane	+	Undecanol	+	Undecylate	+	.	.
12	Dodecane	+	Dodecanol	+	Laurate	+	.	.
13	Tridecane	+	Tridecanol	+	Tridecylate	+	.	.
14	Tetradecane	+	Tetradecanol	+	Myristate	+	.	.
15	Pentadecane	+	Pentadecanol	+	.	.	.	.
16	Hexadecane	+	.	.	Palmitate	+	.	.
18	Octadecane	+	.	.	Stearate	+	.	.
22	.	.	.	.	Behenate	—	.	.

number of aromatic compounds was tested (Table 7) but only relatively few supported growth. The non-aromatic cyclic compounds which served as nitrogen or carbon sources are given in Table 8.

Table 5. *Some aliphatic and related compounds as carbon or nitrogen sources for growth of Bacterium NCIB 8250*

The basal medium contained $\text{KH}_2\text{PO}_4 + \text{MgSO}_4$. A nutrient broth culture was used as inoculum (0.2 %, v/v) and flasks were shaken at 30° for 5 days.

(i) *The organism grew on the following carbon sources (ammonium as nitrogen source):*

Acetoin	Diacetyl	Oxalacetate
Isoamylacetate	Fumarate	2-Oxoadipate
Butane-2,3-diol	3-Hydroxybutyrate	2-Oxoglutarate
Isobutanol	Lactate	Pyruvate
Citrate	Malate	

(ii) *The following compounds were used as nitrogen sources (succinate as carbon source) but not as carbon sources (ammonium as nitrogen source):*

Cyanate	Propionamide
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(iii) *No growth on the following compounds as carbon sources nor, where possible, as nitrogen sources:*

Acetamide	Ethyl acetate	3-Methylvalerate
Acetone	Ethylene glycol	2-Oxobutyrate
Aconitate	3-Ethyl-3-heptanol	Pentane-1,5-diol
Acrylamide	4-Ethyl-3-hexanol	Propane-1,2-diol
Acrylate	3-Ethyl-3-pentanol	Propane-1,3-diol
Adipamide	D,L-Glycerate	Isopropanol
Butane-1,3-diol	Glycollate	Propiolate
Butane-1,4-diol	Glyoxylate	Sarcosine
sec-Butanol	Hexane-1,6-diol	Spermidine
n-Butylamine	Hydroxypyruvate	Spermine
Isobutylamine	Itaconate	Tartrate
sec-Butylamine	Linoleate	Taurine
tert-Butylamine	Linolenate	Thiocyanate
Cadaverine	Maleate	Tris (hydroxymethyl)
Cyanide	2-Methoxyethanol	aminomethane
Ethanolamine	Methylamine	Isovalerate

Table 6. *Purines, pyrimidines and derivatives as nitrogen sources for growth of Bacterium NCIB 8250*

The basal medium contained $\text{KH}_2\text{PO}_4 + \text{MgSO}_4 + \text{succinate}$ as carbon source. A nutrient broth culture was used as inoculum (0.2 %, v/v) and flasks were shaken at 30° for 5 days. None of the compounds served as carbon source when ammonium was the nitrogen source.

	Nitrogen source		Nitrogen source
Adenine	+	Inosine	+
Adenosine	—	Orotate	—
Allantoate	+	Thymidine	—
Allantoin	+	Thymine	+
Barbiturate	—	Uracil	+
Cytidine	—	Urate	+
Cytosine	—	Urea	+
Guanine	+	Uridine	—
Guanosine	+	Xanthine	+
Hypoxanthine	+	Xanthosine	+

DISCUSSION

The examination of the metabolic versatility of *Bacterium* NCIB 8250 involved testing about 450 substances of which over 100 served as sole carbon + energy sources and about 50 as sole nitrogen sources. It is probable of course that wider screening or the use of other basal media and inocula would uncover additional compounds which can support growth. Also some compounds may have failed to give growth because they do not serve as inducers for the appropriate enzymes and furthermore *Bacterium* NCIB 8250 might be impermeable to certain compounds and unable to form permeases for their transport into the bacterium. A number of types of growth response was noted in testing these compounds but the results have been recorded as 'growth' or 'no growth' since it was felt that many of the variations in response were trivial in that they depended on the size and type of inoculum or concentration of nutrient. There is no doubt that some compounds which did not support growth are partially metabolized by *Bacterium* NCIB 8250. An example of this is the oxidation of 3-hydroxy-mandelate to 3-hydroxybenzoate by *Bacterium* NCIB 8250 adapted to grow on mandelate (Kennedy & Fewson, 1966). This type of incomplete oxidation might have considerable ecological significance in the succession of microbial populations in soil (Alexander, 1964).

