JOURNAL OF BACTERIOLOGY, Nov. 1984, p. 797–800
0021-9193/84/110797-04$02.00/0

Vol. 160, No. 2

TOL Plasmid Can Prevent Induction of Chemotactic Responses to Aromatic Acids

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Received 21 May 1984/Accepted 30 July 1984

Growth conditions that elicited positive chemotaxis to benzoate and *m*-toluate in TOL⁻ *Pseudomonas putida* cells failed to elicit taxis to these compounds in TOL⁺ cells. The inability of TOL⁺ cells to respond to these aromatic acids appears to be due to the preferential expression of TOL-encoded genes for aromatic degradation over chromosomally encoded genes. Expression of chromosomal genes for aromatic degradation is required for cells to form β-ketoadipate, the inducer of benzoate and *m*-toluate taxis.

Aromatic acids may be degraded via several different metabolic routes in *Pseudomonas putida*. Chromosomally encoded pathways (6, 8) convert benzoate and *p*-hydroxybenzoate to catechol and protocatechuate, compounds that are cleaved by *ortho*-ring fission and metabolized via the β-ketoadipate pathway (Fig. 1) (11). Some *P. putida* strains carry plasmids encoding catabolic pathways for aromatic compounds (4). The TOL plasmid encodes genes that function in the degradation of benzoate and *m*- and *p*-toluate via a pathway in which catechol is cleaved by *meta*-ring fission (Fig. 1) (14, 15).

A *P. putida* strain (PRS2000) lacking the TOL plasmid has recently been found to exhibit positive chemotaxis to several aromatic acids including *p*-hydroxybenzoate, benzoate, and the methylbenzoates *m*- and *p*-toluate (5). The tactic responses to benzoate and to *m*-toluate are induced by β-ketoadipate, an intermediate in the dissimilation of benzoate by chromosomally encoded genes. This pattern of induction suggests that benzoate and its ring-methylated derivatives are recognized by a common chemoreceptor system. Strain PRS2000 uses benzoate and *p*-hydroxybenzoate as growth substrates, and therefore the ability of these cells to sense and swim up concentration gradients of these compounds can be viewed as an advantageous response. Since *P. putida* strains can utilize toluates as growth substrates only if they carry the TOL plasmid, we compared the chemotactic responses of TOL⁺ and TOL⁻ cells to these and related compounds.

The bacterial strains used in chemotaxis experiments are listed in Table 1. Cells were cultured as described in the accompanying paper (5). The volatile compounds toluene, *m*-xylene, and *p*-xylene were provided as the vapor phase from small glass tubes that were sealed at one end, plugged with cotton, and taped inside the lids of petri dishes. The TOL⁻ strain PaW500 was isolated by a benzoate curing procedure similar to that described by Kunz and Chapman (7). A culture of PaW15 was transferred sequentially six times through liquid growth medium containing 5 mM benzoate and 20 μg of L-leucine per ml. Bacteria were checked for loss of the TOL plasmid by spreading cells on plates of mineral medium containing 5 mM *m*-toluate, 0.5 mM succinate, and 20 μg of L-leucine per ml. Cells lacking the plasmid formed small colonies surrounded by a brown halo (caused by formation of methylcatechol). Single colonies were picked, and their phenotypes were tested.

Conjugation experiments were performed with donor and recipient cells that had been grown overnight at 30°C in 5 ml of 1% yeast extract broth. Samples (0.1 ml) of 10^{-1}, 10^{-2}, and 10^{-3} dilutions of PaW15 were spread with 0.1-ml samples of undiluted cultures of strain PRS2000 on agar plates containing 5 mM *m*-toluate as the sole carbon source. Exconjugants were picked, and their phenotypes were tested. Exconjugants occurred with a frequency of 2.8×10^{-4}. The frequency of prototrophic revertants of PaW15 was 5×10^{-7}.

Cells were prepared for chemotaxis experiments as described in the accompanying paper (5). Chemotactic responses of cells were measured by a quantitative capillary assay similar to that of Adler (1).

