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A PROBABLE PATHWAY IN THE DEGRADATION OF 2-METHOXYETHANOL BY PSEUDOMONAS SPECIES STRAIN VB.

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ABSTRACT

A novel bacterium, *Pseudomonas* species strain VB, able to degrade 2-methoxyethanol was isolated from anaerobic sewage sludge. The bacterium utilizes 2-methoxyethanol as sole source of carbon and energy. In a time course experiment *Pseudomonas* sp. strain VB was grown in 2-methoxyethanol, ethylene glycol, glycollate, glyoxylate, oxalate and methanol. Strain VB was able to utilize all proposed intermediates except methanol. Comparable growth characteristics of *Pseudomonas* sp. strain VB grown in 2-methoxyethanol and proposed intermediates showed significant molar growth yields. These results indicate that ethylene glycol, glycollate, glyoxylate and oxalate might be intermediates in the degradation pathway of 2-methoxyethanol. Thus a reaction sequence: 2-methoxyethanol → ethylene glycol → glycollate → glyoxylate → oxalate is been proposed in this study.

KEYWORDS: Degradation, 2-methoxyethanol, *Pseudomonas* species strain VB, metabolic pathway.

INTRODUCTION

2- Methoxyethanol is a colourless substance that is highly soluble in water and acetone. It has a molecular weight of 76.10g/mol and density of 0.964g/l (Merck Index, 1989). It is used in nail polishes and wood staining process. 2-methoxyethanol serves as a good extracting agent for a mixture of components of polychloroethylene, benzene, toluene and xylene-aromatics (Brau – Stormeyer and Meyer, 1995). It is poisonous when injected orally and has a lethal concentration of 1500ppm for mice in air (ABC Chemie, 1965, Merck Index, 1989).

Marty and Loch –Caruso (1989) reported that 2-methoxyethanol prolonged the gestation period in rodents, due to it's inhibition of the gap junctional communication.

2- methoxyethanol is a member of the organic compounds called glycols, which comprise one unit of ethylene oxide with a -C-O-C- ether linkage terminating with an hydroxyl bond (- OH) at one end (Cox, 1978). The metabolism of 2-methoxyethanol under anaerobic conditions has been extensively investigated and detailed information has been presented on degradation rates and metabolic pathway (Tanaka *et al*; 1986 Tanaka and Pfeninig, 1988). However, the degradation of 2- methoxyethanol under aerobic condition has hardly been previously investigated. A bacterium *Pseudomonas* sp. strain VB isolated from anaerobic sludge was found to degrade 2-methoxyethanol under aerobic conditions (Ekhaise, 2002). The aim of this study is to investigate the probable degradative pathway of 2- methoxyethanol by *Pseudomonas* sp. strain VB under aerobic conditions.

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade (99.9%). 2- methoxyethanol, ethylene glycol, glycollate, glyoxylate, methanol and oxalate were obtained from Fluka (Buchs, Switzerland).

Biodegradation of 2-methoxyethanol and proposed intermediates.

An overnight culture of *Pseudomonas* sp. strain VB was used to inoculate 10 liter of mineral salt medium containing 2-methoxyethanol (126.7mmol). The culture was incubated at 30°C under aerobic conditions. Other cultures in which 2-methoxyethanol was replaced by ethylene glycol, glycollate, glyoxylate, oxalate, methanol, formaldehyde and formate as sole source of carbon and energy were similarly treated and observed for growth. Growth in these compounds was regarded as ability of *Pseudomonas* sp. strain VB to degrade these compounds. The rate of degradation of 2-methoxyethanol, ethylene glycol and methanol was monitored by gas chromatography (Model 430 Packard, Netherlands), while that of glycollate, glyoxylate, oxalate, formaldehyde and formate was monitored colorimetrically by a spectrophotometer (LKB Biochrom, Ultrospec 4050 Cambridge, England) at 530nm, 430nm, 600nm, 500nm and 540nm respectively (Kakac and Vejedelek, 1974).