The experiments described in this paper confirm that *Bacterium* NCIB 8250 is a suitable organism in which to examine the control of catabolic enzyme systems. Growth occurs on a number of diverse compounds and the failure to utilize carbohydrates may simplify studies on catabolite repression. The survey of metabolic versatility has revealed several oxidative sequences which are inducible and amenable to experiments on the mechanism of control. These include the pathways for the degradation of purines, pyrimidines and their ribosides; indole and indolyl-3-acetate; alkanes, alcohols, and acids; butane-2,3-diol, acetoin and diacetyl; several amino acids; quinate and shikimate; the mandelate family. The pathway of oxidation of mandelate and related compounds in *Bacterium* NCIB 8250 has already been elucidated (Kennedy & Fewson, 1966). Work has also been done on other systems such as the oxidation of straight-chain acids (e.g. Callely *et al.* 1958), homogentisate (Chapman & Dagley, 1962) and protocatechuate (Cain, 1961).

The experiments on screening of substrates, in addition to indicating which catabolic systems can be induced in *Bacterium* NCIB 8250, have shown several features of the metabolism of this organism which merit further work. The basis of the inability to grow on carbohydrates should be of considerable interest, especially since members of the *Acinetobacter*-*Moraxella* group show a wide variation in their saccharolytic properties (Steel & Cowan, 1964; Henderson, 1965). It is noteworthy that whereas some *N*-glycosides such as *N*-*o*-carboxyphenyl-D-glucosylamine and the purine ribosides supported growth (presumably at the expense of the non-sugar components) thymidine, uridine and the *O*-glycoside salicin did not give growth even though in these cases also the non-glycosidic moieties were utilized (Tables 6, 7). Another problem is raised by the observation that phenylpyruvate but not phenylalanine served as carbon source (Tables 3, 7). This is surprising since phenylpyruvate can be shown to accumulate in the medium when *Bacterium* NCIB 8250 grows with phenylalanine as nitrogen source (unpublished results). Parallel results were obtained with tryptophan. The explanation would seem to lie in the specificity of enzyme induction and repression.

Table 7. *Aromatic compounds as carbon or nitrogen sources for growth of Bacterium NCIB 8250*

The basal medium contained $\text{KH}_2\text{PO}_4 + \text{MgSO}_4$. A nutrient broth culture was used as inoculum (0.2 %, v/v) and flasks were shaken at 30° for 5 days.

(i) *Compounds used as carbon sources (ammonium as nitrogen source):*

Acetylsalicylate	Ethylphenylacetate	L- or D,L-Mandelate
Benzaldehyde	Homogentisate	Phenol
Benzyl alcohol	2-Hydroxybenzaldehyde	Phenylacetaldehyde
Benzoate	4-Hydroxybenzaldehyde	Phenylacetate
Benzoylformate	2-Hydroxybenzoate	Phenylpyruvate
Catechol	4-Hydroxybenzoate	D,L-Tropate
3,4-Dihydroxybenzaldehyde	2-Hydroxybenzyl alcohol	Vanillate
3,4-Dihydroxybenzoate	4-Hydroxybenzyl alcohol	Vanillin
3,4-Dihydroxymandelate	4-Hydroxymandelate	Vanillyl alcohol
Ethylbenzoate	4-Hydroxyphenylpyruvate	Vanillylmandelate

(ii) *Compounds used as carbon and nitrogen sources:*

2-Aminobenzaldehyde	<i>N</i> -o-Carboxyphenyl-D-glucosylamine
Anthranilate	4-Hydroxy-3-methoxybenzylamine

(iii) *Compounds not used as carbon sources (ammonium as nitrogen source):*

2-Acetamidobenzoate	Ethyl- <i>p</i> -aminobenzoate	2-Nitrophenol
2-Aminophenol	Eugenol	3-Nitrophenol
3-Aminophenol	Isoeugenol	4-Nitrophenol
4-Aminophenol	Ferulate	3-Nitrophthalate
Amygdalin	Gentisate	4-Nitrophthalate
Anisaldehyde	Guaicol	3-Nitrosalicylate
Anisyl alcohol	3-Hydroxybenzaldehyde	5-Nitrosalicylate
Anisate	3-Hydroxybenzoate	2-Nitrosophenol
Atrolactate	3-Hydroxybenzyl alcohol	Orcin
Atropine	3-Hydroxymandelate	Phenoxyacetate
Benzene	3-Hydroxy-4-methoxybenzaldehyde	Phenylacetamide
<i>p</i> -Benzoquinone	3-Hydroxy-4-methoxymandelate	Phenyl- <i>n</i> -butyrate
Benzyl malonate	4-Hydroxy-3-methoxyphenylacetate	2-Phenylethanol
Caffeate	2-Hydroxyphenylacetate	D,L-1-Phenylethanol
2-Carboxybenzaldehyde	3-Hydroxyphenylacetate	3-Phenylpropanol
<i>homo</i> -Catechol	4-Hydroxyphenylacetate	3-Phenylpropionate
Chloramphenicol	4-Hydroxyisophthalate	Phenylsuccinate
Chlorogenate	Mandelamide	Phloretate
Cinnamaldehyde	D-Mandelate	Phloroglucinol
Cinnamate	2-Methoxybenzaldehyde	Isophthalaldehyde
Cinnamyl alcohol	3-Methoxybenzaldehyde	Phthalate
<i>o</i> -, <i>m</i> -, and <i>p</i> -Coumarate	2-Methoxybenzoate	Isophthalate
<i>o</i> -, <i>m</i> -, and <i>p</i> -Cresol	3-Methoxybenzoate	Terephthalate
2,4-Dihydroxybenzaldehyde	3-Methoxybenzyl alcohol	Piperonylate
2,5-Dihydroxybenzaldehyde	4-Methoxyphenol	Pyrogallol
2,3-Dihydroxybenzoate	2-Methoxyphenylacetate	Resorcinol
2,4-Dihydroxybenzoate	Methylbenzoate	Salicin
2,6-Dihydroxybenzoate	Methyl-2-hydroxybenzoate	Sinapate
3,5-Dihydroxybenzoate	Methyl-4-hydroxybenzoate	Sinapinate
3,4-Dihydroxyphenylacetate	Methylsalicylate	Sulphanilamide
3,4-Dihydroxyphenylalanine	1-Naphthol	Syringate
2,4-Dimethoxybenzaldehyde	2-Naphthol	Toluene
3,4-Dimethoxybenzyl alcohol	2-Nitroaniline	<i>o</i> -, <i>m</i> -, and <i>p</i> -Toluate
3,5-Dinitrobenzoate	3-Nitroaniline	3,4,5-Trihydroxybenzoate
2,4-Dinitrophenol	4-Nitroaniline	<i>o</i> -Vanillin
Diphenate	3-Nitrobenzoate	Isovanillin