The chemotactic responses of a TOL-bearing strain (strain PaW15) were examined with cells that had been grown on benzoate or *m*-toluate. *P. putida* strains carrying the TOL plasmid possess genetic information for the utilization of benzoate by either of two pathways that diverge metabolically at the level of catechol (Fig. 1). The enzymes of the plasmid-encoded *meta*-cleavage pathway, used for dissimilation of toluates, are induced directly by benzoate or toluate (3, 9). The induction of the chromosomally encoded enzymes of the *ortho* pathway is less direct, since it requires that the inducer, *cis,cis*-muconate, be formed from benzoate by basal levels of the *ortho*-oxygenase before synthesis of the enzymes of the pathway can be fully elicited (10). Apparently, very little muconate is formed when TOL⁺ cells are grown on benzoate, because the enzymes of the *ortho* pathway are not induced to substantial levels under these circumstances (14). Thus, when TOL-bearing cells are grown on benzoate or *m*-toluate, the *meta* pathway is always synthesized and expressed preferentially to the *ortho* pathway.

PaW15 cells grown on *m*-toluate failed to exhibit significant chemotactic responses to either *m*-toluate or benzoate. PaW15 cells grown on benzoate were only slightly chemotactic to *m*-toluate and benzoate (Fig. 2A and B). These responses were compared with those of PaW15 cells that had been cured of the TOL plasmid (PaW500). Cells lacking the plasmid, and therefore able to metabolize benzoate via the *ortho* pathway, exhibited markedly greater chemotactic responses to *m*-toluate and benzoate than their TOL-bearing counterparts (Fig. 2A and B). PaW15 and PaW500 cells were equally well attracted to Casamino Acids (ca. 35,000 cells

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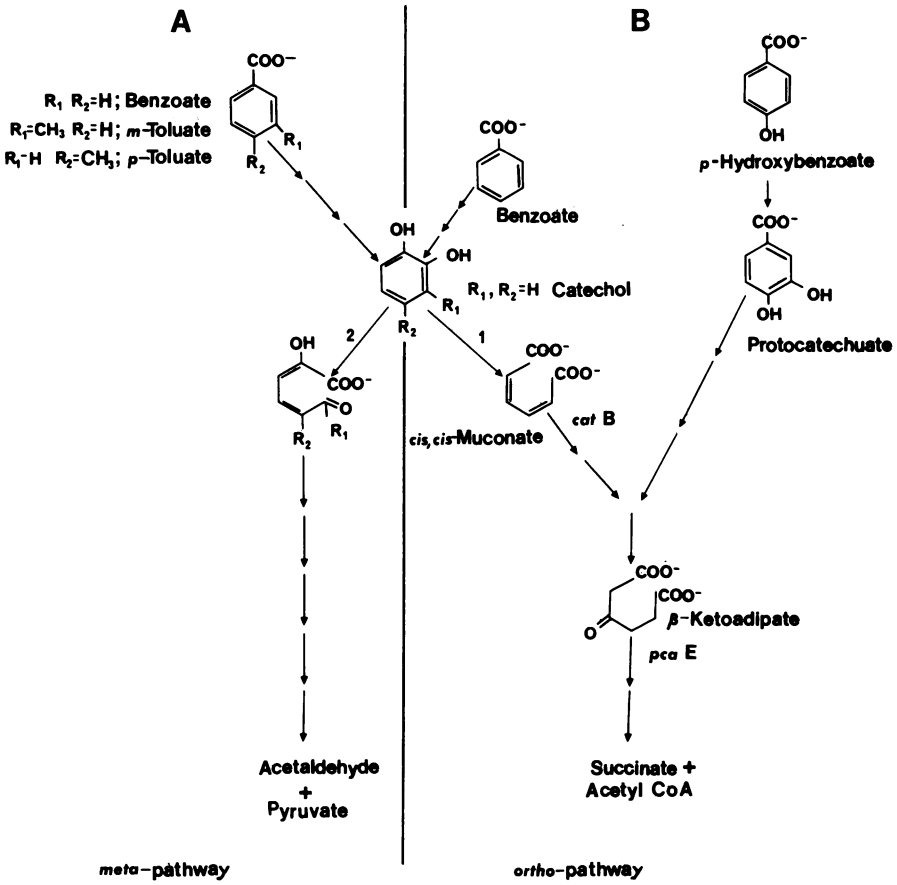

