Bacterial chemotaxis to pollutants and plant-derived aromatic molecules

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There is accumulating evidence that motile bacteria are chemotactically attracted to environmental pollutants that they can degrade. Chemotaxis, the ability of motile bacteria to detect and respond to specific chemicals in the environment, can increase an organism's chances of locating useful sources of carbon, nitrogen and energy, and could thus play an important role in the biodegradation process. Recent evidence demonstrating that chemotaxis and biodegradation genes are coordinately regulated suggests that these processes are intimately linked in nature.

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Abbreviations

2,4-D	2,4-dichlorophenoxyacetate
DCE	*cis*-1,2-dichloroethylene
MCP	methyl-accepting chemotaxis protein
PCB	polychlorinated biphenyl
PCE	perchloroethylene
TCE	trichloroethylene

Introduction

Enzymes and genes required for the bacterial degradation of many kinds of environmental pollutants and other aromatic compounds have been defined over the past two decades [1]. In recent years, increasing effort has been put towards identifying additional features of bacteria that may enhance rates of biodegradation. It is now clear that some flagellated bacteria have specific systems to detect and respond behaviorally to pollutants present in aerobic environments by the process of chemotaxis (Figure 1). In the absence of direct evidence, we can speculate that chemotaxis might accelerate biodegradation in a number of ways. First and most obviously, chemotaxis is capable of bringing cells into contact with the chemical to be degraded. In doing so, limitations in bioavailability due to mass transfer limitations, low solubility or sequestration of a chemical to a matrix surface may be reduced or overcome. In addition, chemotaxis may facilitate the transfer of self-transmissible catabolic plasmids by directing motile bacteria to contaminated sites where strains carrying the relevant catabolic plasmids are likely to be present. We can even visualize a population of chemotactic bacteria following the edge of a moving plume of polluted groundwater (Figure 1). The possibility of a role for chemotaxis in biodegradation and bioremediation processes has been suggested previously [2–4]. In this review, we address what is currently known about chemotaxis to environmentally relevant chemicals and attempt to point out areas that require more intense study.

