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# Tricarboxylic Acid-Cycle and Related Enzymes in Restricted Facultative Methylotrophs

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1. The isolation is described of pure cultures of three non-methane-utilizing methylotrophic bacteria which, together with the previously described *Bacillus* PM6, have a very limited range of growth substrates; these organisms are designated 'restricted facultative' methylotrophs. 2. Two of these isolates, W6A and W3A1, grow only on glucose out of 50 non-$C_1$ compounds tested, whereas the third isolate S2A1 and *Bacillus* PM6 grow on betaine, glucose, gluconate, alanine, glutamate, citrate and nutrient agar, but not on any of a further 56 non-$C_1$ compounds. 3. Crude sonic extracts of trimethylamine-grown and glucose-grown W6A and W3A1 isolates, and of trimethylamine-grown C2A1 (an obligate methylotroph) contain (i) no detectable 2-oxoglutarate dehydrogenase activity, (ii) very low or zero specific activities of succinate dehydrogenase and succinyl-CoA synthetase and (iii) $NAD^+$-dependent isocitrate dehydrogenase activity. 4. Extracts of trimethylamine-grown PM6 and S2A1 methylotrophs have (i) very low 2-oxoglutarate dehydrogenase specific activities, (ii) comparatively high specific activities of succinate dehydrogenase, malate dehydrogenase and succinyl-CoA synthetase and (iii) $NADP^+$-dependent isocitrate dehydrogenase activity but no $NAD^+$-dependent isocitrate dehydrogenase activity. The activities of most of these enzymes are increased during growth on glucose, alanine, glutamate or citrate, but only very low 2-oxoglutarate dehydrogenase activities are present under all growth conditions. 5. The restricted facultative methylotrophs grow on certain non-$C_1$ compounds in the absence of 2-oxoglutarate dehydrogenase and, in some cases, of other enzymes of the tricarboxylic acid cycle; these lesions cannot therefore be the sole cause of obligate methylotrophy.

Two groups of methylotrophic bacteria are recognized at the present time (Colby & Zatman, 1972): (i) obligate methylotrophs that grow non-autotrophically only on $C_1$ compounds, i.e. carbon compounds containing no carbon–carbon bonds; (ii) facultative methylotrophs that can also grow on a variety of non-$C_1$ compounds. The results of studies with the obligate methylotroph 4B6 and with two typical facultative methylotrophs, *Pseudomonas* 3A2 and bacterium 5B1 (Colby & Zatman, 1972), suggested that the absence of 2-oxoglutarate dehydrogenase associated with low specific activities of other tricarboxylic acid-cycle enzymes could explain the inability of obligate methylotrophs to grow on non-$C_1$ compounds.

While attempting to isolate new trimethylamine-utilizing obligate methylotrophs, three organisms were obtained which, together with *Bacillus* PM6 (Myers & Zatman, 1971), are referred to here as 'restricted facultative methylotrophs' because of the very restricted range of non-$C_1$ substrates that support their growth. The restricted facultative methylotrophs (like obligate methylotrophs) constitute a group of micro-organisms with a very restricted growth-substrate range, but they differ from the obligate methylotrophs in their ability to grow on a few non-$C_1$ compounds; they are therefore of considerable relevance to a study of the biochemical basis of obligate methylotrophy. This paper reports the results of our investigations of the activities of the tricarboxylic acid-cycle enzymes during the growth of the restricted facultative methylotrophs together with the corresponding results obtained with the previously described obligate methylotroph bacterium C2A1 (Colby & Zatman, 1973).

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## Materials and Methods

### *Materials*

Dimethylamine hydrochloride, methylamine hydrochloride, and trimethylamine *N*-oxide hydrochloride were obtained from BDH Chemicals Ltd., Poole, Dorset, U.K. Trimethylamine hydrochloride was obtained from Ralph N. Emmanuel Ltd., Wembley, Middx., U.K. and tetramethylammonium chloride was obtained from Kodak Ltd., London

W.C.2, U.K. Other growth substrates were obtained from the usual commercial sources and were the best grade available.

CoA, acetyl-CoA, $NAD^+$, $NADP^+$, 3-acetylpyridine adenine dinucleotide ($APAD^+$), NADH, NADPH, ATP, ADP and isocitrate dehydrogenase (pig heart) were obtained from the Boehringer Corp. (London) Ltd., London W.5, U.K. Thiamine pyrophosphate chloride, 5,5′-dithiobis-(2-nitrobenzoic acid), *cis*-aconitic acid, phenazine methosulphate, 2-oxoglutaric acid, hydroxylamine hydrochloride, GSH, sodium fumarate and sodium pyruvate were obtained from Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey, U.K. Trisodium DL-isocitrate was obtained from Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K. BDH Chemicals Ltd. supplied oxaloacetic acid, L-cysteine hydrochloride, sodium succinate, sodium hydrogen malate and 2,6-dichlorophenol-indophenol.