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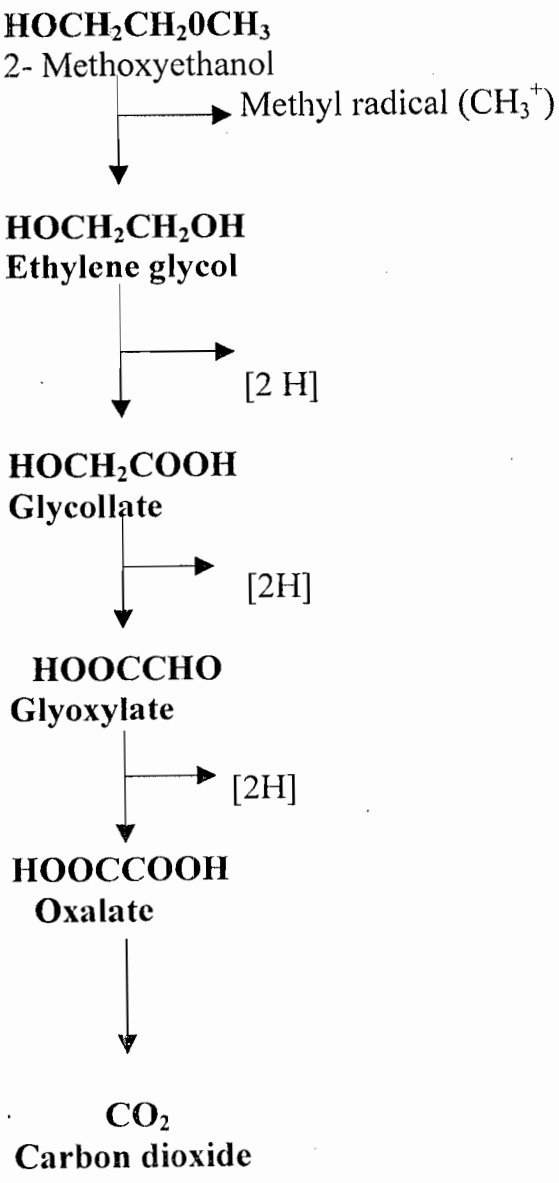

Fig: 1. Proposed reaction pathway in the degradation of 2-methoxyethanol by *Pseudomonas* sp. strain VB

Determination of cell dry weight of bacteria

Dry weight of *Pseudomonas* sp. strain VB was determined by method described by Gerhardt *et al* (1994).

RESULTS

Aerobic transformation of 2-methoxyethanol and hypothetical pathway intermediates.

A typical time course of 2-methoxyethanol and proposed intermediates utilization by *Pseudomonas* sp. strain VB is shown in Fig. 2. Figure 1 shows the putative microbial degradation of 2-methoxyethanol in *Pseudomonas* sp. strain VB under aerobic conditions. Only ethylene glycol, glycollate, glyoxylate and oxalate supported growth of the organism. Growth was not observed with methanol, formaldehyde and formate. The utilization of 2-methoxyethanol was slow decreasing in concentration from 126.7mmol to 39.8mmol after 57h, glycollate decreased rapidly in the first nine hours (9h), reaching a minimum of 43.76mmol after 33h and glyoxylate degradation followed the same pattern. Oxalate decreased in the first 33h from126.7mmol to 42,50mmol. Ethylene glycol was degraded uniformly from 126.7mmolmmol to 42.6mmol at the end of 48h.

Growth characteristics.

Growth (cell density) was highest with 2-methoxyethanol (Fig. 2) followed by glycollate and glyoxylate. However, growth was poor with ethylene glycol and oxalate. Growth peaked at 10h with glycollate and glyoxylate (Fig. 3), which agreed with the high degradation rate noted earlier. Doubling time of 1.739h, 1.937h, 1.198h, 1.408h and 9.231h were observed for 2-methoxyethanol, ethylene glycol, glycollate, glyoxylate and oxalate respectively. The growth yields with 2-methoxyethanol, ethylene glycol, glycollate, glyoxylate and oxalate were 13.728, 1.704, 3.511, 2.454 and 0.308g (cell dry weight/mol substrate) respectively (Table 1). The low growth yield observed with oxalate was probably due to the highly oxidized form of oxalate, which therefore produced an inhibitory effect on the growth (Harder, 1973).

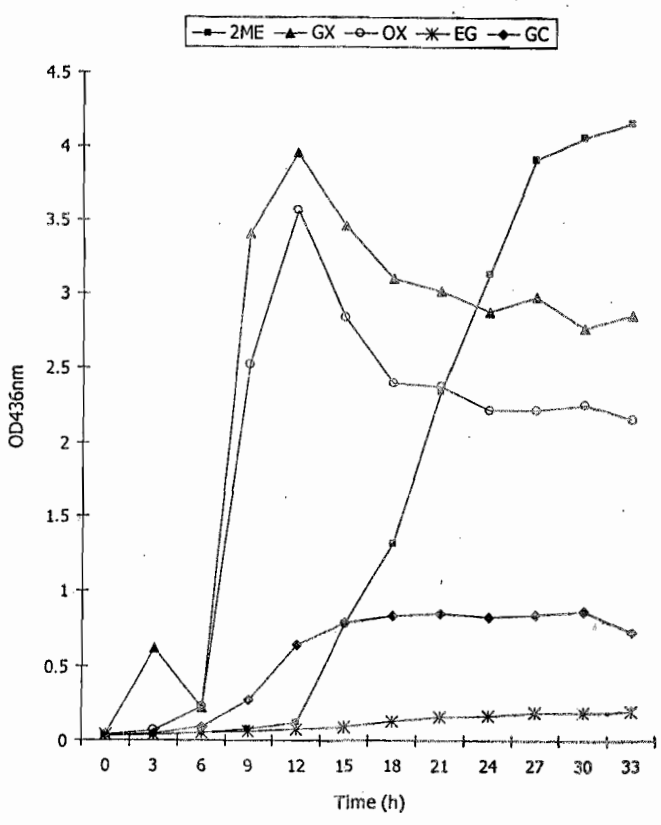

Fig. 2: Growth rate of Pseudomonas SP. strain VB with 2-methoxyethanol and the proposed intermediates.

