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Catechol Synthesis via Demethylation of Guaiacol by Anaerobic Bacterium *Acetobacterium woodii* DSM 1030

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Abstract: The ability of *A. woodii* to synthesise catechol from guaiacol was studied in "Balch" medium containing guaiacol in the presence or absence of fructose. Results showed that *A. woodii* was able to grow in both media containing guaiacol with or without fructose to produce catechol. Cultures incubated with a mixture of guaiacol and fructose gave higher concentration of cells than cultures grown with guaiacol alone. The highest concentration of cells (0.75 g L^{-1} cells dry weight) was found in a culture containing 5 g L^{-1} fructose plus 0.6 g L^{-1} guaiacol. Although cells production was relatively low in cultures with guaiacol compared with cultures with guaiacol and fructose, catechol synthesis was higher in the former cultures.

Key words: *Acetobacterium woodii*, demethylation, demethylase, catechol

Introduction

There is a growing interest in demethylation reactions in recent years. Some of the possible applications of demethylations are in the modification of natural products and also in drug synthesis (Page *et al.*, 1988; Sewell *et al.*, 1984; Davis *et al.*, 1985). Both aerobic fungi and bacteria can demethylate the methyl-substituted aromatic compounds. The fungi have doubling times of several days or weeks, whilst aerobic bacteria such as Pseudomonas have rapid growth. The disadvantages in using these organisms includes low reaction rates and the products produced after demethylation are subjected to further degradation (Cartwright and Smith, 1967). Anaerobic bacteria such as *A. woodii* grow rapidly with a doubling time of few hours, and this may offer rapid transformations (Balch *et al.*, 1977). Furthermore, this bacteria perform a single-step reaction and only transform the methyl substituents of the aromatics. Therefore, the products produced will be accumulated. In O-demethylation, the methyl group attached to an oxygen atom (R-OCH_3) is removed, whilst, in N-demethylation the methyl group attached to a nitrogen atom (R-N-CH_3) is removed. 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). Bache and Pfennig (1981) reported that growing cultures of *A. woodii* were able to perform O-demethylation of several methoxy aromatics, but the cultures were unable to perform either N- or S-demethylation (Kalil and Stephens, 2001). An oxygen sensitive compound such as catechol is hard to produce in aerobic condition due to auto-oxidation reaction when it exposed to air. Therefore, we would like to find an alternative method for catechol production by using anaerobic bacterium to avoid the product being auto-oxidized if produced aerobically. This research was aimed to study catechol synthesis via O-demethylation of guaiacol by growing cultures of *A. woodii*.

Materials and Methods

Acetobacterium woodii DSM 1030 was grown an-aerobically in an anaerobic jar at 30°C for 2-3 days. The bacterium was grown and maintained in both solid and liquid medium described by Balch *et al.* (1977) using fructose (5 g L^{-1}) as a carbon source. The medium was supplemented with selenite-tungstate solution (Tschech and Pfennig, 1984). The medium was made anaerobic before sterilization (Hungate, 1969) and sterile medium components were mixed together and dispensed in the anaerobic cabinet. The ability of *A. woodii* to demethylate guaiacol to catechol was assessed by measuring catechol produced using a method developed by LaRue (1964). Growth was monitored by measuring the optical density (OD) of the culture at 660 nm. Substrate inhibition (toxicity) was assessed by growing the culture in "Balch" medium containing fructose and the compound tested.

Results and Discussion

Catechol synthesis in cultures containing guaiacol as a single organic carbon for growth: The biomass increased with the increase in concentration of guaiacol from 0.6 to 1.2 g L^{-1} and there was only a small increase in biomass when 1.8 g L^{-1} guaiacol was used (Fig. 1). Cultures grown with 0.6 g L^{-1} guaiacol were growing at a limiting concentration of guaiacol and there was something else limiting growth at higher concentration of guaiacol. When the culture was provided with high concentration of guaiacol, acetate and catechol production were also high. It was found that *A. woodii* produces 0.228 and 0.432 g L^{-1} acetate when grown on 0.6 and 1.2 g L^{-1} guaiacol respectively. Whilst, the concentrations of catechol produced were 0.48, 0.8 and 0.94 g L^{-1} in cultures grown on 0.6, 1.2 and 1.8 g L^{-1} guaiacol. High concentration of acetate causes a low pH value of the culture, while catechol may be toxic to the cells. Therefore, the growth of the culture may be limited by the low pH value or by the toxic product (catechol) when high concentration of guaiacol was used. It was found that increasing guaiacol concentration up to 1.8 g L^{-1} did not increase the growth of *A. woodii*. This may suggest that either product or substrate inhibited the growth, since the compounds may be toxic to the cells (Bache and Pfennig, 1981). Therefore, we investigated the effect of guaiacol (substrate) and catechol (product) on growth of *A. woodii* on fructose. Guaiacol did not inhibit growth on fructose when provided at 0.6 g L^{-1} , while catechol (0.6 g L^{-1}) only caused slight inhibition (Table 1). However, both catechol and guaiacol inhibited the growth when the concentration was higher. Guaiacol was less inhibitory than catechol since *A. woodii* only grew to 13% of the optical density (OD) of the control culture when 2.4 g L^{-1} catechol was present. In contrast, the presence of 2.4 g L^{-1} guaiacol in cultures grown on 5 g L^{-1} fructose resulted in the production of 67% of the OD of the control culture.

It is believed that catechol production by *A. woodii* can be improved by several ways. For example, by using a non-toxic concentration of guaiacol. The toxic effect from accumulated catechol in the batch culture can be avoided if the product is removed from the culture by using a product removal system. Other method to improve catechol production is by using a chemostat culture operating with a limiting concentration of fructose and guaiacol. In the chemostat culture cells are uniform and cells are in the exponential stage of growth, so that they may have high demethylase activity.