## *Methods*

*Isolation and characterization of trimethylamine-utilizing bacteria.* The methods used were those described by Colby & Zatman (1973). Potential sources were screened for trimethylamine-utilizers by liquid enrichment and by direct streaking, and the ability of each isolate to grow on a range of organic compounds was determined. In addition to the characterization methods of Colby & Zatman (1973), stationary-phase cultures of the isolates were stained for endospores by the method of Conklin (1934) and the ability of a nutrient-broth (0.5% Evans peptone, 0.5% Lab Lemco, 0.5% NaCl) culture of organism S2A1 to remain viable after heating at 80°C for 10min was tested. GC (DNA base ratios) values of trimethylamine-grown organisms were determined by the $T_m$ method by Dr. D. M. Gibson at the Torry Research Station, Aberdeen, U.K.

*Bulk growth of organisms for the preparation of crude sonic extracts.* Although organisms W6A, W3A1 and S2A1 were isolated in a medium containing a mixture of vitamins, later experiments showed that these isolates grew well in the absence of any added vitamin; similarly isolate C2A1 (Colby & Zatman, 1973) and *Bacillus* PM6 (Myers, 1971) do not require added vitamins for growth. Organisms for the preparation of crude sonic extracts were grown in 2-litre flasks each containing 1 litre of medium incorporating 0.2% of growth substrate. The flasks were inoculated with 1% of an exponential-phase culture and incubated on an orbital shaker at 30°C. The cultures were harvested in mid-exponential phase by centrifugation at 2°C and washed twice with ice-cold 50mM-sodium phosphate buffer, pH7.5. Cells were stored as pellets at −20°C until required.

*Preparation of crude sonic extracts.* All operations were performed at 0–4°C. Washed suspensions of the isolates in 50mM-sodium phosphate buffer, pH7.5, were sonicated as described by Colby & Zatman (1972). Crude sonicated suspensions were centrifuged at 10000*g* for 20min and the supernatants used for the determination of NADH- and NADPH-oxidizing activity, 2-oxoglutarate dehydrogenase and succinate dehydrogenase. Other enzymes were assayed in the supernatants obtained after centrifuging the crude sonicated suspensions at 35000*g* for 30min.

*Protein estimations.* The concentration of protein in crude sonic extracts was determined with the Folin–phenol reagent (Kennedy & Fewson, 1968) with crystalline bovine plasma albumin (Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, U.K.) as the standard.

*Enzyme assays.* All spectrophotometric assays were done at 30°C in a Hitachi Perkin–Elmer 124 double-beam grating spectrophotometer (Perkin–Elmer Ltd., Beaconsfield, Bucks., U.K.) fitted with a constant-temperature cuvette housing and coupled to a Servoscribe chart recorder (Smiths Industries Ltd., Wembley, Middx., U.K.). The assay methods used for citrate synthase, $NAD^+$- and $NADP^+$-dependent isocitrate dehydrogenases, aconitate hydratase, succinyl-CoA synthetase, succinate dehydrogenase, fumarate hydratase, malate dehydrogenase, glutamate dehydrogenases and isocitrate lyase were those described by Colby & Zatman (1972, 1973). Because of the high rates of NADPH oxidation catalysed by extracts of trimethylamine-grown S2A1 and PM6 isolates (see Table 4), the NADP–isocitrate dehydrogenase and aconitate hydratase activities of these extracts were assayed anaerobically by the technique described below for the anaerobic assay of 2-oxoglutarate dehydrogenase. NADH- and NADPH-oxidizing activities were assayed spectrophotometrically as described by Smith *et al.* (1967). Alanine dehydrogenase activity was measured in the direction of alanine synthesis as described by Yoshida & Freese (1965). 2-Oxoglutarate dehydrogenase was generally measured by the method of Mukherjee *et al.* (1965) with $NAD^+$, but under anaerobic conditions; this method gave the best results with all crude sonic extracts except those obtained from isolate C2A1. Extracts from the latter isolate catalysed a high endogenous rate of $NAD^+$ reduction and this difficulty was overcome by using the method of Amarasingham & Davis (1965) with $APAD^+$ for these extracts. The anaerobic modification of the method of Mukherjee *et al.* (1965) was as follows. Each assay was done in a semi-micro cuvette (10mm light-path, maximum vol. 1.5ml) fitted with a Teflon cap through which a small hole, sufficiently large to allow the insertion of a syringe needle, had been bored. The crude sonic extract and the substrate solution were both degassed by evacuation in a Thunberg tube just before the experiment and kept under $N_2$. The remaining assay constituents were