Table 7 (cont.)

(iv) *Compounds not used as either carbon or nitrogen sources:*

2-Aminobenzoate	Aniline	2-Nitrobenzaldehyde
4-Aminobenzoate	Benzamide	3-Nitrobenzaldehyde
3-Amino-4-hydroxybenzoate	Benzylamine	4-Nitrobenzaldehyde
D,L- α -Aminophenylacetate	2-Carboxyphenylglycine	2-Nitrobenzoate
4-Aminophenylacetate	Hippurate	4-Nitrobenzoate
4-Aminosalicylate	Mandelonitrile	Phenylhydrazine

Table 8. *Heterocyclic, alicyclic and related compounds as carbon or nitrogen sources for Bacterium NCIB 8250*

The basal medium contained $\text{KH}_2\text{PO}_4 + \text{MgSO}_4$. A nutrient broth culture was used as inoculum (0.2 %, v/v) and flasks were shaken at 30° for 5 days.

(i) *Compounds used as carbon sources (ammonium as nitrogen source):*

Quinate	Shikimate
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(ii) *Compounds used as carbon and nitrogen sources:*

Indole	D,L-Kynurenine
Indolyl-3-acetate	

(iii) *Compound used as nitrogen source but not as carbon source:*

Xanthurenate

(iv) *Compounds not used as carbon sources or, here possible, as nitrogen sources:*

Adamantanecarboxylate	2-Hydroxycyclohexanone
Adrenaline	Indolyl-3-acetamide
Noradrenaline	Indolyl-3-butyrate
Aneurine	Indolyl-3-propionate
Biotin	Indolyl-3-pyruvate
Camphor	Isatin
2,6-Dipicolinate	Kojate
Folate	Nicotinate
2-Furoate	Pyridoxine
Cyclohexane	Skatole
Cyclohexanecarboxylate	Urocanate
Cyclohexanol	

The pathways of degradation of some of the purines and pyrimidines are also in doubt (Table 6). Adenine appears to be oxidized via xanthine and uric acid to urea since all these compounds served as nitrogen but not carbon sources. In the metabolism of uracil, however, it would seem that the dihydrouracil pathway (White, Handler & Smith, 1964) did not function since the product would be β -alanine which unlike uracil was a carbon and a nitrogen source. The barbiturate pathway (Schulman, 1961) did not seem to operate since bacterium NCIB 8250 did not grow on barbiturate.

The central role of the Krebs cycle in the metabolism of bacterium NCIB 8250 has been indicated by previous workers (Dagley & Rodgers, 1953; Dagley & Patel, 1955; Calley *et al.* 1958). It may also be presumed that the organism possesses a glyoxylate cycle since it grows on acetate (Table 4), and possesses isocitratase and malate synthetase (Calley *et al.* 1958). The failure to grow on glycollate or glyoxylate (Table 5) might be caused by the lack of a suitable anaplerotic sequence (Kornberg, 1966), but neither glycerate nor glycerate with glyoxylate, glycollate or glycine supported growth. Another possible cyclic system of Bacterium NCIB 8250 is the butane-2,3-diol cycle (Juni & Heym, 1956) for the dissimilation of acetoin, diacetyl and butane-2,3-diol.

All these compounds supported growth (Table 5); an inducible specific enzyme which oxidises butane-2,3-diol to acetoin is at present under investigation.

The range of compounds which can support the growth of *Bacterium* NCIB 8250 is considerable, and perhaps, as suggested in a preliminary and unpublished communication by Stanier & Palleroni, the utilization of some of the compounds tested in this work may serve as a basis for classification of the *Acinetobacter*-*Moraxella* group. The range of versatility also helps to explain the apparent ubiquity of this group in nature (Henderson, 1965), especially since many of the compounds metabolized, such as vanillin, are derivatives of common organic polymers found in soil.

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