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BIOTRANSFORMATIONS IN ORGANIC SOLVENTS: A DIFFERENCE BETWEEN GRAM−POSITIVE AND GRAM−NEGATIVE BACTERIA

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Hydroxylation of naphthalene by Gram−negative *Pseudomonas putida* can be performed in a wider range of organic solvents with lower log P values than can the steroid Δ^1−dehydrogenation by Gram−positive *Arthrobacter simplex*, as measured in two−liquid phase bioreactors. This observed Gram−positive/Gram−negative difference may be a key factor in catalyst selection.

Most of the studies in chemical transformations by intact organisms have been carried out in aqueous solution but there is now a considerable interest in reactions involving non−polar organic compounds. We have been concerned with developing two−liquid phase bioreactors in which compounds only sparingly soluble in water can be dissolved to high concentrations in water−immiscible organic solvents so as to bring about a bioconversion in a two−liquid phase environment (Lilly, 1983). There are many potential advantages of operating in the presence of a second liquid phase (Lilly and Woodley, 1985); however, there are drawbacks, including the possible inactivation of the biocatalyst by the organic solvent (Lilly *et al.*, 1987). With the development of this new technology there comes an increasing need to understand how the catalyst interacts with its environment during the biotransformation and in particular the effect of the potentially toxic organic phase on catalytic activity.

Pseudomonas putida UV4 performs the microbial hydroxylation of naphthalene to naphthalene 1,2−dihydrodiol at 100% of the rate of the aqueous control in the presence of solvents with log P values greater than 4 (Fig.1). However, the Δ^1−dehydrogenation of hydrocortisone to prednisolone by *Arthobacter simplex* NCIB 8929, at 100% of the aqueous control, is only observed with solvents of log P above 9.8. With solvents of log P less than 9.8 there is a reduction in rate with a linear relationship between product formed and decreasing solvent log P down to a log P of 3, below which no activity is observed (Hocknull and Lilly, 1987).