placed in the cuvette and $N_2$ was bubbled through the mixture for about 1 min via a syringe needle inserted through the cap. The crude sonic extract was then transferred to the cuvette and the endogenous rate of $NAD^+$ reduction recorded. Finally the substrate solution was added and the rate of $NAD^+$ reduction again determined.

*Enzyme units.* One munit of enzyme is defined as the quantity that catalyses the transformation of 1 nmol of substrate or the formation of 1 nmol of product/min at 30°C in the assays described above.

## Results and Discussion

### *Growth-substrate specificity of the isolates*

Trimethylamine-utilizing bacterial isolates were obtained from river-, ditch- and pond-water, fish and soil by liquid enrichment and by direct streaking using trimethylamine basal media. Most grew on a wide range of $C_1$ and non-$C_1$ growth substrates and were considered to be typical facultative methylotrophs. Two isolates, W3A1 and W6A, grew only on glucose of those non-$C_1$ compounds tested, whereas another isolate S2A1, and an earlier isolate *Bacillus* PM6 (Myers & Zatman, 1971), grew only on betaine, D-glucose, gluconate, L-glutamate, citrate, L-alanine and nutrient agar (Table 1). Isolates W3A1, W6A, S2A1 and PM6 were tested and failed to grow on the following compounds, in addition to those given in Table 1: trimethylsulphonium chloride, methane, formate, triethylamine, diethylamine, ethylamine, choline, ethanolamine, putrescine, tyramine, benzylamine, ethanol, acetate, glycollate, glyoxalate, oxalate, glycine, sarcosine, L-serine, L-aspartate, L-lysine, L-arginine, 4-aminobutyrate, 2-oxoglutarate, malonate, DL-glycerate, L-malate, succinate, fumarate, pyruvate, DL-lactate, glycerol, D-fructose, D-galactose, D-mannose, L-sorbose, D-ribose, D-xylose, D-sorbitol, D-mannitol, glucose 6-phosphate, fructose 6-phosphate, 6-phosphogluconate, benzoate, 4-hydroxybenzoate, L-mandelate. Isolates S2A1 and PM6 were also tested, and failed to grow on L-leucine, L-isoleucine, L-proline, L-hydroxyproline, L-methionine L-histidine, DL-tryptophan, L-phenylalanine, L-valine, L-threonine, L-cysteine, L-tyrosine and β-methyl-DL-aspartate.

Table 1. *Substrates supporting the growth of the restricted facultative methylotrophs*

All substrates except nutrient agar were tested at 0.1% on solidified basal medium. The test plates were inoculated with trimethylamine-grown organisms and then incubated at 30°C and examined for growth after 3, 7 and 14 days incubation. +, Growth; −, no growth; N.T. not tested. Details of the other substrates tested and definition of types M and L are given in the text.

| | Type M | | Type L | |
|---|---|---|---|---|
| Isolate ... / Substrate | W3A1 | W6A | S2A1 | PM6 |
| $C_1$ compounds | | | | |
| Tetramethylammonium | − | − | − | + |
| Trimethylamine | + | + | + | + |
| Trimethylamine *N*-oxide | − | − | + | + |
| Dimethylamine | + | + | + | + |
| Methylamine | + | + | + | + |
| Methanol | + | + | − | − |
| Non-$C_1$ compounds | | | | |
| Glucose | + | + | + | + |
| Gluconate | − | − | + | + |
| Citrate | − | − | + | + |
| Glutamate | − | − | + | + |
| Alanine | N.T. | N.T. | + | + |
| Betaine | − | − | + | + |
| Nutrient agar | − | − | + | + |

Growth of isolates W3A1 and W6A on glucose was extremely slow with mean doubling times, in shaken glucose–mineral medium cultures, of 38 and 32 h respectively compared with 2.5 h on trimethylamine; no growth occurred on prolonged incubation of these organisms in mineral medium without added substrate. Despite their slow rate of growth on glucose, quite good yields of the organisms (43 g dry wt./mol of glucose utilized) were obtained.

