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14.
Degradation of fluorobenzene by a *Rhizobiales* strain F11 via *ortho* cleavage of 4-fluorocatechol and catechol

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The aerobic metabolism of FB was investigated using a pure bacterial culture that was isolated from a consortium enriched from a contaminated site in northern Portugal. This strain, designated F11 and phylogenetically related to the *Rhizobiales* order, can grow on FB as a sole carbon and energy source under aerobic conditions. To investigate the catabolic route for FB, activities of enzymes of the *ortho*- and *meta*-cleavage pathways were measured in cell-free extracts of strain F11 grown on FB. Activity for the key enzyme of the *meta*-cleavage pathway – catechol 2,3-dioxygenase – was never

detected, while enzymes of the *ortho*-cleavage pathway – catechol 1,2-dioxygenase, muconate cycloisomerase, maleylacetate reductase and 3-oxoadipate:succinyl-coenzyme A (CoA) transferase – were present in the cell-free extracts. This indicated that FB is converted via *ortho*-cleavage pathway. HPLC and LC–MS analysis showed that both 4-fluorocatechol and catechol were transiently present in the culture medium during the early phase of fluorobenzene degradation by F11 cell suspensions. Inhibition experiments with 3-fluorocatechol showed that this compound is a strong inhibitor of FB degradation and of catechol 1,2-dioxygenase activity, whereas 4-fluorocatechol supported growth and was converted by cell-free extracts. The results suggest that fluorobenzene is degraded by initial dioxygenation to yield either 4-fluorocatechol after subsequent reduction, or catechol with concomitant defluorination. The main metabolite is proposed to be 4-fluorocatechol, which is converted by *ortho* cleavage to 3-fluoro-*cis,cis*-muconate and subsequent defluorination yields maleylacetate that is channeled into the tricarboxylic acid cycle via 3-oxoadipate.

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