Catechol synthesis in cultures containing guaiacol and fructose: Initial results indicated that *A. woodii* grew better on fructose and produce high concentration of cells. Since demethylase enzyme is produced inside the cells via tetrahydrofolate (THF) pathway during growth, it is possible to increase catechol production by increasing the cells produced. It was found that the demethylase

Table 1: Effect of guaiacol and catechol on growth of *A. woodii*

Concentration of catechol or guaiacol (g L^{-1})	Effect of guaiacol		Effect of catechol	
	Specific growth rate (μ) h^{-1}	Inhibition (%)	Specific growth rate (μ) h^{-1}	Inhibition (%)
0.0	0.129	0.0	0.129	0.0
0.6	0.123	0.0	0.12	7.0
1.2	0.111	17.0	0.09	26.0
1.8	0.091	29.3	0.051	44.9
2.4	0.065	33.4	not determined	87.0

[This experiment was done in order to find the suitable concentration of substrate (guaiacol) or product (catechol) for catechol synthesis by growing cultures of *A. woodii*.]

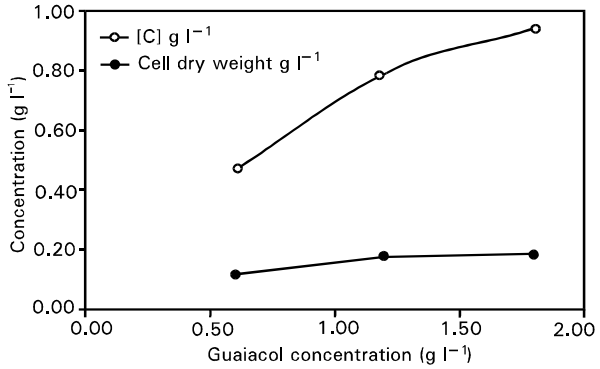

Fig. 1: Catechol synthesis in cultures containing guaiacol as a single organic carbon source [C] Catechol concentration

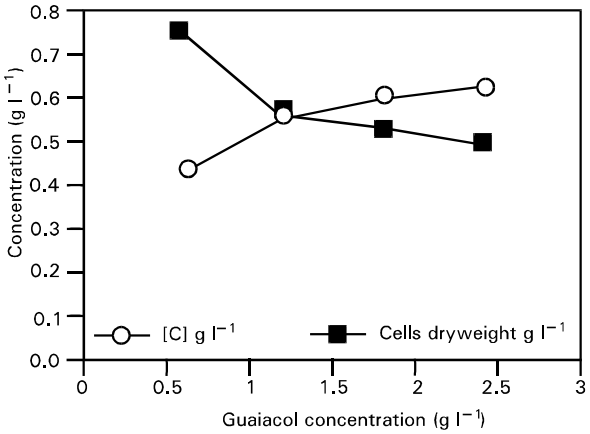

Fig. 2: Catechol synthesis in cultures containing guaiacol and fructose (5 g L^{-1}) [C] catechol concentration

was induced during growth on fructose in the presence of guaiacol since catechol was produced (Fig. 2).

Although *A. woodii* can demethylate guaiacol during growth on fructose, the concentrations of catechol produced were less than those obtained in cultures grown on guaiacol alone. Thus, cultures grown on fructose (5g L^{-1}) plus 0.6, 1.2 and 1.8 g L^{-1} guaiacol could only produced 91.1% [i.e. (0.4373 g L^{-1}/0.48 g L^{-1}) X 100%], 68.2% [i.e. (0.546 g L^{-1}/0.80 g L^{-1}) X 100%] and 64% [i.e. (0.602 g L^{-1}/0.94 g L^{-1}) X 100%] of catechol in cultures grown with similar concentrations of guaiacol without fructose respectively, although the former cultures had been incubated for 17.5 h longer. This suggested that the presence of high concentration of fructose (such as 5 g L^{-1}) in the culture may repress demethylase production in *A. woodii*. Therefore, we tried to minimize the fructose concentration to a level that was sufficient to achieve a maximum cell concentration. We found that cultures grown with limited concentration of fructose (2 g L^{-1}) plus guaiacol produced higher concentration of catechol compared with cultures grown with guaiacol alone or cultures grown with 5 g L^{-1} fructose plus guaiacol. Thus, 97.4% [i.e. (0.589 g L^{-1}/0.6 g L^{-1}) X 100%] of the substrate (guaiacol) was transformed to product (catechol) in a culture provided with 0.6 g L^{-1} guaiacol plus 2 g L^{-1} fructose.

The highest concentration of cells was obtained in a culture provided with 5 g L^{-1} fructose plus 0.6 g L^{-1} guaiacol (i.e. 0.75 g L^{-1} cells dry weight) while the lowest cells concentration was found in the cultures provided with 0.6 g L^{-1} guaiacol without fructose (i.e. 0.115 g L^{-1} cells dry weight). Although the presence of 5 g L^{-1} fructose increased cells concentration to 6.5 folds, less catechol was produced. However, the highest concentration of catechol can be achieved by using limiting fructose concentration.

Results showed that *A. woodii* was able to grow on guaiacol with or without fructose. Growth on guaiacol was due to the ability of the bacterium to remove the *O*-methyl group of guaiacol. The methyl group released is used by the cells as an organic carbon source for growth to produce catechol and acetate (Bache and Pfennig, 1981). Although a high concentration of fructose such as 5 g L^{-1} increased cells production, it repressed demethylase synthesis in cultures containing fructose and guaiacol. Therefore, a limited concentration of fructose should be used together with guaiacol in order to get better transformation of guaiacol to produce catechol.

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