

KONFERENCE

GENTEKNOLOGI

- ET MIDDEL TIL FORURENINGSBEKÆMPELSE

Kan gensplejsede mikroorganismer benyttes ved oprensning af kemikalieforurennet jord ?

Genetic engineering - a tool for pollution abatement.
Can genetic engineered microorganisms be used to clean up soil polluted with chemicals ?

Torsdag den 15. marts 1990. kl. 13.00 - 17.00

MODIFICATION OF EFFECTOR AND SUBSTRATE SPECIFICITIES OF REGULATORS AND ENZYMES OF AROMATIC-DEGRADING PSEUDOMONAS AND POSSIBLE CONTAINMENT BY INSERTION OF SUICIDE GENES.

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Pollution by chemical compounds is a problem of considerable magnitude. The genetic manipulation of catabolic pathways of microorganisms offers a powerful experimental approach to evolving such pathways and achieving the elimination of recalcitrant compounds.

The Pseudomonas TOL plasmid is the most extensively characterized catabolic plasmid. It encodes enzymes for

the mineralization of toluene, m- and p-xylene, m-ethyltoluene and 1,2,4-trimethylbenzene. In the degradation of these compounds the methyl group at carbon 1 in the aromatic ring is sequentially oxidized to yield the corresponding carboxylic acids (upper pathway). The carboxylic acids are then oxidized to catechols, which are further metabolized to amphibolic intermediates (meta-cleavage pathway).

We are directing our efforts to the manipulation of such pathways to allow the metabolism of recalcitrant alkyltoluenes and alkylbenzoates, as i.e. p-ethyltoluene and p-ethylbenzoate. The laboratory evolution of TOL plasmid catabolic pathways involves the acquisition of new enzymatic activities and/or regulatory specificities (for substrates and effectors, respectively) through the mutational alteration of existing proteins. This evolution is possible thanks to the considerable plasticity exhibited by TOL-encoded enzymes and regulators in broadening their specificities without loss of function.

The TOL plasmid pWWO-EB62 is a genetically engineered plasmid derived from the natural TOL plasmid pWWO, which allows bacteria to grow on p-ethyltoluene. pWWO-EB62 exhibits three independent mutations. *xylS2*, encodes for a mutant regulator able to activate the meta-cleavage pathway with p-ethylbenzoate; while *xylE6*, that encodes for a catechol 2,3-dioxygenase resistant to inactivation by its substrate, p-ethylcatechol. These two mutations on the TOL plasmid allow the bacteria to grow on p-ethylbenzoate, and, together with a third mutation in the toluene oxidase genes, allow the bacteria to grow on p-ethyltoluene.

A containment system based on the TOL plasmid regulatory elements and the *hok* (host-killing) genes of *E. coli* is being developed to control, via substrate availability, bacterial populations bearing manipulated genes.