Aromatic Acids Are Chemoattractants for *Pseudomonas putida*

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A quantitative capillary assay was used to show that aromatic acids, compounds that are chemorepellents for *Escherichia coli* and *Salmonella* sp., are chemoattractants for *Pseudomonas putida* PRS2000. The most effective attractants were benzoate; 2-hydroxybenzoate; the methylbenzoates; n-, p-, and o-toluate; salicylate; DL-mandelate; β-phenylpyruvate; and benzoylformate. The chemoattractant responses to these compounds were inducible. Taxis to benzoate and n-toluate was induced by β-ketoadipate, a metabolic intermediate formed when benzoate is dissimilated via enzymes specified by chromosomal genes. Benzoylformate taxis was induced by benzoylformate and L(+) mandelate. Taxis to mandelate, benzoylformate, and β-phenylpyruvate was exhibited by cells grown on mandelate, but not by cells grown on benzoate. Cells grown on benzoate were chemoattractant to benzoate, the toluates, 2-hydroxybenzoate, and salicylate. These results show that *P. putida* synthesizes at least two distinct chemoreceptors for aromatic acids. Although DL-mandelate was an effective attractant in capillary assays, additional experiments indicated that the cells were actually responding to benzoylformate, a metabolite formed from mandelate. With the exception of mandelate taxis, chemotaxis to aromatic acids was not dependent on the expression of pathways for aromatic degradation. Therefore, the chemoattractant responses exhibited by cells cannot be attributed to an effect of the oxidation of aromatic acids on the energy metabolism of cells.

Motile bacteria sense and respond to changes in the concentrations of chemicals present in their natural environments by changing the direction of their movement. Bacteria swim toward compounds that serve as chemoattractants and away from compounds that are chemorepellents. This behavioral response, bacterial chemotaxis, has been studied extensively in the enteric bacteria *Escherichia coli* and *Salmonella* sp., and a great deal is now known about the biochemical and genetic basis for behavior in these organisms. Bacterial chemotaxis involves detection of chemicals present in the external environment by chemoreceptor proteins, integration of the information by sensory transducers called the methyl-accepting chemotaxis proteins, and transmission of this information to effect a change in the direction of rotation of bacterial flagella (5, 12).

The capacity of a particular compound to elicit a positive chemotactic response is sometimes related to its nutritional value. *E. coli* and *Salmonella* sp., for example, are attracted to many kinds of sugars and to some amino acids (2, 13). Similarly, *Pseudomonas aeruginosa* responds favorably to an array of organic acids, compounds that this species favors as growth substrates (14, 15).

In some cases the survival value of negative chemotaxis has been established. The membrane permeant aromatic acids benzoate and salicylate are chemorepellents for *E. coli* and *Salmonella* sp. (26). These weak acids carry protons into cells, and the negative behavioral responses of enteric bacteria to benzoate and salicylate represent a response to a lowered cytoplasmic pH, rather than a negative response to the aromatic structure of the compounds per se (9, 22, 24). Since extremes of intracellular pH can impair numerous metabolic processes, the migration of cells away from an acid environment can be viewed as an advantageous behavioral response.

*P. putida* differs markedly from *E. coli* and *Salmonella* sp. in its nutritional properties. Unlike the enteric bacteria, *P. putida* is able to utilize a wide array of aromatic acids as sole carbon and energy sources for growth (25). Aromatic compounds are dissimilated by *Pseudomonas* via several different metabolic routes. Genes for the metabolism of many aromatic compounds, including mandelate, 2-hydroxybenzoate, and benzoate, are chromosomally encoded in *P. putida* (8, 10). These compounds are degraded to form diphenolic intermediates, which are then further dissimilated by either the catechol or the protocatechuate branches of the ortho-ring fission (β-ketoadipate) pathway (Fig. 1) (6, 17). *P. putida* degrades other aromatic acids, including salicylate and toluates, with enzymes that are encoded by genes carried on catabolic plasmids (4).

A consideration of its nutritional capabilities suggested to us that aromatic acids might be chemoattractants for *P. putida*. The results reported in this paper show that *P. putida* is attracted to metabolizable as well as nonmetabolizable aromatic acids. We found that the chemoattractant responses to aromatic acids were inducible, and that at least two chemoreceptors are involved in the detection of aromatic acids. Induction of the tactic responses to benzoate and methylbenzoates was elicited by β-ketoadipate, whereas chemotaxis to benzoylformate was induced by benzoylformate and L(+) mandelate.