Chemotaxis

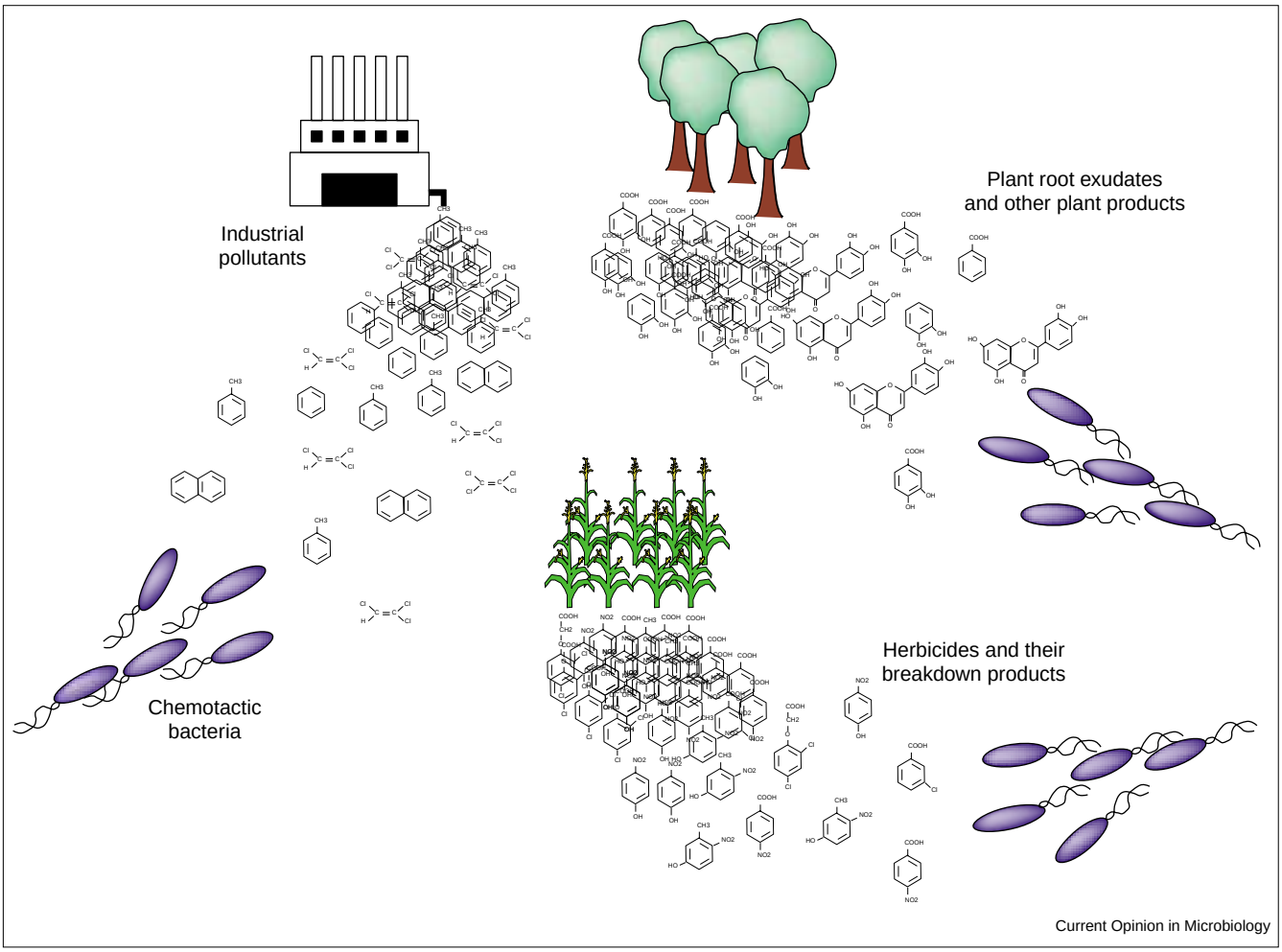
The directed movement towards or away from chemicals in the environment by the process called chemotaxis is a behavioral response exhibited by many, if not all, flagellated bacteria. Chemotaxis has been well studied in *Escherichia coli* and *Salmonella* spp., where it has served as a paradigm for bacterial two-component signal transduction pathways [5–7]. These species sense amino acids or sugars by means of cell surface receptors called methyl-accepting chemotaxis proteins (MCPs). Upon binding an attractant, an MCP undergoes a slight conformational change that is communicated to the flagellar motor via a series of physically associated chemotaxis proteins and phosphorylation events. The change in the direction of flagellar rotation that occurs as a consequence of the signaling cascade is manifested as chemotaxis. The chemotaxis machinery of *E. coli* includes five MCPs and six chemotaxis proteins. Recent genome sequence information and experimental work indicates that other bacteria have more complex chemotactic signaling systems [8]. Multiple sets of chemotaxis genes are present in many species and several of these, including *Pseudomonas putida*, have 25 or more MCP genes. Despite these complexities, the fundamental characteristics of signal reception and transduction that occur during chemotaxis by *E. coli* are probably conserved among bacteria [9].

Chemotaxis assays

Bacteria sense and swim up gradients of chemicals to accumulate near the source of an attractant. Several assays for chemotaxis are based on this fundamental characteristic of the chemotactic response.

The soft-agar swarm plate assay (Figure 2a) requires that bacteria create their own chemical concentration gradient by metabolizing carbon compounds present in soft agar media. Cells are stabbed into the center of a petri dish containing growth medium solidified with a low percentage of agar (typically 0.3%). Chemotaxis is visualized as a sharp ring of growth that forms and spreads to the edge of the plate as cells swim through the agar following the gradient of attractant created as they metabolize the compound. The swarm plate assay is well-suited for the enrichment and identification of chemotaxis mutants [10]. A drawback is that only metabolizable compounds can be tested as chemoattractants.

Figure 1



Schematic representation of chemotactic bacteria sensing and swimming up concentration gradients of industrial pollutants (top left), plant root exudates and other plant products, such as lignin monomers (top right), and herbicides and their breakdown products (bottom). Overlapping structures represent gradients of chemical attractants.

