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Transformation of *E. coli* with plasmids coding for degradation of aromatic structure of phenols

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Recently much attention is paid to the ability of microorganisms to degrade and detoxify large amounts of aromatic compounds entering the environment as a result of man's industrial and agricultural activity [1]. Different genera of bacteria are able to metabolize a vast majority of natural and synthetic aromatic compounds [2]. These microorganisms are capable of inducing synthesis of catabolic enyzmes that initiate degradation of several phenolic compounds. The versality of the biodegradation processes suggests the dependence of metabolic regulation by extra-chromosomal elements. Recent investigations evidenced the presence of degradative plasmids in numerous strains of bacteria with inducible capacity for efficient metabolism of aromatic compounds served as a sole carbon and energy source [3, 4].

In the present work two bacterial strains were used: Gram (+) strain of *Micrococcus sedentarius* and Gram (–) *Pseudomonas vesicularis*. These strains, derived from a mixed population of activated sludge microorganisms, were adapted to 10 mM phenol. The isolated strains were subjected to a secondary adaptation which resulted in selection of the straines able to degrade 4 mM phenol (Fig. 1). Both strains proved to bear plasmids. It was found that, in the presence of phenol, *Micrococcus sedentarius* and *Pseudomonas vesicularis* induced 2,3-catechol dioxygenase (EC 1.13.1.2) initiating extradiol splitting pathway. The synthesis of this enzyme seemed to be plasmid-dependent [4–6].

In order to confirm the assumption that the isolated plasmids coded for biosynthesis of the enzymes responsible for the *meta*-cleavage of phenol aromatic ring, the cells of *E. coli* JM 105 were transformed with plasmid DNA isolated from the cells of either bacteria strains: the competent cells of *E. coli* JM 105, not bearing plasmids, without capacity to degrade phenol, were subjected to transformation with plasmids of different size isolated either from Gram (+) strain of *Micrococcus sedentarius* or Gram (–) of *Pseudomonas vesicularis*. After 5 days of culturing in the medium containing 4 mM phenol cells of the transformed strain of *E. coli* acquired a stable ability to degrade phenol within 24 h (Fig. 2).

It should be noted that there was no difference in transfer of plasmid material from either strain. The plasmids were transferred from the donor to the recipient cells as a full entity to the new host.

Induction of 2,3-dioxygenase by *E. coli* was confirmed by showing that degradation of phenol proceeded *via* the *meta*-cleavage pathway, proving finally that the new metabolic feature was acquired by transformation of plasmid material, derived from *Micrococcus sedentarius* or *Pseudomonas vesicularis* to *E. coli*. Curing of degradative plasmids by mitomycin C from the transformed cells of *E. coli* JM 105 appeared more difficult compared to the cells of parent strains of bacteria.

The presented genetic manipulation enables to obtain new artificially constructed strains of *E. coli* capable of degrading the aromatic struc-

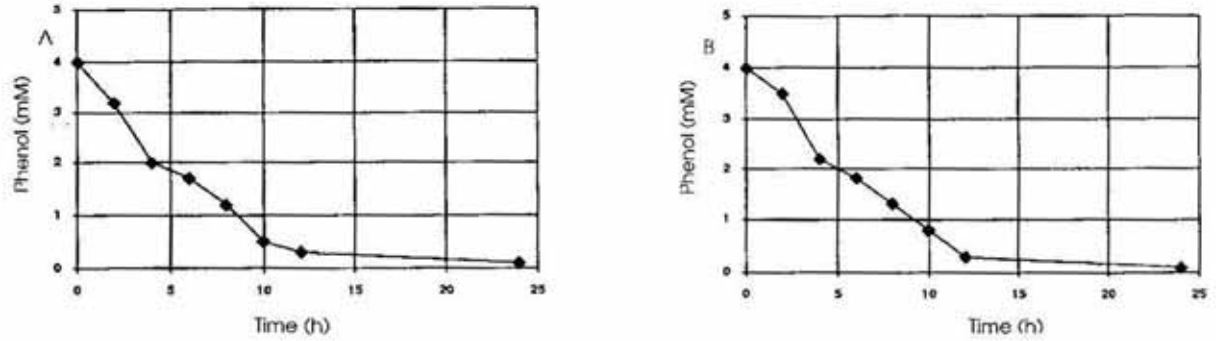

Fig. 1. Time course of biodegradation of 4 mM phenol by Micrococcus sedentarius (A) or Pseudomonas vesicularis (B).

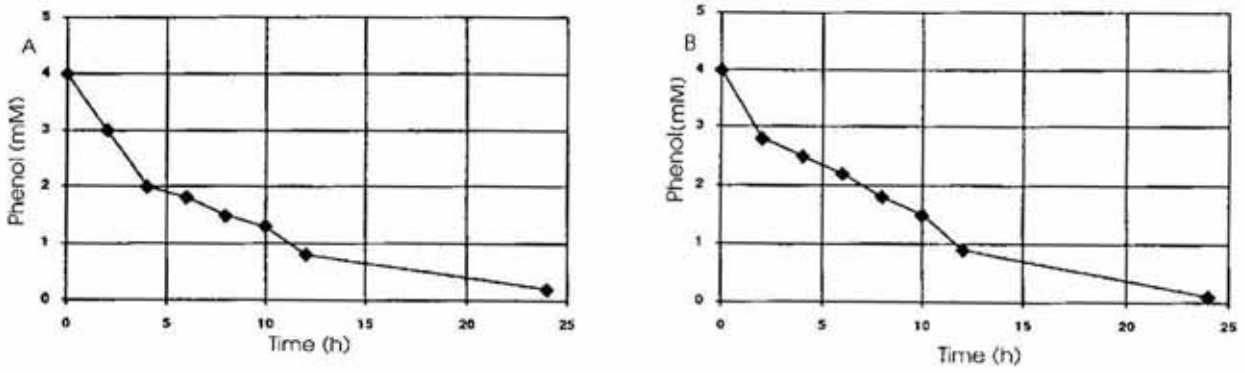

Fig. 2. Time course of biodegradation of 4 mM phenol by E. coli JM 105 transformed with plasmid DNA derived from Micrococcus sedentarius (A) or Pseudomonas vesicularis (B).

ture of phenols and use them as a sole carbon and energy source.

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