**MATERIALS AND METHODS**

**Media and culture conditions.** All strains were cultivated in defined mineral medium (20). All carbon sources were prepared as 0.5 M concentrated solutions and filter sterilized separately. Concentrated solutions of all aromatic acids and β-ketoadipate were prepared as their sodium salts and neutralized to a pH of 7.0. Solutions of β-ketoadipate were filter sterilized and stored at −20°C until used. Carbon sources were added to the culture media to a final concentration of 5 mM. Liquid cultures of 10 ml were grown in 50-ml Erlenmeyer flasks with constant aeration provided by a gyratory
New Brunswick environmental shaker at 30°C. Mineral agar plates were solidified with 1.5% purified agar (Oxoid Ltd.). At the end of chemotaxis assays bacterial plate counts were performed by spreading cells on plates of L agar medium that contained 1.0 g of tryptone (Difco Laboratories), 0.5 g of yeast extract (Difco), 0.5 g of NaCl, 0.1 g of D-glucose, and 1.5 g of agar (Difco) per 100 ml of distilled water.

Bacterial strains. The bacterial strains used in this study are listed in Table 1. Cultures of each strain were enriched for actively motile cells at the onset of this investigation by allowing cells to swarm, as described by Adler (1), on plates of semisolid, dilute L agar. This medium was of the same chemical composition as the L agar described above, except that all carbon compounds were included at 1/10 the final concentration. In addition, the medium was solidified with 0.3%, rather than 1.5%, Difco agar.

Preparation of cell suspensions. Cells for chemotaxis experiments were harvested by centrifugation from 10-ml cultures in the early to mid-logarithmic phase of growth. The inocula for these cultures were from similar cultures that had been inoculated from single colonies. The harvested cells were washed once in chemotaxis buffer (50 mM potassium phosphate buffer, pH 7.0, containing 10 μM disodium EDTA) prepared with glass-distilled water. The chemotaxis buffer was aerated immediately before use by vigorous shaking. The washed cells were suspended to a density of approximately $6 \times 10^7$ cells per ml, unless specified otherwise, for use in chemotaxis experiments. The chemotactic responses to 0.1% (wt/vol) Casamino Acids (Difco) were found to be directly proportional to cell density over a range of $10^7$ to $10^8$ cells per ml.

Microscopic examination revealed that cells of all P. putida strains used in this investigation were motile throughout the logarithmic phase of growth in all growth media used to prepare cells for chemotaxis assays. After being washed and suspended in chemotaxis buffer, approximately 60 to 80% of the cells used in our experiments were motile. These cells retained their motility for at least 2 h.

Chemotaxis assays. Chemotaxis was measured by a quantitative capillary assay similar to that described by Adler (1).
Suspensions of motile cells containing approximately \( 6 \times 10^7 \) cells per ml were placed in a small chamber formed by placing a U-shaped glass tube between a microscope slide and cover slip. Chemotactic behavior was measured by placing the open end of a 1-\( \mu \)l capillary tube containing an attractant dissolved in chemotaxis buffer into the pool of cells in the chamber. After incubation for 30 min at 30°C, the contents of the capillaries were transferred to tubes of mineral medium. Appropriate dilutions were prepared, and then 0.1-ml samples were spread on plates of L agar. Colonies were counted after the plates had been incubated at 30°C overnight. Results are based on averages of duplicate plate counts on each of two separate capillary assays and are expressed in terms of the number of cells per capillary. In all experiments, a blank (no attractant present) and a positive control (0.1% [wt/vol] Casamino Acids) were included. In 14 assays performed with PRS2000 cultures grown on seven different days, the range of responses to this concentration of Casamino Acids was between \( 12.3 \times 10^4 \) and \( 22.4 \times 10^4 \) cells per ml. The standard deviation was 15%.

To normalize for differences in motility between strains, the chemotactic response was also expressed as a ratio of the accumulation in attractant capillaries to that of control capillaries (the relative response).

The terms concentration response curve, peak response, peak concentration, and threshold concentration used in this paper are as defined by Mesibov and Adler (13). A compound was considered to be an effective attractant if it had a threshold concentration of \( 5 \times 10^{-3} \) M or lower.

**Chemicals.** Sodium benzoate was from Matheson, Coleman and Bell; \( m \)-toluic acid, \( p \)-toluic acid, and \( p \)-hydroxybenzoic acid were from Aldrich Chemical Co., Inc.; benzaldehyde was from Fisher Scientific Co.; and \( o \)-toluic acid was from Eastman Kodak Co. All other compounds tested as chemotactants were from Sigma Chemical Co.