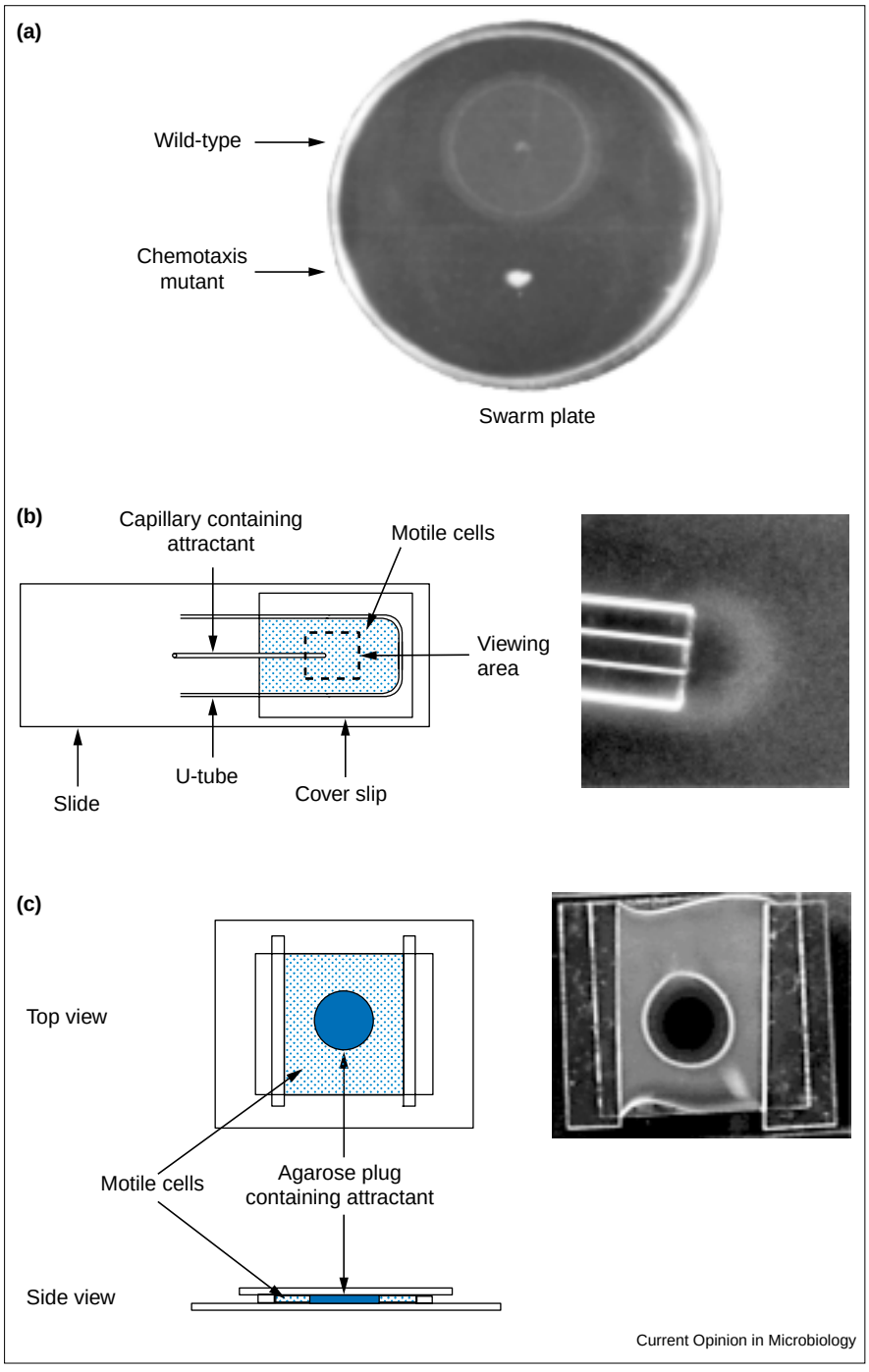
Chemotactic behavior can also be measured with a capillary assay [11]. A microcapillary tube containing a solution of attractant is placed into a suspension of motile bacteria. Chemotactic cells respond to the concentration gradient that is formed as the attractant diffuses from the mouth of the tube by swimming up the gradient and into the tube. The tube is then removed and the number of cells it contains counted. A strong chemotactic response is visible with a phase-contrast microscope, and sometimes even to the naked eye, as a cloud of cells that accumulates around the mouth of the capillary. Thus, one can also use the capillary assay to qualitatively assess chemotaxis by direct observation (Figure 2b). The qualitative capillary assay has worked particularly well to test the ability of sparingly soluble compounds, such as naphthalene, to serve as chemoattractants [12].

