BACTERIAL COMETABOLISM OF CHLORINATED PHENOLS AND ANILINES : BIOTECHNOLOGICAL IMPLICATIONS OF THE PHENOMENON

D. JANKE and W. JHN
Central Institute of Microbiology and Experimental Therapy (ZIMET), Jena, G.D.R.

ABSTRACT :
Model experiments have been conducted on cometabolic biotransformation of selected monochloroaromatic non-growth substrates by various aromatics-degrading Rhodococcus wildtype-strains. The results obtained clearly indicate that under controlled process conditions the phenomenon of microbial cometabolism can be employed as a special approach in the field of environ mental biotechnology.

INTRODUCTION :

Microbial cometabolism, i.e. "transformation of a non-growth substrate in the obligate presence of a growth substrate or another transformable compound" (DALTON and STIRLING 1982) is a whole-cell phenomenon physiologically based on coupling of different catabolic pathways at the cellular level (through transfer of reducing equivalents and/or energy). It is frequently observed in transformation of xenobiotic non-growth substrates by individual microbial species (e.g. pseudomonads, rhodococci) with appropri ate non-specific enzymes of the peripheric cellular metabolism and usually leads to the accumulation of intermediate and/or dead-end products in the reaction medium (JANKE and FRITSCHE 1985).
Several Rhodococcus strains proved able to utilize unsubstituted phenol and aniline as sole sources of carbon and energy by means of inducible enzymes of the β-ketoadipate pathway while failing to grow with any of the monochlorinated phenols or anilines (JANKE et al. 1986, 1988a). The present work summarizes the results of experiments on cometabolic biotransfor mation of selected monochlorophenols (MCPs) and monochloroanilines (MCAs) by different aromatics-degrading Rhodococcus wildtype-strains and points out possible biotechnological implications of processes of this type.

EXPERIMENTAL PART :

Isolation and description of Rhodococcus sp. An117 and An213, have been re ported by KAMINSKI et al. (1983) and JANKE et al. (1988a), respectively. R. rubropertinctus IMET7481 was obtained from the IMET Culture Collection

located in the ZIMET. The conditions used for maintenance and submerged precultivation (with either phenol or aniline as the sole source of carbon and energy) of the said bacterial strains were those described previously (JANKE et al. 1988a). Analytical methods used for (i) biomass estimation, (ii) substrate analysis, and (iii) isolation, identification and quantification, respectively, of the intermediate and dead-end products formed during the biotransformation processes have been reported by JANKE et al. (1988b, 1989) and IHN et al. (1989).

RESULTS AND DISCUSSION :

As shown in Table 1, in the presence of glucose as the additional energy-providing carbon substrate turnover of 3-MCP, 4-MCP, 2-MCA and 3-MCA, respectively, by resting pre-adapted cells of the Rhodococcus test strains occurred with four- to ninefold increased rates (corresponding to 31-59% as compared to the unsubstituted aromatic compound). Under the conditions used, only negligible increases (5-20%) in biomass concentration were observed during incubation of the different cell preparations.

Table 1 : Effect of glucose (1 g/l) on turnover of selected monochlorinated phenols and anilines by cells of the Rhodococcus strains An117, An 213 and IMET7481, respectively, in 0.1 M phosphate buffer (pH 6.9)1)

Substrate used for cell precultivation	Test substrate (1 mM)	Turnover rate2) An117 -glc	An117 +glc	An213 -glc	An213 +glc	IMET7481 -glc	IMET7481 +glc
Phenol (6 mM)	3-MCP	0.04	0.26	0.05	0.21	NT	NT
	4-MCP	0.06	0.33	0.06	0.24	NT	NT
Aniline (5 mM)	2-MCA	0.04	0.29	0.06	0.27	0.02	0.10
	3-MCA	0.04	0.38	0.05	0.26	0.02	0.09

1) initial biomass conc., 0.5-1.0 mg cell dry weight per ml

2) expressed as umoles of test substrate removed per hour per mg cell dry weight

Detailed time course studies on removal of 3-MCP, 4-MCP, 2-MCA and 3-MCA, respectively, revealed that cometabolic biotransformation of these monochloroaromatic non-growth substrates by resting pre-adapted cells of either strain An117 or An213 is characterized by three principle events :

(i) complete removal of the test substrate within 4-6 hours of incubation,

(ii) transient accumulation of considerable amounts of either 3-chlorocatechol (3-MCC) or 4-chlorocatechol (4-MCC), and (iii) gradual increase of the chloride level (with the maximum concentration corresponding to 0.85-0.95 umoles of chloride released per umole of the respective test substrate removed) and coincident building-up of a certain level (0.25-0.42 umoles per umole of test substrate removed) of cis-4-carboxymethylenebut-2-en-4-olide (cis-4-CMB) in the incubation medium (for a typical kinetic curve, see Fig. 1).