**RESULTS**

Attraction to benzoate and other aromatic acids. Benzoate elicited a positive tactic response from \( P. \) putida PRS2000 cells grown on benzoate (Fig. 2). In 16 assays performed with 8 different benzoate-grown cultures on 8 different days, the range of responses to 5 mM benzoate was between \( 4.2 \times 10^4 \) and \( 10.5 \times 10^4 \) bacteria per capillary, and the standard deviation was 23%. When benzoate was present in the capillary and the bacterial suspension at equal concentrations (5 mM), there was only slight accumulation of \( P. \) putida over the background accumulation. Therefore the response exhibited by cells was a behavioral response to a concentration gradient of the attractant and was not merely due to a stimulation of cell motility in the presence of benzoate. Since benzoate was provided as the sodium salt, we tested the response of bacteria to sodium ions alone. \( P. \) putida did not exhibit a response to NaCl when this salt was present in capillaries over the concentration range of 0.5 to 30 mM. Therefore chemotaxis toward sodium benzoate is due to detection of the aromatic acid and does not represent a response to the sodium ions.

The chemotactic response of cells was induced by the presence of benzoate in the growth medium. There was a negligible response to benzoate when cells were grown with D-glucose as the carbon and energy source (Fig. 2). The response of cells grown in the presence of both D-glucose and benzoate was the same as the response exhibited by cells grown at the expense of benzoate alone. Therefore

![FIG. 2. Concentration response curves for benzoate taxis by \( P. \) putida PRS2000 cells grown on benzoate (■), glucose (●), or glucose plus benzoate (▲).](http://jb.asm.org/)

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**TABLE 1. Pseudomonas putida strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant phenotypea</th>
<th>Relevant genotypeb</th>
<th>Parent strain</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRS2015</td>
<td>Ben&quot; Pob&quot; Tal&quot;</td>
<td>pcaE</td>
<td>PRS2000</td>
<td>(19)</td>
</tr>
<tr>
<td>PRS2241</td>
<td>Ben&quot; Pob&quot; Tal&quot;</td>
<td>m- and p-toluate utilization; Sal, salicylate utilization.</td>
<td>PRS2015</td>
<td>(21)</td>
</tr>
<tr>
<td>PRS2178</td>
<td>Ben&quot; Pob&quot; Tal&quot;</td>
<td>βKa, β-ketoadipate utilization.</td>
<td>PRS2000</td>
<td>(7)</td>
</tr>
<tr>
<td>PRSB3</td>
<td>Ben&quot; Dmdl&quot; Lmdl&quot;</td>
<td>mtlA</td>
<td>PRS2000</td>
<td>(7)</td>
</tr>
<tr>
<td>PRS61</td>
<td>Ben&quot; Dmdl&quot; Lmdl&quot;</td>
<td>mtlB</td>
<td>PRS2000</td>
<td>(7)</td>
</tr>
</tbody>
</table>

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a Phenotype abbreviations: Ben, benzoate utilization; Dmdl, (±)-mandelate utilization; Lmdl, l(+)-mandelate utilization; Pob, \( p \)-hydroxybenzoate utilization; Tal, \( m \)- and \( p \)-toluate utilization; βKa, β-ketoadipate utilization; Sal, salicylate utilization.

b Genotypes: catB, cis,cis-muconate lactonizing enzyme negative; pcaE, \( β \)-ketoadipate succinyl coenzyme A transferase negative; mtlA, mandelate racemase negative; mtlB, mandelate dehydrogenase negative.
failure of glucose grown cells to respond to benzoate was due to the absence of an appropriately induced response, rather than to a repression of the tactic response to benzoate by glucose.

Various other aromatic acids were surveyed for their ability to attract P. putida PRS2000 (Table 2). Peak concentrations, peak responses, and threshold concentrations for each of the compounds screened were determined from concentration response curves like those shown in Fig. 2. Threshold concentrations were also determined by extrapolating double-log plots of response versus attractant concentration (13).

In addition to benzoate, salicylate and the methylbenzoates m-toluate, p-toluate, and o-toluate were effective attractants for benzoate-grown cells. Salicylate and methylbenzoates do not support the growth of strain PRS2000. Therefore, the complete metabolism of aromatic compounds is not required for chemotaxis.