Chemotaxis can also be visualized qualitatively by eye with the agarose plug assay (Figure 2c) [13]. In this, a drop of melted agarose mixed with the chemical to be tested is placed on a microscope slide, and a coverslip supported by two plastic strips is then placed on top to form a chamber. A suspension of motile cells is flooded into the chamber. A chemotactic response is visualized by the accumulation of a band of cells around the perimeter of the agarose plug. This assay is useful for testing responses to compounds, such as toluene, that are soluble in aqueous medium but are also very volatile [14••]. Such a compound cannot be lost by volatilization, as the system is almost completely closed.

Chemotaxis to plant-derived aromatic compounds

Various aromatic acids are breakdown products of lignin, a main structural polymer of plants. The earliest report of aromatic compounds as chemoattractants was of the attraction of *P. putida* PRS2000 to aromatic acids (Table 1) [15,16]. Benzoate and 4-hydroxybenzoate were attractants for cells grown on aromatic acids. The inducer of the chemotactic response was found to be β-ketoadipate, a

Figure 2



Qualitative chemotaxis assays. (a) Swarm plate assay. Wild-type bacteria and a chemotaxis mutant were stabbed into a semi-solid medium containing 4-hydroxybenzoate as the attractant. Bacteria that degrade 4-hydroxybenzoate generated a concentration gradient of 4-hydroxybenzoate and swam outward from the point of inoculation toward higher concentrations. This assay requires metabolism of the attractant and cell growth. Chemotactic swarms can be observed after 6–24 hours, depending on the growth rate of the bacterial strain on the substrate provided. The mutant strain grows at the point of inoculation but does not form a swarm. (b) Modified capillary assay. In this assay, the attractant (toluene) present in the microcapillary tube diffuses out into the pool of motile cells suspended in buffer. The cells respond to the gradient of attractant and congregate at the mouth of the capillary. The response takes place over 5–30 minutes, does not require cell growth or attractant metabolism, and is observed under 40X magnification by dark field microscopy. (c) Agarose plug assay. A solution of low-melting-temperature agarose containing the attractant (in this case, toluene) was allowed to solidify between a slide and cover slip. Motile bacterial cells suspended in buffer were introduced into the chamber and the attractant diffused out into the cell suspension. The cells respond to the gradient of attractant and a distinct band of cells that surround the agarose plug is typically observed within 5 minutes. Growth and metabolism are not required.

metabolite formed during benzoate and 4-hydroxybenzoate degradation. β-Ketoadipate is also the inducer of many of the biodegradation genes. Mutant strains that were unable to metabolize these compounds, due to defects in the β-ketoadipate pathway [17], still demonstrated a chemotactic response when they were grown in the presence of β-ketoadipate or its non-metabolized analog, adipate. The growth substrates mandelate and benzoylformate were also attractants for *P. putida*, and the chemotactic responses to these compounds were also inducible. In addition, several non-metabolizable attractants were identified (Table 1) and the response to these compounds was also induced by β-ketoadipate [15]. The chemoreceptor for 4-hydroxybenzoate was identified as PcaK. A member of the major facilitator superfamily of transport proteins [18], PcaK is a dual-function membrane protein that is required for both transport of and chemotaxis to 4-hydroxybenzoate [10,19–21]. The *pcaK* gene is located in a cluster of genes necessary for aromatic acid degradation by *P. putida*. PcaR, the activator of several gene clusters involved in the degradation of

Table 1

Chemoattractants sensed by plant-associated and pollutant-degrading bacteria.