FIG. 1. TOL plasmid-encoded (A) and chromosome-encoded (B) pathways of aromatic metabolism in TOL$^+$ *P. putida*. Enzymes: 1, catechol 1,2 dioxygenase (*ortho*-oxygenase); 2, catechol 2,3-dioxygenase (*meta*-oxygenase).

per capillary). These results are consistent with the results of similar experiments carried out with PRS2000 cells containing a TOL plasmid that was introduced by conjugation with PaW15 (strain PCH1). TOL-bearing PRS2000 cells grown on either benzoate or *m*-toluate also failed to respond chemotactically to either of these aromatic acids (data not shown).

It seemed likely that TOL$^+$ strains grown on benzoate or *m*-toluate failed to exhibit chemotaxis to these aromatic acids because the metabolic pool of β-ketoadipate present in the cells was too low to induce the chemoreceptor system for benzoate and its methyl derivatives. That the failure of TOL$^+$ cells to respond to benzoate or *m*-toluate is due to the absence of appropriately induced chemotaxis systems and not to some repressive effect of the TOL plasmid on chemotaxis was shown by experiments with PaW15 cells grown on *p*-hydroxybenzoate. *p*-Hydroxybenzoate is metabolized via the protocatechuate branch of the *ortho* pathway to form β-ketoadipate as an intermediate (Fig. 1). The pattern of induction for this pathway is such that it is fully expressed in TOL-bearing cells (10). When strain PaW15 was grown with 5 mM *p*-hydroxybenzoate, cells exhibited positive chemotaxis to 5 mM benzoate (8,425 cells per capillary), 5 mM *m*-toluate (4,802 cells per capillary), and 5 mM *p*-toluate (1,078 cells per capillary).

Cells present in capillaries at the end of all chemotaxis experiments carried out with PaW15 were checked for the maintenance of the TOL plasmid by plating capillary contents on appropriate media. All of the cells that were tested were found to retain the plasmid. Therefore, the attraction to benzoate and methylbenzoates exhibited by *p*-hydroxybenzoate-grown PaW15 cells did not represent chemotaxis by cells cured of the TOL plasmid. Similarly, the slight chemotactic response of benzoate-grown PaW15 cells to benzoate (Fig. 2A) was not due to a response by plasmid-cured cells. Rather, it may have reflected a chemotactic response that had been partially induced by a low level of endogenous

TABLE 1. *P. putida* strains

Strain	Relevant phenotype[a]	Relevant genotype (plasmid)	Parent strain	Source or reference
PRS2000	Ben$^+$ Tal$^-$ Xyl$^-$ Tln$^-$	Wild type		(12)
PCH1	Ben$^+$ Tal$^+$ Xyl$^+$ Tln$^+$	Wild type (TOL)	PRS2000	By conjugation with PaW15, this study
PaW15	Ben$^+$ Tal$^+$ Xyl$^+$ Tln$^+$ Leu$^-$	*leu-1* (TOL)	PaW1	(14)
PaW500	Ben$^+$ Tal$^-$ Xyl$^-$ Tln$^-$ Leu$^-$	*leu-1*	PaW15	Benzoate curing, this study

[a] Phenotype abbreviations: Ben, benzoate utilization; Tal, *m*- and *p*-toluate utilization; Tln, toluene utilization; Xyl, *m*- and *p*-xylene utilization; Leu$^-$, requires leucine.

β-ketoadipate generated in cells by the activities of partially induced enzymes of the *ortho* pathway.

Our data indicate that the TOL plasmid does not carry genetic information that either induces or represses chemotaxis to aromatic acids. However, because the TOL plasmid in effect takes carbon that would ordinarily flow through the catechol branch of the *ortho* pathway and diverts it so that it flows through the *meta* pathway, cells fail to form β-ketoadipate, the inducer of the benzoate chemoreceptor. Thus, expression of the TOL encoded genes can, under certain circumstances, strongly impair the chemotactic responses of *P. putida* cells to benzoate and *m*-toluate.