The four isolates W3A1 ,W6A, S2A1 and PM6, thus have a very restricted range of substrates that will support their growth. This clearly distinguishes them from such typical facultative methylotrophs as those isolated by Colby & Zatman (1973) and from *Pseudomonas* AM1 (Peel & Quayle, 1961) and *Pseudomonas* M27 (Anthony & Zatman, 1964) and they are therefore referred to as restricted facultative methylotrophs. For convenience the organisms with the more-restricted range of growth substrates (W3A1 and W6A) are designated 'type M', and the less-restricted organisms (S2A1 and PM6) are designated 'type L'. The only methylotrophs known to the authors, which have a similarly restricted growth-substrate specificity, are the hyphomicrobia (Hirsch & Conti, 1964; Quayle, 1972). Attwood & Harder (1973, 1974) tested their strains of hyphomicrobia on a variety of $C_2$, $C_3$, $C_4$, $C_5$ and $C_6$ compounds and only ethanol (all strains), acetate (most strains) or β-hydroxybutyrate supported growth. These data suggest that hyphomicrobia are restricted facultative methylotrophs.

### *Characterization of the restricted facultative methylotrophs*

Some morphological and physiological properties of isolates S2A1, W3A1 and W6A are presented in Table 2 together with the corresponding properties of *Bacillus* PM6 as determined by Myers (1971). The

Table 2. *Some morphological and physiological properties of the restricted facultative methylotrophs*

All the isolates are motile, non-pigmented, obligately aerobic, oxidase-positive, catalase-positive, rod-shaped bacteria. The following symbols are used: N.G., no growth in the test medium; A, short regular rods; B, large regular rods; +, positive; −, negative; N.T. not tested. Details of differences between isolates W6A and W3A1 in liquid and on solid media are given in the text.

| Source ... | Pond-water | River-water | Soil | Soil* |
|---|---|---|---|---|
| Isolate ... | W6A | W3A1 | S2A1 | PM6 |
| Morphology | A | | B | B |
| Gram reaction | − | | + | + |
| Slime produced | + | | − | − |
| Nitrate reduction | N.G. | | − | + |
| Acid produced from glucose aerobically | + | | + | + |
| Survival after 10min at 80°C | N.T. | | + | + |
| Endospores | − | | + | + |
| GC ratio (mol of G + C/100 mol) | 49.0–49.5† | | 34.9 | 36.1 |
| Probable genus | ? | | *Bacillus* | *Bacillus* |

* Data of Myers (1971), except for the GC ratio.
† Values for isolates 4B6 and C2A1 are 48.1 and 52.0 respectively.

properties of isolate S2A1 given in Table 2 suggest that it is a member of the genus *Bacillus*. Isolates W3A1 and W6A do not grow on complex laboratory media, precluding the use of most routine physiological tests as aids to identification, and have not been assigned to genera. The properties of isolates W31A and W6A given in Table 2 are identical, as are the growth substrates that will support their growth. They differ, however, in at least two respects: (i) organism W6A shows much more severe clumping towards the end of exponential phase in shaken trimethylamine liquid culture; (ii) on solid media, isolate W6A but not W3A1 forms colonies that are difficult to disperse.

*Specific activities of enzymes of the tricarboxylic acid cycle and of other enzymes associated with the cycle in extracts of methylotrophs*

Table 3 shows that, when grown on trimethylamine, all the methylotrophs have zero or insignificant 2-oxoglutarate dehydrogenase activity and low specific activities of at least some of the other enzymes of the cycle. These data are consistent with the tricarboxylic acid-cycle reactions playing an anabolic rather than an amphibolic role during bacterial growth on trimethylamine (cf. Colby & Zatman, 1972). The possibility that the deficient 2-oxoglutarate dehydrogenase step in these organisms could be by-passed by isocitrate lyase (Kelly, 1971) is precluded by the finding of very low or zero specific activities of this enzyme under all growth conditions tested (Table 3; Colby & Zatman, 1972).