Bacterial strain	Attractants detected	Receptor	Receptor gene location	Reference(s)
Pseudomonas putida G7	Salicylate* (I) Naphthalene* (I) Biphenyl (I)	*nah*Y	NAH7 plasmid	[12,32]
Pseudomonas sp. NCIB 9816-4	Salicylate* (I) Naphthalene* (I)	Unknown	Unknown	[12]
P. putida RKJ1	Salicylate* Naphthalene*	Unknown	Unknown	[29]
Azospirillum brasilense sp. 7 and CD	Benzoate* (C) Catechol* (C) Protocatechuate* (C) 4-Hydroxybenzoate* (C)	Unknown	Unknown	[24]
Azospirillum lipoferum sp. 59b	Benzoate* (C) Catechol* (C) Protocatechuate* 4-Hydroxybenzoate* (I)	Unknown	Unknown	[24]
P. putida PRS2000	Benzoate* (I) 4-Hydroxybenzoate* (I) *m*-Toluate (I) *p*-Toluate (I) Salicylate (I) 3-Chlorobenzoate (I) 4-Chlorobenzoate (I) DL-mandelate* (I) β-Phenylpyruvate (I) Benzoylformate* (I)	Unknown PcaK Unknown Unknown Unknown Unknown Unknown Unknown Unknown Unknown	*pca* cluster (β-ketoadipate pathway genes)	[10,15,16,36–38]
Ralstonia sp. SJ98	3-Methyl-4-nitrophenol* 4-Nitrophenol* 4-Nitrocatechol* *o*-, *p*-Nitrobenzoate*	Unknown	Unknown	[41•,42]
Ralstonia eutropha JMP134(pJP4)	2,4-D* (I)	TfdK	In *tfd* cluster (2,4-D degradation genes) on pJP4	[35•]
Ralstonia pickettii PKO1	Toluene* (I)	Unknown	Unknown	[14••]
Burkholderia cepacia G4	Toluene* (I)	Unknown	Unknown	[14••]
P. putida F1	Toluene* (I) Benzene* Ethylbenzene* Isopropyl benzene *cis*-1,2-DCE TCE PCE Naphthalene α,α,α-Trifluorotoluene	Unknown	Unknown	[14••]

benzoate and 4-hydroxybenzoate, is also required for induction of *pcaK* [10].

Numerous flavonoid compounds present in plant root exudates act as signals that induce the expression of bacterial genes involved in the formation of specific plant–bacteria associations. One example of this is the formation of root nodules on legumes by rhizobial species. Strains of *Rhizobium*, *Bradyrhizobium*, *Agrobacterium* and *Azospirillum* are attracted to various aromatic acids and plant-derived flavonoid compounds (Table 1). Members of these genera are known to form symbiotic or pathogenic associations with specific plants. It is tempting to speculate that chemotaxis stimulates these processes by bringing bacteria into close proximity with plant roots [22–28].

Chemotaxis to aromatic hydrocarbons

Aromatic hydrocarbons are components of petroleum and petroleum products. As a result of man's activities in the extraction, transportation, refinement and use of petroleum, aromatic hydrocarbons have become serious environmental pollutants. It is relatively easy to isolate aerobic bacteria that degrade simple aromatic hydrocarbons such as benzene, toluene or naphthalene from just about any environment.

Chemotaxis to naphthalene

P. putida G7, *Pseudomonas* sp. strain NCIB 9816-4 and *P. putida* RKJ1 grow on naphthalene via a well-characterized pathway in which salicylate is both an intermediate metabolite and the inducer of the naphthalene degradation genes. These three strains are chemotactically attracted to naphthalene [12,29], and the responses of strains G7 and NCIB 9816-4 are induced during growth with naphthalene (and in the case of *P. putida* G7, with salicylate) [12]. In addition, induced *P. putida* G7 cells are also attracted to the related aromatic hydrocarbon biphenyl, a compound that does not serve as a growth substrate for this strain [12].

Table 1 continued

Chemoattractants sensed by plant-associated and pollutant-degrading bacteria.

Bacterial strain	Attractants detected	Receptor	Receptor gene location	Reference
Bradyrhizobium japonicum I-110	Vanillate* β-Ketoadipate* (C) *p*-Coumarate* 4-Hydroxybenzoate* Protocatechuate (I) Quinate* (I) Salicylate Shikimate* (I)	Unknown	Unknown	[22]
Rhizobium trifolii 2066	Gallate Shikimate (I) Quinate* Protocatechuate* *p*-Coumarate* 4-Hydroxybenzoate*	Unknown	Unknown	[22]
Rhizobium leguminosarum biovar phaseoli RP8002†	Vanillyl alcohol 4-Hydroxybenzoate Protocatechuate Acetosyringone Apigenin Luteolin Umbelliferone	Unknown	Unknown	[28]
Agrobacterium tumefaciens A348 and A136(Ti^-)	Catechol (C) Gallate (C) 4-Hydroxybenzoate* (C) Protocatechuate* (C) Quinate* (C) β-Resorcylate (C) Shikimate* (C)	Unknown	Unknown	[23]
A. tumefaciens C58C†	Acetosyringone Sinapinic acid Syringic acid Vanillin Ferulate Protocatechuate Catechol 4-Hydroxybenzoate Vanillyl alcohol 3,4-Dihydroxybenzaldehyde	Unknown	Unknown	[25]

*Growth substrates; C, constitutive; I, chemotactic-response-inducible. †Growth substrates not reported.