A quite different type of kinetics was observed during cometabolic 2-MCA turnover by strain IMET7481 : In this case, no significant release of chloride ions occurred and removal of the test substrate resulted in the accumulation of nearly stoichiometric amounts of 2-chloro-cis,cis-muconic acid (2-CMA; Fig. 2).

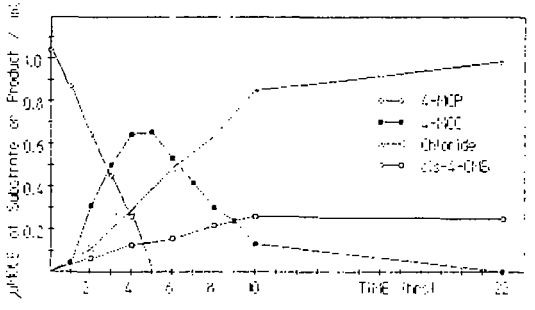

FIG.1: Kinetics of 4-MCP turnover in the presence of glucose (initial conc., 0.5 g/l) by phenol-grown cells of P. sp. ANJP (initial biomass conc., 0.6 mg cell dry weight/ml) in 0.1 M phosphate buffer, pH 6.9

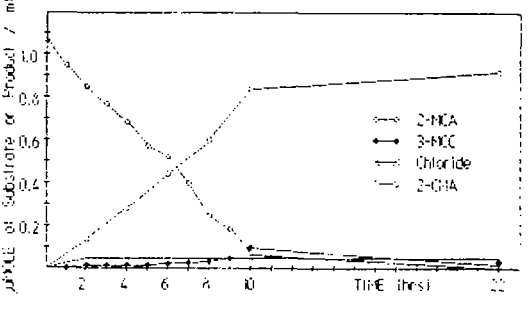

FIG.2: Kinetics of 2-MCA turnover in the presence of glucose (initial conc., 1 g/l) by aniline-grown cells of P. rubropertinctus IMET7481 (initial biomass conc., 1 mg cell dry weight/ml) in 0.1 M phosphate buffer, pH 6.9

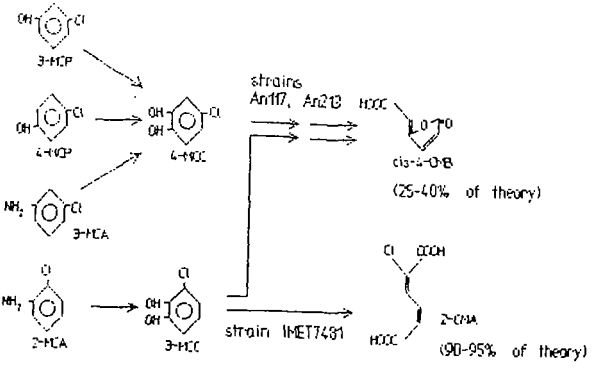

FIG.3: Intermediate and dead-end products formed during cometabolic turnover of different monochloroaromatic non-growth substrates by resting preadapted cells of the test strains

Cometabolic biotransformation of all monochloroaromatic test substrates by *Rhodococcus* sp. An117 and An213 converged at the level of cis-4-CMB (Fig. 3). This points out the possibility to simultaneously transform different monochloroaromatic non-growth substrates by using appropriate mixtures of pre-adapted resting cells of strain An117 and/or An213. Preliminary experiments (to be published) from our laboratory confirmed the validity of this presumption.

Together, the results obtained in this work provide clear evidence that the phenomenon of cometabolism might be a useful approach in biotechnology to extend the range of biotransformable xenobiotic compounds and to achieve under controlled process conditions (i.e. high concentration of pre-adapted cells, appropriate substrate mixtures) a high-rate detoxification of riskful pollutants with concomitant accumulation of certain (commercially unavailable) biochemical products at high yield. The almost stoichiometric accumulation of 2-CMA during cometabolic 2-MCA turnover by R. rubropertinctus IMET7481 (patent application under consideration) may serve to illustrate this suggestion.

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