Cells for the p-hydroxybenzoate and DL-mandelate experiments (Table 2) were grown at the expense of the compound for which the tactic response was tested. To determine whether p-hydroxybenzoate, and DL-mandelate were required for the induction of these chemotactic responses, cells were also grown at the expense of alternative carbon and energy sources and tested for chemotaxis. Cells grown at the expense of benzoate did not recognize DL-mandelate as an attractant (Fig. 3). Cells grown on p-hydroxybenzoate also failed to respond to DL-mandelate (data not shown). Thus it appears that mandelate or a metabolite formed in the metabolism of mandelate to benzoate (6) induces mandelate taxis in P. putida. The response to p-hydroxybenzoate was induced when cells were grown on either benzoate or p-hydroxybenzoate (Fig. 4).

The chemotactic responses of glucose-grown PRS2000 cells to aromatic acids were also tested. With the exception of p-toluate, there was only a slight response (ca. 3,000 cells per capillary) to each of the compounds listed in Table 2 when they were present in capillaries at their peak concentrations. Approximately 14,000 cells accumulated in capillaries containing $5 \times 10^{-2}$ M p-toluate.

**β-Ketoadipate induces taxis to benzoate and m-toluate.** To determine whether benzoate itself or a metabolite of benzoate induced the tactic response to benzoate in P. putida, we examined tactic responses to benzoate of cells grown on several different carbon and energy sources as well as the responses of mutant cells blocked in different steps of benzoate metabolism. We were also interested in elucidating the basis for induction of the chemotactic response to m-toluate, since this compound is not catabolized by strain PRS2000.

Wild-type cells grown on glucose in the presence of m-toluate were not attracted to either benzoate or m-toluate
The ratio of the number of cells that accumulated in attractant capillaries to the number of cells that accumulated in no attractant control capillaries. Benzoate and m-toluate were used at a concentration of 5 × 10⁻³ M.

(19). When strain PRS2178 was grown on β-ketoacidapate, chemotactic responses to benzoate and m-toluate were elicited (Table 3).

The finding that benzoate, p-hydroxybenzoate, and β-ketoacidapate each elicited chemotaxis to benzoate and m-toluate indicated that β-ketoacidapate was the inducer for these tactic responses. This conclusion was reinforced by experiments with a strain (PRS2241) blocked in the metabolism of β-ketoacidapate. When strain PRS2241 was grown under conditions where β-ketoacidapate accumulated in cells, cells were tactic to benzoate and m-toluate. The induction effected by β-ketoacidapate is not likely to be due to its conversion to a metabolic precursor because the enzyme reaction that forms β-ketoacidapate is the highly exergonic hydrolysis of an enol-lactone. Furthermore, a strain (PRS2015) that lacked the ability to form β-ketoacidapate from benzoate failed to exhibit any chemotactic responses to benzoate or m-toluate (Table 3).

**Induction of taxis to benzyloformate.** Wild-type cells grown on DL-mandelate or benzyloformate were attracted to benzyloformate, whereas cells grown on benzaldehyde or benzoate were not attracted to this compound (Table 4). Therefore possible inducers of the chemotactic response include D(-)-mandelate, L(+)-mandelate, and benzyloformate (Fig. 1). D(-)-mandelate is nonmetabolizable in strain PRSB3; in the absence of its metabolism, D(-)-mandelate does not induce benzyloformate chemotaxis (Table 4). Both L(+)-mandelate and benzyloformate induce the chemotactic response, and evidence that each compound can act independently as an inducer derives from the observation that either substrate can induce chemotaxis in a mutant strain (PRS61, Table 4) blocked in their interconversion. L(+)-mandelate and benzyloformate are also inducers of the catabolic enzymes for benzyloformate degradation (7), and it might be argued that taxis to benzyloformate is due to the detection of a metabolite of this compound by the benzoate chemoreceptor. That this is not the case is shown by the observation that PRS61 cells grown in the presence of L(+)-mandelate are attracted to benzyloformate even though the benzoate chemoreceptor is not induced (Table 4).

**Taxis to mandelate and β-phenylpyruvate.** Although experiments with wild-type P. putida cells seemed to indicate that both D(-)- and L(+)-mandelate were effective chemotacticants, data obtained with mutant strains blocked in steps of mandelate metabolism showed that D(-)-mandelate and L(+)-mandelate were effective chemotacticants only for those strains that had the enzymatic capability to convert these compounds to benzyloformate (Table 4). For example, a mutant (PRS3B) blocked in the conversion of D(-)-mandelate to L(+)-mandelate was not attracted to D(-)-mandelate after it had been grown on L(+)-mandelate, a growth condition that was sufficient to induce taxis to D(-)-mandelate in wild-type cells (data not shown). Similarly, a mutant (PRS61) blocked in the conversion of L(+)-mandelate to benzyloformate was not attracted to D(-)- or L(+)-mandelate after grown on benzyloformate, even though benzyloformate-grown wild-type cells were attracted to the mandelates (Table 4).