The specific activities of tricarboxylic acid-cycle enzymes present in trimethylamine-grown methylotrophs (Table 3; Colby & Zatman, 1972) indicate a marked resemblance between the type M restricted facultatives and the obligates 4B6 and C2A1. Thus in both the obligates and the type M organisms when grown on trimethylamine, (i) 2-oxoglutarate dehydrogenase is not detectable, (ii) succinate dehydrogenase, malate dehydrogenase and succinyl-CoA synthetase are either undetectable or are present at very low specific activity, and (iii) $NAD^+$-dependent isocitrate dehydrogenase is present at relatively high specific activity whereas NADP-dependent isocitrate dehydrogenase is present at low specific activity. Further, when the type M organisms are grown on glucose the specific activities of tricarboxylic acid-cycle enzymes are virtually unchanged apart from the specific activity of malate dehydrogenase which increases significantly. These observations suggest that the type M organisms, and perhaps the obligates that they resemble, contain invariable low specific activities of most of the tricarboxylic acid-cycle enzymes. However, as the type M organisms grow extensively, albeit slowly, on glucose, such a deficient tricarboxylic acid cycle cannot be the sole explanation of the inability of the obligates to grow on glucose. This leads to the conclusion that the obligates are impermeable to glucose, and/or they lack a mechanism, alternative to the tricarboxylic acid cycle, for the provision of assimilatory power from glucose; such an alternative pathway must exist in the type M organisms.

In contrast with the obligate and the type M organisms, trimethylamine-grown type L restricted facultatives have (i) very low specific activities of 2-oxoglutarate dehydrogenase, (ii) relatively high specific activities of succinate dehydrogenase, malate dehydrogenase and succinyl-CoA synthetase, the values for which are comparable with those found

Table 3. *Specific activities of enzymes of the tricarboxylic acid-cycle and related enzymes in extracts of methylotrophs*

All values are expressed as nmol/min per mg of protein, and were determined as described in the 'Materials and Methods' section. N.T. Not tested; TMA, trimethylamine; Glu, glutamate; Cit, citrate; Glc, glucose; Ala, alanine.

| | Obligate | Type M | | | | Type L | | | | | | Typical facultative | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Organism ... | C2A1 | W3A1 | | W6A | | S2A1 | | | PM6 | | | 5B1 | |
| Growth substrate ... | TMA | TMA | Glc | TMA | Glc | TMA | Ala | Glc | TMA | Cit | Glut | TMA | Glu |
| Mean generation time (h) ... | 2.5 | 2.5 | 38 | 2.5 | 32 | 3 | 4 | 3 | 3 | 4 | 12 | 4 | 4.5 |
| Citrate synthase | 13 | 13 | 14 | 23 | 27 | 33 | 160 | 145 | 39 | 25 | 13 | 41 | 58 |
| Aconitate hydratase | 28 | 52 | 27 | 2 | 4 | 36 | 153 | 87 | 62 | 89 | 63 | 400 | 590 |
| Isocitrate dehydrogenase ($NADP^+$) | 11 | 9 | 6 | 3 | 4 | 34 | 24 | 29 | 26 | 32 | 2 | 220 | 685 |
| Isocitrate dehydrogenase ($NAD^+$) | 52 | 43 | 36 | 39 | 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2-Oxoglutarate dehydrogenase ($NAD^+$) | N.T. | 0 | 0 | 0 | 0 | 2 | 2 | 1 | 1 | 3 | 0 | N.T. | 79 |
| 2-Oxoglutarate dehydrogenase ($APAD^+$) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | N.T. |
| Succinyl-CoA synthetase | 3 | 2 | 5 | 4 | 2 | 10 | 27 | 30 | 16 | 41 | 27 | 14 | 55 |
| Succinate dehydrogenase | 0 | 1 | 0 | 0 | 0 | 14 | 152 | 42 | 30 | 105 | 157 | 24 | 79 |
| Fumarate hydratase | 0 | 30 | 20 | 0 | 0 | 57 | 121 | 264 | 79 | 338 | 240 | 210 | 165 |
| Malate dehydrogenase | 6 | 7 | 40 | 5 | 32 | 164 | 1050 | 640 | 680 | 646 | 549 | 675 | 1170 |
| Glutamate dehydrogenase ($NADP^+$) | 345 | 0 | 0 | 0 | 0 | 95 | 1500 | 810 | 79 | 742 | 48 | 109 | 210 |
| Glutamate dehydrogenase ($NAD^+$) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Alanine dehydrogenase ($NAD^+$) | N.T. | N.T. | N.T. | N.T. | N.T. | 0 | 2550 | 0 | 0 | 270 | 0 | N.T. | N.T. |
| Isocitrate lyase | 2 | 3 | 6 | 1 | 2 | 0 | 0 | 0 | 1 | 3 | 2 | 8 | 0 |

