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N- and *S*-methyl aromatics as carbon source for growth of *Acetobacterium woodii*

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Introduction

Previous reports showed that many acetogenic bacteria including *A.woodii* can grow and demethylate several *O*-methyl substituted aromatic compounds to produce their corresponding hydroxy derivatives and acetate. The acetogenic bacteria uses the methyl groups released as a carbon and energy source for growth (Sembiring and Winter, 1990; Bache and Pfennig, 1981; Tschech and Pfennig, 1984; Krumholz and Bryant, 1986). The acetogens are also expected to perform *N*- or *S*-demethylation by a mechanism analogous to *O*-demethylation. Only few reports on *N*-demethylation by bacteria found in literature, they are *N*-demethylation by Streptomyces platensis (Davis *et al.*, 1979), S.griseus (Davis *et al.*, 1977), *S.linoclensis, Srimosus* and *S.paucisporogenes* (Sewell *et al.*, 1984). They performed N-demethylation of lergotrile, glaucine, metazocine and codeine, respectively, to the *N*-desmethyl products. *S.griseus* showed both *N*- and *O*-demethylations, as it transformed glucine to norglaucine and *2-O*-demethyl gloucine (Davis *et al.*, 1977). There was no report on *S*-demethylation in the literature. Demethylation of a compound such as methyl-N-methylanthranilate was important, since this may provide a route for the synthesis of methylanthranilate, which is useful in the food industry as a component in food flavourings (Page *et al.*, 1988). In this study, the ability of *A.woodii* to grow and demethylate aromatic compounds substituted with *N*- and *S*-methyl groups was assessed.

Materials and Methods

A.woodii DSM 1030 was grown and maintained at 30°C in an anaerobic cabinet (Don Whitely Scientific Ltd. U.K.) in "Balch" medium (Balch *et al.*, 1977) supplemented with selenite-tungstate solution (Tsechech and Pfennig, 1984). The medium was made anaerobic before sterilisation (Hungate, 1969) and sterile medium components were mixed together and dispensed in the anaerobic cabinet. The ability of *A.woodii* to demethylate *N*- and *S*-methyl substituted aromatic compounds was assessed by assessing growth of the bacterium on the test substrates at 5 mM concentration as the organic carbon source. Growth was monitored by measuring the optical density (OD) of the culture at 660 nm. Substrates inhibition (toxicity) was assessed by growing the culture on fructose plus the test compound.

Results and Discussion

None of *N*- or *S*-methyl aromatics and methylesters substrates tested supported growth of *A.woodii* (Table 1).

Although *A.woodii* was unable to grow on *N*-methylaniline and *N*-methylanthranilate, these compounds were less toxic than the ester, methyl-N-methylanthranilate. Growth on fructose plus 5 mM methyl-*N*-methylanthranilate resulted in 15.6% of the OD obtained in a culture grown with fructose alone. This compound was tested again at lower concentrations to find out the non-toxic concentration for growth. The maximum OD of the cultures grown on fructose plus methyl-*N*-methylanthranilate decreased with increasing concentration of methyl-*N*-methylanthranilate. Further incubation caused the OD of the cultures containing methyl-*N*-methylanthranilate to decrease significantly except the culture containing 0.5 mM of the compound, suggesting that the cells may have been killed due to the longer exposure to the compound. It was found that 0.5 mM of the compound was not toxic. However, growth of the organism on the compound at a non-toxic concentration was not done because it was not possible to assess growth on such low concentrations of the test substrate. Although the organism was able to grow on fructose in the presence of low concentrations of methyl-*N*-methylanthranilate, GCMS analysis showed that the compound was not demethylated. When the concentration of *4*-methylthiobenzoic acid was reduced from 5 mM to 2 mM, it wa still inhibitory. Therefore, it does not seem to be possible to assess growth at a lower concentration. *4*-Methylthiobenzylalcohol and *4*-methylmercaptophenol were not toxic, but did not support growth.

A.woodii seems to be unable to perform either *N*- or *S*-demethylation as indicated by the inability of the organism to grow on *N*- and *S*-methyl-substituted aromatic compounds. The *N*- and *S*-methyl compounds tested may be unable to induced the demethylase enzyme when the organism was grown on these compounds as the organic carbon source. Therefore, it will be necessary to test for demethylation of these compounds by using cells which have been induced for demethylation. Although most of these compounds were not toxic, some of them were inhibitory, such as methyl-*N*-methylanthranilate and *4*-methylthiobenzoic acid. However, the non-toxic concentration of both compounds seemed to be too low to be used for growth. Therefore, the use of non-growing cells induced for demethylation will be more convenient for testing for demethylation of these compounds.

Table 1: Growth of *A.woodii* on *N*- and *S*-methyl substituted aromatic compounds

Substrates	Max.OD 660nm	Toxicity (% growth)
Control (no substrate)	0.1	-
N-methyl aromatics		
N-methylaniline	0.05	81.3
N,N-dimethylanile	0.06	nd
N-methylanthranilate	0.075	98.5
Methyl-*N*-methylanthranilate	0.03	15.6
S-methyl aromatics		
Thioanisole	0.08	41.9
4-methylthiobenzoic acid	0.035	15.9
4-methylthiobenzylalcohol	0.08	98.5
4-methylmercaptophenol	0.035	94.7

It was reported that methyl-*N*-methylanthranilate was also toxic to *Sporomusa ovata* (Tsagourno, 1992) and many other microorganisms (Page *et al.*, 1988). It can be concluded that growing cultures of *A.woodii* are not suitable for demethylation of *S*- or *N*-methyl aromatics although they may be used for demethylation of *O*-methyl aromatics.

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