Salicylate also serves as a chemoattractant for all three strains. The response in all three strains is dependent on the presence of the resident naphthalene catabolic plasmid: cured strains are not attracted to naphthalene [12,29]. A mathematical model describing chemotaxis to naphthalene by *P. putida* G7 was developed on the basis of quantitative capillary assay data [30,31]. This model accurately predicted the concentration of substrate and bacteria at various points in a gradient and may serve as a prototype for more sophisticated models that will be needed to predict bacterial behavior in the field. The naphthalene chemoreceptor in *P. putida* G7, NahY, is a MCP that is encoded downstream of the naphthalene catabolic genes on the NAH7 plasmid [32]. *nahY* is part of an operon that contains genes for salicylate degradation (the naphthalene lower pathway). As such, it is coordinately regulated with the naphthalene and salicylate degradation genes.

To our knowledge, the only report clearly demonstrating that chemotaxis enhances biodegradation was carried out with *P. putida* G7. Marx and Aitken [33••] demonstrated that naphthalene was degraded more rapidly by the wild-type strain than by a non-motile mutant or a mutant specifically non-chemotactic to naphthalene.

Chemotaxis to toluene and related compounds

We recently demonstrated that toluene is a good chemoattractant for three toluene-degrading bacterial strains (*P. putida* F1, *Ralstonia pickettii* PKO1 and *Burkholderia cepacia* G4), each with a different toluene degradation pathway [14••]. In each strain, chemotaxis to toluene was induced during growth in the presence of toluene. *P. putida* F1 was also shown to respond to the growth substrates benzene and ethylbenzene, as well as to aromatic hydrocarbons, such as isopropyl benzene and naphthalene (Table 1), that do not serve as its growth substrates. An analysis of a series of catabolic mutants of *P. putida* F1 that are blocked at various steps in the toluene degradation pathway demonstrated that toluene itself and not a metabolite is directly detected. In addition, regulatory

mutants of *P. putida* F1 that lack the two-component regulatory system required for induction of the toluene degradation genes were not chemotactic to toluene.

Chemotaxis to chlorinated herbicides and related compounds

Many chlorinated aromatic compounds are man-made and are resistant to biodegradation. Chlorinated herbicides and polychlorinated biphenyls (PCBs) persist in the environment and are toxic. Chlorobenzoates are breakdown products of PCBs.

2,4-Dichlorophenoxyacetate chemotaxis

2,4-Dichlorophenoxyacetate (2,4-D) is a widely used man-made herbicide that is degraded by specific strains of bacteria. The best studied 2,4-D-degrading strain is *Ralstonia eutropha* JMP134(pJP4). The pathway for 2,4-D degradation is encoded on the catabolic plasmid pJP4 [34]. Recent work has demonstrated that 2,4-D is a good chemoattractant for JMP134(pJP4) and that the 2,4-D permease TfdK acts as a receptor in the chemotactic response to 2,4-D [35•]. *tfdK* is located on plasmid pJP4 within the cluster of *tfd* genes that are required for 2,4-D degradation. A plasmid-cured strain and a *tfdK* mutant were not attracted to 2,4-D, and wild-type JMP134(pJP4) only responded to 2,4-D after growth in the presence of this substrate [35•]. TfdK is the second member of the aromatic-acid–H^+ symporter family of the major facilitator superfamily of transport proteins [18] that has been shown to be involved in both chemotaxis and transport of its substrate. TfdK is closely related to PcaK, the permease/chemoreceptor for 4-hydroxybenzoate from *P. putida* [10,19].

Chemotaxis to chlorobenzoates

Chlorobenzoates are metabolites that are formed during the degradation of PCBs and other chlorinated aromatic compounds. It was found that *P. putida* PRS2000 is attracted to 3- and 4-chlorobenzoate after growth on benzoate or 4-hydroxybenzoate [36,37]. This inducible response to compounds that are not growth substrates for this particular strain represents an example of fortuitous chemoattraction. Given that pathways for the degradation of chlorobenzoates are commonly found on self-transmissible catabolic plasmids, it is possible that this chemotactic response facilitates the transfer of catabolic plasmids by bringing strains lacking the appropriate catabolic genes to environments that are contaminated with chlorobenzoates. This response would bring the chemotactic organism into close proximity with strains carrying catabolic plasmids that encode pathways for the degradation of chlorobenzoates [38].

