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Isolation and Growth Rates of Methanol Utilizing Rhodospirillaceae

H. A. DOUTHIT and N. PFENNIG

Institut für Mikrobiologie der Gesellschaft für Strahlen- und Umweltforschung m.b.H. München, Grisebachstr. 8., D-3400 Göttingen, Federal Republic of Germany

Abstract. 38 pure culture strains belonging to seven species of the Rhodospirillaceae were isolated from 39 methanol enrichment cultures inoculated with water and mud samples of different habitats. None of the strains exhibited doubling times shorter than 10 h in methanol-bicarbonate media.

Key words: Methanol enrichment cultures – Rhodospirillaceae – *Rhodopseudomonas acidophila* – Growth rates.

Quayle and Pfennig (1975) described optimal growth conditions for the cultivation of strains of the phototrophic Rhodospirillaceae in simple methanol-bicarbonate media. As a readily available and inexpensive organic carbon source and hydrogen donor, methanol is of interest for the production of single cell protein. The use of phototrophic bacteria as animal food supply was experimentally supported by the work of Kobayashi (1973). The strains of the Rhodospirillaceae studied so far have shown generation times of 13 h and more. We were interested, therefore, in searching for new, faster growing strains using enrichments in simple methanol-bicarbonate media.

38 water and mud samples collected from ditches, ponds, lakes and rivers in Ireland and Germany, were inoculated as promptly as possible after collection into 50 ml srew-cap bottles completely filled with methanol-bicarbonate medium (Quayle and Pfennig, 1975). A relatively high methanol concentration (0.3%) was used for the enrichments and all subsequent media. Cultures were incubated at 28° C and 500–1000 lux light intensity from a 100 watt tungsten lamp. Secondary to quarternary enrichments were inoculated when growth of phototrophic bacteria in the previous enrichment became apparent.

Final enrichments were streaked on plates using the same medium plus 2% agar and 3.7 mM phosphate buffer at pH 6.8; the plates were incubated in anaerobic jars under an atmosphere of 95% nitrogen and 5% CO_2 for 4–7 days. Pigmented colonies of phototrophic bacteria were restreaked on second plates from which pure cultures could be isolated. Stock cultures were maintained in 50 ml screw-cap bottles with succinate medium (Quayle and Pfennig, 1975). 38 pure cultures capable of growing in the presence of 0.3% methanol were thus obtained. As can be seen from Table 1, the newly isolated strains belong to seven species of the Rhodospirillaceae, of which *Rhodopseudomonas (R.) palustris* and *R. acidophila* were the most frequently isolated species.

Growth rate determinations were carried out with several of the fastest growing isolates after selection from the more slowly growing strains. The seven most promising isolates and two strains from the stock culture collection (10050 and TL 1) were pregrown in methanol-bicarbonate medium and transferred to screw-cap test tubes containing the same medium. The turbidity of the cultures was recorded at 650 nm using a Bausch and Lomb Spectronic 70 photometer. The maximal growth rates and doubling times given in Table 2 were calculated from the early portion of semilogarithmic plots of E_{650} as a function of time.

Although the ability to utilize methanol for growth is widespread among Rhodospirillaceae (Table 1), it is apparent that only very few strains – all belonging to the one species *R. acidophila* – show fairly good doubling times around 10–15 h. None of all the strains isolated by methanol enrichment grew faster than *R. acidophila* strain 10050, an organism from the culture collection which had not been isolated on methanol.

The results of our extensive enrichment efforts may indicate that there are hardly any phototrophic bacteria in nature which are particularly well adapted

Table 1. Taxonomic affiliation of the newly isolated methanol-utilizing Rhodospirillaceae strains

Species	Number of strains isolated
Rhodopseudomonas palustris	16
Rhodopseudomonas acidophila	9
Rhodospeudomonas sphaeroides	4
Rhodopseudomonas gelatinosa	4
Rhodopseudomonas capsulata	1
Rhodomicrobium vannielii	3
Rhodospirillum fulvum	1

Table 2. Doubling times and growth rates of the fastest-growing methanol-utilizing Rhodospirillaceae

Species	Strain designation	Doubling time (h)	Maximum growth rate (h^{-1})
Rhodopseudomonas acidophila	10050	10.6	0.094
Rhodospeudomonas acidophila	GD-14	12.1	0.083
Rhodospeudomonas acidophila	GD-24	12.3	0.082
Rhodopseudomonas acidophila	TL 1	15.2	0.066
Rhodopseudomonas palustris	GD-39	39.4	0.025
Rhodopseudomonas acidophila	GD-9	44.4	0.022
Rhodopseudomonas acidophila	GD-16	50.6	0.02
Rhodomicrobium vannielii	GD-31	51.0	0.019
Rhodopseudomonas acidophila	GD-27	81.8	0.012

to the utilization of methanol. This assumption is supported by the studies of Sahm et al. (1975) who demonstrated for the fast growing *R. acidophila* strain 10050 that it utilizes methanol autotrophically. The authors characterized methanol, formaldehyde and formate dehydrogenase which oxidize methanol to CO_2 and thus provide the necessary reducing equivalents for autotrophic CO_2 fixation; Ribulosediphosphate carboxylase was present at a specific activity sufficient to account for the actual growth rate of the organism. The authors obtained no evidence for the operation of any known reduced C_1-fixation sequence. For growth on formate the same situation was found by Yoch and Lindstrom (1967) and Stokes and Hoare (1969) in *R. palustris*. Under autotrophic growth conditions with molecular hydrogen as the electron donor, *R. acidophila* strain 10050 exhibits a minimal doubling time of 4.4 h (maximum growth rate 0.157 h^{-1}) (Göbel, personal communication). It is therefore most likely that the relatively long doubling time of 10.6 h on methanol is not due to growth rate limitation by the rate of the autotrophic CO_2-fixation reactions but rather by the rates of the dehydrogenations of methanol, formaldehyde and formate.

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