Key: 2-ME (MG) = 2-Methoxyethanol, EG = Ethylene glycol, GK(GC) = Glycollate, GX= Glyoxylate and OX = Oxalate.

TABLE 1: Growth yield of *Pseudomonas* sp. strain VB

Substrate	Molar growth yield (cell dry weight/mol. Substrate)	Growth rate (Generation time in hr)
2-methoxyethanol	13.728	1.739
Ethylene glycol	1.704	1.937
Glycollate	3.511	1.198
Glyoxylate	2.454	1.408
Oxalate	0.308	9.231

DISCUSSION

In the proposed reaction pathway (Fig, 1, the degradation of 2-methoxyethanol was initiated by oxidative decarboxylation with methanol as a by-product. *Pseudomonas* sp. strain VB was however unable to utilize methanol. This may be because it ends up as a methyl radical $-CH_3^+$ (Diekert, 1992). The ethylene glycol resulting from above decarboxylation is converted to oxalate through glycollate, glyoxylate by oxidative dehydrogenation. Finally oxalate is converted to carbon and water. All these intermediates were actively degraded by *Pseudomonas* sp. strain VB. The higher rate of degradation of the ethylene glycol, glycollate, glyoxylate and oxalate compared to 2-methoxyethanol appears to support the proposal that they are intermediates in the reaction pathway. However, the high growth yield for glycollate and glyoxlate (compared to ethylene glycol) seems to show that there might be an alternative pathway which may involve a reduction reaction. This theory is supported by the report of Clarke and Ornston (1975), who found that glyoxylate formed from a number of alternative pathways by species of *Pseudomonas* when grown on glycollate, glycine and oxalate. The utilization of glycollate and glyoxylate as substrates by *Escherichia coli* K-12 involves a series of similar reactions. The glycollate and glyoxylate are interconverted by distinct enzymes, glycollate oxidase and glyoxylate reductase (Ornston and Ornston, 1969).. The GC analysis showed only the peak of 2-methoxyethanol during the period of degradation. In the anaerobic degradation of 2-methoxyethanol by *Acetobacterium malicum*, ethylene glycol was suggested as one of the intermediates but could not be detected by GC (Tanaka *et al.*, 1986), indicating the transcient nature of these intermediates. Further work to confirm the reaction pathway involving the isolation of enzymes involved in the degradation pathway, the application of nuclear magnetic resonance(NMR), gas chromatography(GC) and mass spectrometry are currently being investigated.

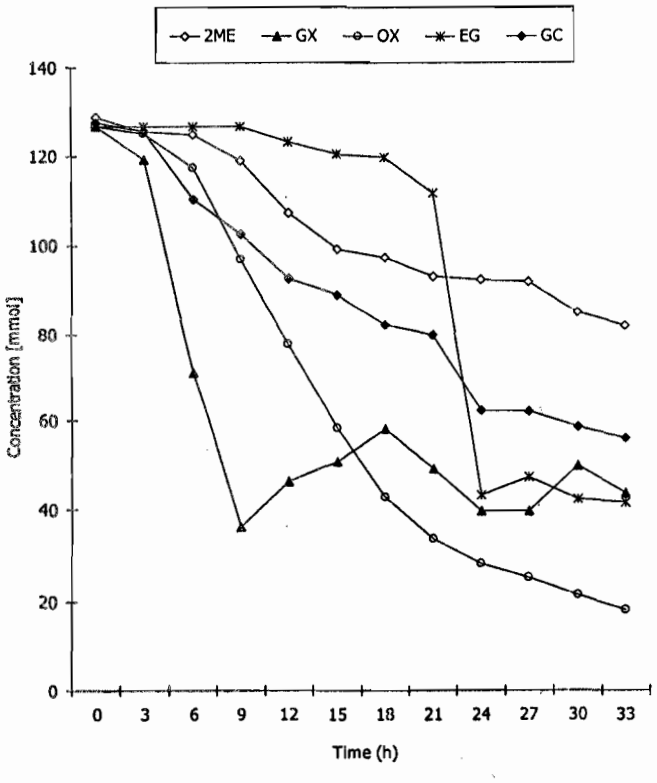

Fig. 3: Degradation of 2-methoxyethanol and proposed Intermediate compounds in the reaction pathway.

Key: 2-ME (MG) = 2-Methoxyethanol, EG = Ethylene glycol, GK(GC) = Glycollate, GX= Glyoxylate and OX = Oxalate.

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