The most straightforward explanation for these observations is that P. putida cells do not synthesize a chemo-receptor that specifically recognizes either D(-)- or L(+)-mandelate. Instead, they synthesize a benzyloformate chemoreceptor that recognizes the benzyloformate that is formed endogenously when mandelate is degraded by cells. Thus, the apparent recognition of mandelates by cells in capillary assays would appear to actually represent a response to the benzyloformate that is formed when cells metabolize the mandelate they encounter in their external environment. The benzyloformate chemoreceptor(s), in addition to recognizing exogenous benzyloformate and endogenously generated benzyloformate, appears also to recognize β-phenylpyruvate, a compound that is closely related structurally to benzyloformate (Fig. 1). Taxis to β-phenylpyruvate was exhibited by DL-mandelate-grown cells, but not by...
TABLE 4. Taxis of P. putida strains to mandelate and benzoylformate

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant trait</th>
<th>Carbon source(s) supplied</th>
<th>Chemotaxis&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>d(-)-Mandelate</td>
</tr>
<tr>
<td>PRS2000 Wild type</td>
<td>DL-Mandelate</td>
<td>40,000</td>
<td>39,000</td>
</tr>
<tr>
<td></td>
<td>Benzoilformate</td>
<td>35,000</td>
<td>27,000</td>
</tr>
<tr>
<td></td>
<td>Benzaldehyde</td>
<td>1,000</td>
<td>1,000</td>
</tr>
<tr>
<td></td>
<td>Benzotate</td>
<td>1,000</td>
<td>1,000</td>
</tr>
<tr>
<td>PRSB3 mdIA</td>
<td>d(-)-Mandelate, glucose</td>
<td>1,000</td>
<td>1,000</td>
</tr>
<tr>
<td>PRS61 mdIB</td>
<td>L(+)-Mandelate, glucose</td>
<td>2,000</td>
<td>1,000</td>
</tr>
<tr>
<td></td>
<td>Benzoylformate, glucose</td>
<td>1,000</td>
<td>1,000</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of bacteria attracted per capillary. Values are not corrected for background accumulation (ca. 1,000). The aromatic acids were used at a concentration of 5 \times 10^{-2} M.

cells grown on \(\beta\)-phenylpyruvate (Fig. 5) or benzoate (data not shown).

DISCUSSION

The results described in this paper show that P. putida is attracted to 9 of 11 aromatic acids tested as chemotacticants. To our knowledge this is a first report of chemotaxis to aromatic acids by a procaryote. P. putida is found widely distributed in soil and freshwater environments where aromatic compounds are likely to be present as components of degrading plant materials, as plant root exudates, or as components of toxic wastes (11, 23). Since P. putida can use many of these compounds as sole sources of carbon and energy (25), positive chemotaxis toward aromatic acids can be viewed as an advantageous behavioral response.

Negative chemotaxis elicited by aromatic compounds in the enteric bacteria is the consequence of lowered cytoplasmic pH caused by diffusion of lipophilic acids across the cell membrane (9, 22, 24). The positive chemotactic responses of P. putida do not appear to have such a general basis, because they are induced by specific metabolites that are intermediates in pathways of aromatic acid degradation; the tactic response to benzoylformate is induced by L(+)-mandelate and benzoylformate, and the tactic responses to benzoate and tolulates are induced by \(\beta\)-ketoadiapate.

Based on patterns of induction, the aromatic acid attractants tested fall into two groups that can be categorized as being recognized by a benzoate chemoreceptor and a benzoylformate chemoreceptor (Table 5). Note that this represents the minimum number of chemoreceptors that must be involved in aromatic acid taxis. For example, although taxis to methyl- and hydroxyl-substituted benzoates was exhibited by cells grown on benzoate, that these responses were induced specifically by \(\beta\)-ketoadiapate was established rigorously only for benzoate and m-toluate. It is possible that more than one inducer and more than one chemoreceptor are responsible for taxis to substituted benzoates. It is also possible that benzoylformate and \(\beta\)-phenylpyruvate are detected by more than one chemoreceptor. To firmly establish the number of aromatic acid chemoreceptors and their effector specificities, it will be necessary to isolate and analyze mutants that are specifically nonchemotactc to one or more aromatic acids.

