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The *ortho* cleavage of catechol by *Azotobacter chroococcum*

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Azotobacter chroococcum **utilized catechol as sole carbon source. Maximum growth was supported by 2 mM. Catechol was cleaved by the *ortho* pathway mediated by catechol 1, 2-dioxygenase.**

In an effort to isolate efficient strains of *Azotobacter* that would rapidly cleave phenolic waste, we isolated a strain of *Azotobacter chroococcum* from Kalakad Reserve Forest, Tamil Nadu. Among the dihydric phenols which are substrates for ring cleavage, catechol is the most prominent one[1]. Azotobacters are known to cleave catechol by the *meta* pathway[2]. A study[3] of benzoate catabolism in *Azotobacter* sp. established the oxalocrotonate branch for *meta* cleavage of catechol.

A. chroococcum strain MSB1 was grown in nitrogen-free growth medium (mannitol, 5 g; K_2HPO_4, 0.03 g; $MgSO_4 2H_2O$, 0.2 g; $Na_2SO_4 10H_2O$, 0.2 g; $CaCl_2$, 0.01 g; $Na_2MoO_4 2H_2O$, 0.005 g; $FeSO_4 7H_2O$, 0.002 g; distilled water 1000 ml; pH 7.0) to which filter sterilized catechol at different concentrations was added. The cells were grown at 30°C at 125 rpm in an orbital shaker. After 3 days of incubation the mode of ring cleavage was determined by Rothera's reaction[4] and by the assay of catechol 1,2-dioxygenase[5].

Catechol was utilized by *A. chroococcum* after 8 h. Catechol at 2 mM was a better carbon substrate than at 5 mM; 10 mM was inhibitory, 20 mM was toxic (Figure 1). The generation time of the bacterium in 2 mM catechol was 4.7 h. Rothera reaction with catechol-grown cells yielded deep purple colour indicating the *ortho* cleavage of catechol and the presence of keto compounds. Catechol-grown cells displayed catechol 1,2-dioxygenase activity as evidenced by decrease in O.D. at 278 nm, the absorption maxima of catechol and increase in 258 nm, indicating *cis*,-*cis* muconic acid formation (Figure 2, *a*). The specific activity of the

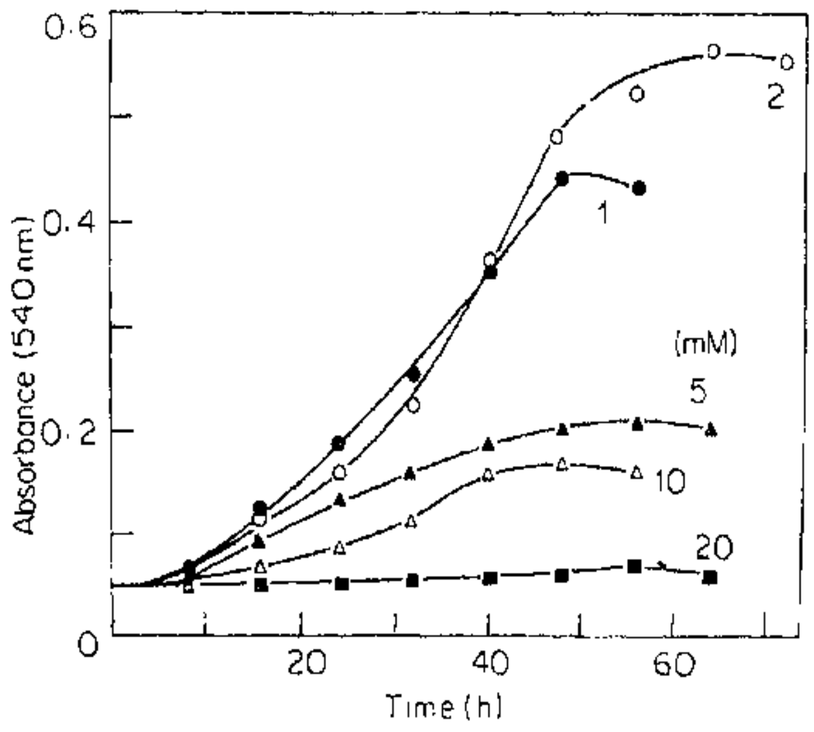

Figure 1. Growth of *A. chroococcum* in catechol.

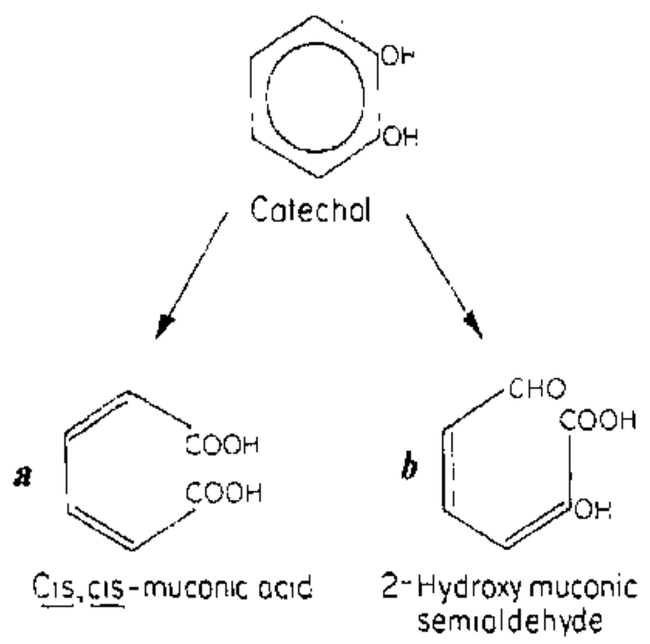

Figure 2. Ring cleavage of catechol. *a*, *Ortho* cleavage. *b*, *meta* cleavage.

enzyme was 1.8. No catechol 2,3-dioxygenase activity was detected.

This is the first report on the *ortho* cleavage of catechol by *A. chroococcum* in contrast to the existing reports on the *meta* cleavage (Figure 2, *b*). Not surprisingly this bacterium also efficiently cleaved 2,4-D (ref 6), which could be attributed to the presence of the *ortho* enzyme, since *meta* enzyme is irreversibly inactivated[7].

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