Chemotaxis to chlorinated aliphatic compounds

When grown in the presence of toluene, *P. putida* F1 was attracted to trichloroethylene (TCE), *cis*-1,2-dichloroethylene (DCE) and perchloroethylene (PCE) [14••]. Chlorinated alkenes like TCE, DCE and PCE are very common groundwater pollutants that are used as solvents and cleaning agents in dry cleaning and other industries. Although none of these compounds are growth substrates for *P. putida* F1, TCE is oxidized by toluene dioxygenase, the enzyme that catalyzes the first step in toluene degradation in *P. putida* F1 [39,40]. One can speculate that, at a contaminated site with a moving plume of TCE, an organism like *P. putida* F1 could detect and follow a gradient of TCE. In the presence of a co-substrate that provides carbon and energy (and induces the toluene degradation pathway), TCE would be oxidized and detoxified. A variation of this bioremediation strategy involving chemotactic organisms has been proposed in a United States patent [3]. To our knowledge, however, application of the proposed technology has not been reported.

Chemotaxis to nitroaromatic compounds

The majority of nitroaromatic compounds are man-made and most are difficult to degrade. Various nitroaromatic compounds are used as pesticides, herbicides, dyes and explosives, and as precursors in polymer production. Samanta *et al.* [41•] used a chemotactic enrichment technique to isolate *Ralstonia* sp. SJ98 from pesticide-contaminated agricultural soil. The strain was initially grown on *p*-nitrophenol and was found to also grow on 4-nitrocatechol, 3-methyl-4-nitrophenol and *o*- and *p*-nitrobenzoate [41•,42]. The strain was also chemotactic to these nitroaromatic growth substrates [41•,42]. At this time, it is not clear whether these chemotactic responses are inducible, and the identity of the chemoreceptor(s) is currently unknown.

Conclusions and future research

On the basis of available data, several generalizations are apparent. In cases in which the chemoreceptor genes have been identified (*nahY*, *pcaK* and *tfdK*), the genes are located within biodegradation gene clusters and are coordinately regulated with these genes. In other cases in which chemoreceptors have not yet been identified, chemotaxis to aromatic compounds and pollutants is known to be inducible. In the *P. putida* F1 toluene chemotaxis system, the same *trans*-acting regulatory elements are required for induction of both chemotaxis and biodegradation. The close proximity of chemotaxis and biodegradation genes and their coordinate expression implies a natural link between chemotaxis and biodegradation.

In some cases, chemotactic responses could actually be energy taxis. That is, during metabolism of a substrate, the cell derives energy and responds to the increased energy level, as opposed to a specific chemical [43]. In some cases, chemotaxis is linked with attractant transport. However, specific metabolism is definitely not necessary in many cases. Metabolism of aromatic acids by *P. putida* and other strains is not required for chemotaxis, and toluene degradation by *P. putida* F1 is also not necessary. In addition, many examples of non-metabolizable attractants have been described (Table 1).

In our experience, we have found that a strong qualitative tactic response indicates a 'real' response, even in the

absence of quantitative data. This is particularly important to point out, because chemotaxis assays are especially difficult when the attractants being tested are sparingly soluble in aqueous solution and/or highly volatile, as are many aromatic pollutants.

In the future, studies will be needed to identify the chemoreceptors involved in chemotaxis to aromatic compounds and pollutants and to characterize the mechanisms of chemotaxis. It is not clear, at this time, whether most receptors will be typical MCPs, major facilitator superfamily permeases or novel receptors. Although it is becoming increasingly apparent that biodegradation of aromatic compounds under anaerobic conditions does occur and is probably quite important, essentially nothing is known about chemotaxis in the absence of oxygen. The ever-increasing wealth of genome sequence data desperately needs to be mined in order to understand the functions of the many chemotaxis operons and genes that have been annotated. Finally, we need better data in order to confidently state that chemotaxis does indeed stimulate biodegradation. More data are also required to prove that chemotaxis guides the development of plant-root–bacteria associations in the rhizosphere. This may require the interaction of environmental engineers with microbiologists and of plant biologists with microbiologists in what could be very productive and interesting collaborations.

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