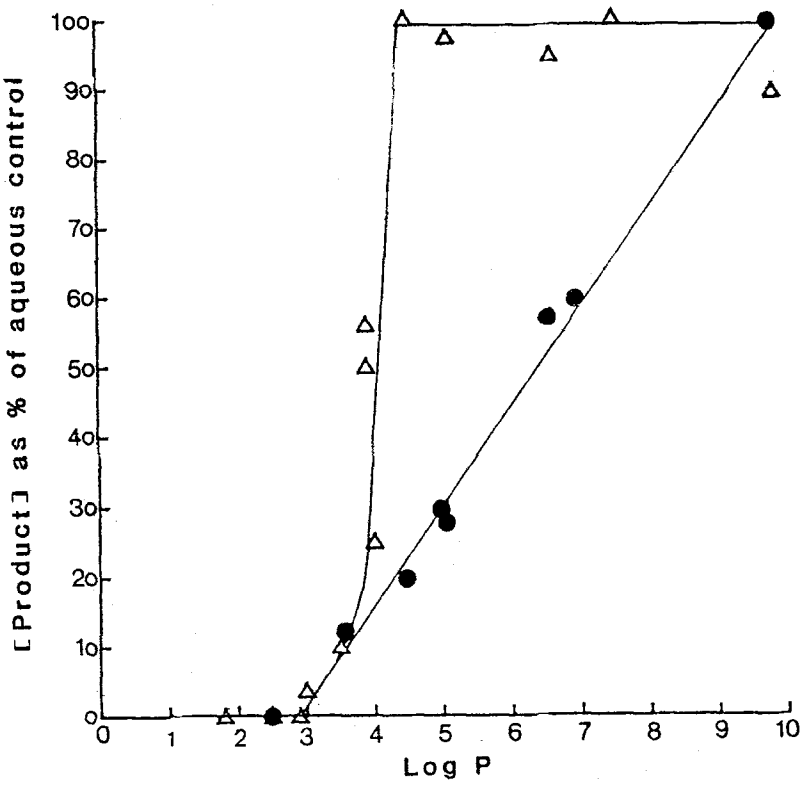

Figure 1
Product formed by *P. putida* (△) and *A. simplex* (●) in two−liquid phase stirred tank reactors (50% v/v solvent) over 2 hours as a percentage of aqueous control, against organic solvent log P. Biotransformations were done in 100 ml stirred tank reactors (working volumes 60 ml and 70 ml and stirrer speeds 800 rpm and 750 rpm for *P. putida* and *A. simplex* respectively). Both systems were operated under non−oxygen limiting conditions. For *P. putida* the rates of product formation were linear over the 2 hour period, even with the more toxic low log P solvents. Biotransformations took place using non−growing bacteria in phosphate buffer (50mM, pH 7) and Tris−HCl buffer (50mM, pH 7.8) for *P. putida* and *A. simplex* respectively. Organic solvents in both cases were saturated with substrate. Log P is defined as the logarithm of the solvent partition coefficient in a standard octanol−water two−phase system.

The presence of a liquid−liquid interface appears to be important in bringing about inactivation of the steroid Δ^1−dehydrogenation system of *A. simplex*. Immobilisation in calcium alginate brings about a normalised rate−log P profile similar to that of free *P. putida* in the stirred tank (Hocknull, 1989). However, the liquid−liquid interface does not

seem to play any significant part in the inactivation of the hydroxylation system of *P. putida*, possibly due to the shielding effect of the outer cell membrane, a structure lacking in Gram−positive organisms.

The presence of the outer membrane in Gram−negative bacteria has been reported to confer increased resistance to both hydrophobic antibiotics and dyes (Leive, 1974; Gustafsson *et al.*, 1973). The disorganisation of this barrier by various chemical treatments results in increased sensitivity to hydrophobic antibiotics and greater permeability to hydrophobic dyes. Some mutants with altered outer membranes behave in a similar way. The lipopolysaccharide (LPS) contained within the outer membrane is thought to constitute the main penetration barrier, its partial removal from the outer membrane by treatment with ethylenediaminetetracetic acid (EDTA) resulting in increased antibiotic sensitivity and permeability to hydrophobic dyes (Nikaido, 1976; Leive, 1965). Susceptibility to hydrophilic antibiotics appears to be unaffected by the disorganisation of the outer membrane (Roantree *et al.*, 1977). The polysaccharide moiety of the LPS is negatively charged and hydrophilic in character (Luderitz *et al.*, 1982), and may play a similar role to calcium alginate in shielding against the toxic effects observed for *Arthrobacter simplex* in the presence of organic solvents. Shielding may also be enhanced by the absence of phospholipids on the external surface of the outer membrane (Nikaido and Nakae, 1979).

The finding that *P. putida* UV4 is tolerant to a wider range of organic solvents of lower log P than is *A. simplex* is in agreement with the results of Inoue & Horikoshi (Inoue and Horikoshi, 1989). They examined a range of bacteria and found that some Gram−negative bacteria including strains of *P. putida, P. fluorescens* and *E.coli* were able to grow in the presence of solvents of lower log P, down to 2.4 for a novel *P. putida* isolate, whereas the Gram−positive bacterium *Bacillus subtilis* could not grow in the presence of solvents of log P less than 4.9 (Inoue and Horikoshi, 1989). Earlier, we showed that for alkene epoxidation by *P. putida* PpG6 there was a sharp transition in activity between cyclohexane (log P = 3.2) and tetrachlomethane (log P = 3.0) (Harbron *et al.*, 1986), which also confirms this Gram−positive/Gram−negative difference. It is possible that some of the observed differences in product formation rates during biotrans−formation may be related to a difference in the oxidation/reduction systems we have examined.

P. putida, both in our study and that of Inoue and Horikoshi 1989, was found to be actively motile in solvents of low log P indicating the presence of an intact cytoplasmic membrane, a proton motive force being required for motility, and presumably an intact electron transport chain. *A. simplex*, in contrast, in a two−liquid reaction system loses the function of its electron transport chain at log P values less than 9.8, being more pronounced at lower log P values (Hocknull and Lilly, 1987).

This Gram−positive/Gram−negative dif[illegible]nt tolerance is currently under

investigation with the development of broad host range vectors to enable transfer of the *nahA* gene, encoding naphthalene dioxygenase, responsible for the hydroxylation of naphthalene, into other Gram−negative and Gram−positive bacteria. Broad host range vectors of this type will also allow examination of the solvent resistance during biotransofrmation for both yeasts and filamentous fungi. We believe that this information will be of assistance in biocatalyst selection for biotransformations in the presence of potentially toxic organic solvents.

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