Although DL-mandelate was an effective attractant in capillary assays, experiments with mutants blocked in mandelate degradation indicated that the cells were actually responding to benzoylformate, a metabolite formed from mandelate (Fig. 1, Table 4). A similar observation made with E. coli indicated that lactose metabolism was prerequisite for lactose taxis (2). The conclusion that cells respond to endogenously generated benzoylformate suggests that the chemotactic response could be mediated by recognition at a receptor site inside cells. Alternatively, it is possible that endogenous benzoylformate is excreted by cells into the medium and then recognized by a receptor domain present on the outer cell surface.

Available evidence indicates that chemotaxis in the pseudomonads and the enteric bacteria has a similar biochemical basis (3). However, at present it is not known whether

TABLE 5. Aromatic acid chemoreceptors

<table>
<thead>
<tr>
<th>Chemoreceptor</th>
<th>Attractants detected</th>
<th>Inducer(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoate</td>
<td>Benzoate</td>
<td>(\beta)-Ketoadiapate</td>
</tr>
<tr>
<td></td>
<td>m-Toluate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-Toluate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\alpha)-Toluate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salicylate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-Hydroxybenzoate</td>
<td></td>
</tr>
<tr>
<td>Benzoilformate</td>
<td>Benzoilformate</td>
<td>L(+)-Mandelate</td>
</tr>
<tr>
<td></td>
<td>(\beta)-Phenylpyruvate</td>
<td>Benzoilformate</td>
</tr>
</tbody>
</table>
sensory information from aromatic acids is received by conventional receptors and processed through methyl-accepting chemotaxis proteins or whether behavioral stimuli are processed through some other type of pathway.

With the above-mentioned exception of mandelate taxis, the expression of tactic responses to aromatic acids does not require that the enzymes specifying the degradation of these compounds also be expressed. Chemotaxis toward β-phenylpyruvate, for example, is expressed independently of the pathway for β-phenylpyruvate catabolism (Fig. 5). Similarly, cells grown on p-hydroxybenzoate or β-ketoadipate sense and respond to benzoate (Table 3), even though the enzymes required for benzoate metabolism are not induced (16). In addition, strain PRS2000 cells are strongly attracted to salicylate and m-toluolate (Table 2), even though this strain lacks the catabolic plasmids that encode the enzymes required for the degradation of these compounds. This shows that chemotaxis to aromatic acids is not caused by a flux in proton motive force or some other form of metabolic energy caused by the oxidation of chemoattractants.

Although the expression of genes for the degradation of aromatic acids is not required for chemotaxis toward most aromatic acids, it is interesting to note that the compounds that we identified as the inducers of the aromatic acid chemoreceptors are also the inducers of certain of the enzymes required for aromatic acid breakdown. For example, β-ketoadipate, the inducer of the benzoate chemoreceptor, is a cardinal regulatory metabolite that elicits the synthesis of two of the nine enzymes that convert benzoate to tri-carboxylic acid cycle intermediates and four of the six enzymes that convert p-hydroxybenzoate to central metabolites (16). Analysis of mutant strains should reveal the extent to which the syntheses of various proteins induced by β-ketoadipate are governed by the same or separate genetic elements (18). We tested the chemotactic responses of strain PRS2178, a regulatory mutant that constitutively forms the transport system for β-ketoadipate and three of the enzymes ordinarily induced by β-ketoadipate (21). We found that, whereas PRS2178 cells that were grown on β-ketoadipate were attracted to benzoate (Table 3), cells grown on glucose were not (data not shown). Therefore, the benzoate chemoreceptor does not appear to share a common regulatory element with most of the proteins induced by β-ketoadipate. It is not known whether the benzoate chemoreceptor and a fourth enzyme induced by β-ketoadipate, β-ketoadipate succinyl coenzyme A transferase, are subject to coordinate regulation.

Benzoyleformate is a regulatory metabolite that elicits the synthesis of all five of the enzymes required to convert D(+) mandelate to benzoate (7) as well as the synthesis of the benzyloformate chemoreceptor. Synthesis of the benzyloformate chemoreceptor and the mandelate catabolic enzymes is not subject to exactly the same regulatory control because benzyloformate, L(+)-mandelate, and D(+) mandelate are equipotent inducers of the catabolic enzymes, whereas only benzyloformate and L(+)-mandelate induce the benzyloformate chemoreceptor.

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LITERATURE CITED


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