in the typical facultatives *Pseudomonas* 3A2 and bacterium 5B1 (Colby & Zatman, 1972), and (iii) $NADP^+$-dependent, but no $NAD^+$-dependent isocitrate dehydrogenase. The type L organisms possess the ability to increase the specific activities of at least some of the tricarboxylic acid-cycle enzymes when grown on non-$C_1$ compounds with the perhaps significant exceptions of 2-oxoglutarate dehydrogenase, which remains at a very low value, and isocitrate dehydrogenase. The absence of 2-oxoglutarate dehydrogenase and virtual absence of isocitrate dehydrogenase in glutamate-grown isolate PM6 precludes the tricarboxylic acid cycle as the catabolic route for glutamate, and some other, at present unknown, catabolic route must therefore be present. Similarly an alternative route to the tricarboxylic acid cycle must be present during the growth of organism PM6 on citrate, and of isolate S2A1 on alanine and glucose in view of the very low 2-oxoglutarate dehydrogenase activities present during growth on these substrates.

It is concluded that all the obligate and restricted facultative methylotrophs have a deficient tricarboxylic acid cycle whether grown on $C_1$ or non-$C_1$ substrates. Except for the results of Davey *et al.* (1972), obtained with the type II methane-oxidizing bacteria, all the obligate methylotrophs that have been examined possess a deficient tricarboxylic acid cycle (Table 3; Colby & Zatman, 1972; Davey *et al.*, 1972; Dahl *et al.*, 1972; Patel *et al.*, 1969). This can no longer be accepted as the sole cause of obligate methylotrophy because of the existence of the restricted facultative methylotrophs which, despite the presence of a deficient tricarboxylic acid cycle, are nonetheless able to grow on certain non-$C_1$ substrates. As discussed above, hyphomicrobia can also be regarded as restricted facultative methylotrophs. Attwood & Harder (1973, 1974) have demonstrated the virtual absence of the pyruvate dehydrogenase complex in their strains. This lesion prevents the conversion of non-$C_1$ compounds, except for acetate and its precursors, into acetyl-CoA and the hyphomicrobia can therefore also be regarded as having a deficient tricarboxylic acid cycle.

### *NAD(P)H-oxidizing activity in extracts of methylotrophs*

The ability of extracts of methylotrophs to catalyse NAD(P)H oxidation was measured in view of the original proposal of Smith *et al.* (1967) that the absence of NADH oxidase could explain the phenomenon of obligate autotrophy (see the discussion by Kelly, 1971). The results in Table 4 show that extracts of all the methylotrophs examined catalyse NADH oxidation and in all but bacterium 4B6 this rate of oxidation is significantly decreased by 1 mM-cyanide suggesting that cytochrome oxidase is involved; such a $CN^-$-sensitive system could therefore mediate ATP synthesis during electron transport. The significance of the relatively high amounts of NADPH-oxidizing activity in extracts of organisms S2A1 and PM6 grown on trimethylamine is at present obscure (but see Colby & Zatman, 1975).

Table 4. *NAD(P)H-oxidizing activity in extracts of methylotrophs*

All values are expressed as nmol/min per mg of protein and were determined spectrophotometrically as described in the Materials and Methods section. When used, cyanide was present at 1 mM.

| | | NADH oxidation | | NADPH oxidation | |
|---|---|---|---|---|---|
| Isolate | Growth substrate | $-CN^-$ | $+CN^-$ | $-CN^-$ | $+CN^-$ |
| 4B6* | Trimethylamine | 12 | 12 | 2 | 2 |
| C2A1 | Trimethylamine | 133 | 19 | 6 | 5 |
| W6A | Trimethylamine | 73 | 38 | 4 | 4 |
| | Glucose | 60 | 43 | 2 | 3 |
| W3A1 | Trimethylamine | 50 | 16 | 11 | 11 |
| | Glucose | 50 | 21 | 0 | 0 |
| S2A1 | Trimethylamine | 31 | 10 | 134 | 107 |
| | Glucose | 87 | 8 | 0 | 0 |
| PM6 | Trimethylamine | 41 | 7 | 173 | 125 |
| | Citrate | 74 | 27 | 20 | 0 |
| 3A2* | Trimethylamine | 35 | 3 | 3 | 3 |
| | Succinate | 77 | 7 | 7 | 3 |
| 5B1* | Trimethylamine | 162 | 10 | 7 | 5 |
| | Glutamate | 140 | 15 | 5 | 3 |

* Isolate 4B6 is an obligate methylotroph; isolates 3A2 and 5B1 are typical facultative methylotrophs (Colby & Zatman, 1972, 1973).

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