It is important to note that the TOL plasmid does not influence the flow of carbon through the protocatechuate branch of the *ortho* pathway. Because the protocatechuate and catechol branches of the *ortho* pathway converge to form β-ketoadipate, the benzoate chemoreceptor (and chemotaxis to *m*-toluate) is fully induced in TOL-bearing cells that are exposed to *p*-hydroxybenzoate and, presumably, hydroaromatic compounds (e.g., quinate and shikimate) that are also metabolized to form β-ketoadipate. Hydroaromatic compounds are components of decaying plant material (i.e., lignin), and plant root exudates and *P. putida* cells are readily isolated from soil and aquatic habitats where these compounds exist (2, 13). Accordingly, it is possible that the benzoate chemoreceptor is induced for a substantial portion of the life span of *P. putida* cells present in nature.

One might ask what possible survival advantage could be associated with the ability of toluates to serve as chemoattractants for TOL$^-$ *P. putida* cells, since these cells are unable to catabolize toluate. Because benzoate and *m*-toluate are detected by the same chemoreceptor, it could be argued that taxis to *m*-toluate is fortuitous and merely a consequence of the mechanism that has evolved for expression of benzoate taxis. Although toluate taxis may indeed have evolved fortuitously, a positive survival value of this response can be envisioned if one considers that taxis to *m*- or *p*-toluate will allow motile bacteria lacking the TOL plasmid, and therefore lacking the genetic information required for the degradation of *m*- and *p*-toluate, to migrate to areas in nature, for example, oil spills, where these compounds exist. Such a behavioral response would be expected to bring *P. putida* cells that lack the TOL plasmid into close proximity with TOL-bearing cells already present in these areas and thereby increase the likelihood of the formation of cell aggregates required for cell-to-cell plasmid transmission. Thus, chemotaxis may play a role in facilitating the transfer of catabolic plasmids between bacteria present in low numbers in natural environments.

