

Growth of *Derxia gummosa* and *Azospirillum* spp. on C₁-Compounds *

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Bacterial growth on C₁-compounds requires *de novo* synthesis of carbon-to-carbon bonds which are lacking in these compounds [1]. Growth on methane and other C₁-compounds by certain N₂-fixing bacteria has been shown by Davis *et. al* [2] and Bont [3]. This work reports the ability of *Derxia gummosa*, *Azospirillum brasilense* and *A. lipoferum* to grow on methane, methanol and formate as sole carbon sources.

Azospirillum lipoferum (strains 208, BC3, BR17, 209, RG20), *Azospirillum brasilense* (strain Sp 7, 107) and *Derxia gummosa* were grown in the liquid medium of Pedrosa *et al.* [4] where the FeSO₄·7H₂O was chelated by nitrilotriacetic acid (0.0014 g.l⁻¹) and lactate (1.0 g.l⁻¹) was used as carbon source. Potassium nitrate (0.5 g.l⁻¹) was used as the N source. For methane and other C₁-compound dependent growth, lactate was omitted. Growth was tested in "suba seal" sealed test tubes containing 3 ml liquid medium under atmospheres of O₂:N₂ (2:98, v/v) or O₂:CH₄:N₂ (2:20:78, v/v). Formate or methanol were added at concentrations of 0.2% (w/v). All experiments were incubated at 30°C under static conditions. Growth was estimated by turbidity at 560 nm and by total protein by Lowry [5].

Radioactivity of ¹⁴C was measured in a Beckman Scintillation counter using Bray's scintillation fluid (10 ml). The composition of the gas phases was measured either using a Poropak Q or a Molecular Sieve 5A column fitted in a Varian Gas-chromatograph with a

T.C. detector and was corrected whenever necessary.

The three species grew on lactate-free medium when methane, methanol or formate were present (Table I). The doubling times were not significantly different when compared with growth on lactate, this being sub-optimum due to the low oxygen content. Formate was the best C₁-source for the three bacteria (protein yields and doubling time).

Results on Table I show that *D. gummosa* and *Azospirillum* spp. can be classified as facultative methylotrophs, however an interesting feature of these organisms is that they grew on formate and also on methane. According to Anthony [1] no facultative methylotroph would be able to grow on methane.

TABLE I

Growth (Protein Yields*) of *Derxia gummosa*, *Azospirillum brasilense* (Strain Sp 7) and *A. lipoferum* (Strain Sp 208) on Lactate and C₁-Compounds

	METHANE	METHANOL	FORMATE	LACTATE	-CONTROL
<i>D. gummosa</i>	174	97	340	308	65
<i>A. brasilense</i>	164	212	244	348	63
<i>A. lipoferum</i>	235	124	270	495	65

* The initial protein values were 24, 20 and 30 µg per ml. respectively for the three organisms (yields in µg. ml⁻¹. per 96 h).

Growth of 5 different *Azospirillum* spp. strains under CH₄ and ¹⁴CO₂ was accompanied by incorporation of ¹⁴C from CO₂ even though the major percentage of the C seemed to come from CH₄. Experiments with strains Sp 7 and Sp 208 using ¹⁴CH₄ however showed neither significant incorporation of

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¹⁴C into biomass nor was it oxidized to CO₂. Experiments to clarify this apparent controversy are under way.

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