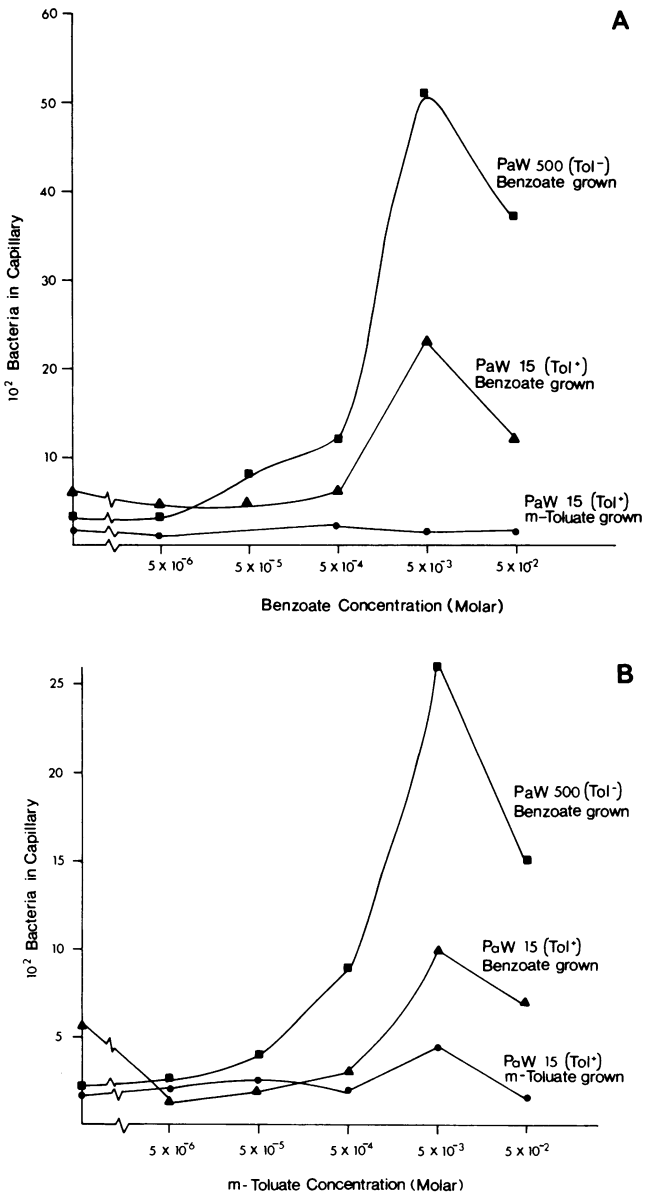

FIG. 2. Concentration response curves for taxis to aromatic compounds by *P. putida* PaW15 cells grown on benzoate (▲) or *m*-toluate (●) and PaW500 cells grown on benzoate (■). (A) Concentration response curves for benzoate taxis. (B) Concentration response curves for *m*-toluate taxis.

We thank Robert M. Macnab for helpful discussions and Anne T. Harrison for excellent clerical assistance.

This research was supported by Celanese Research Co.

LITERATURE CITED

1. **Adler, J.** 1973. A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by *Escherichia coli*. J. Gen. Microbiol. **74**:77–91.
2. **Cain, R. B.** 1979. Uptake and catabolism of lignin-related compounds and their regulation in microorganisms, p. 21–60. *In* T. K. Kirk et al. (ed.), Lignin biodegradation: microbiology, chemistry and potential applications. CRC Press, Inc., Boca Raton, Fla.
3. **Feist, C. F., and G. D. Hegeman.** 1969. Regulation of the *meta* cleavage pathway for benzoate oxidation by *Pseudomonas putida*. J. Bacteriol. **100**:1121–1123.
4. **Haas, D.** 1983. Genetic aspects of biodegradation by pseudomonads. Experientia **39**:1199–1213.
5. **Harwood, C. S., M. Rivelli, and L. N. Ornston.** Aromatic acids are chemoattractants for *Pseudomonas putida*. J. Bacteriol. **160**:622–628.
6. **Holloway, B. W., V. Krishnapillai, and A. F. Morgan.** 1979. Chromosomal genetics of *Pseudomonas*. Microbiol. Rev. **43**:73–102.
7. **Kunz, D. A., and P. J. Chapman.** 1981. Isolation and characterization of spontaneously occurring TOL plasmid mutants of *Pseudomonas putida* HS1. J. Bacteriol. **146**:952–964.
8. **Leidigh, B. J., and M. L. Wheelis.** 1973. The clustering on the *Pseudomonas putida* chromosome of genes specifying dissimilatory functions. J. Mol. Evolution **2**:235–242.
9. **Murray, K., C. J. Duggleby, J. M. Sala-Trepat, P. A. Williams.** 1972. The metabolism of benzoate and methylbenzoates via the *meta*-cleavage pathway by *Pseudomonas arvilla* mt-2. Eur. J.

Biochem. **28**:301–310.
10. **Ornston, L. N.** 1966. The conversion of catechol and protocatechuate to β-ketoadipate by *Pseudomonas putida*. IV. Regulation. J. Biol. Chem. **241**:3800–3810.
11. **Ornston, L. N.** 1971. Regulation of catabolic pathways in *Pseudomonas*. Bacteriol. Rev. **35**:87–116.
12. **Ornston, L. N., and D. Parke.** 1976. Properties of an inducible uptake system for β-ketoadipate in *Pseudomonas putida*. J. Bacteriol. **125**:475–488.
13. **Schroth, M. N., and J. G. Hancock.** 1982. Disease-suppressive soil and root-colonizing bacteria. Science **216**:1376–1381.
14. **Williams, P. A., and K. Murray.** 1974. Metabolism of benzoate and the methylbenzoates by *Pseudomonas putida* (*arvilla*) mt-2: evidence for the existence of a TOL plasmid. J. Bacteriol. **120**:416–423.
15. **Wong, C. L., and N. W. Dunn.** 1974. Transmissible plasmid coding for the degradation of benzoate and *m*-toluate in *Pseudomonas putida*. Genet. Res. **23**:227–232.