Part 8

Cellular Ecophysiology: Uptake

49 Chemotaxis

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Abstract: Chemotaxis is the ability of organisms to move toward or away from chemical gradients in the environment. Hydrocarbon compounds, which are sources of carbon and energy for many bacterial species, have been shown to be chemoattractants for specific organisms. While much is known about catabolic pathways for the degradation of hydrocarbons and related compounds, few details are currently known about the molecular basis for chemotactic responses to these volatile and toxic chemicals.

1 Introduction

Chemotaxis is the active movement of cells along chemical gradients in the environment. This behavioral response allows bacteria to move toward or away from specific chemicals in order to locate an optimal environment for growth and survival. Chemotaxis has been characterized in detail in the enteric bacteria *Escherichia coli* and *Salmonella enterica* serovar Typhimurium where it serves as a paradigm for signal transduction (Armitage, 1999; Bourret et al., 2002; Bourret and Stock, 2002; Wadhams and Armitage, 2004). The chemotaxis machinery in *E. coli* consists of six cytoplasmic chemotaxis proteins that transmit signals from four membrane-bound methyl-accepting chemotaxis proteins (MCPs) to the flagellar motors (*Fig. 1*). Each MCP detects a specific set of chemicals via a periplasmic sensing domain. Binding of a chemoeffector (or a chemoeffector bound to a periplasmic binding protein) causes the MCP to undergo a conformational change that is transmitted across the membrane to the cytoplasmic signaling domain. The signal is transmitted to the flagella via CheW and the sensor histidine kinase CheA, which is capable of phosphorylating the response regulator CheY. CheY-P controls swimming behavior by binding to the flagellar switch to reverse the direction of flagellar rotation. This signaling cascade results in directed movement toward or away from the source of the attractant or repellent. Adaptation is mediated by methylation and demethylation of specific glutamate residues on the MCPs by the methyltransferase CheR and the methylesterase CheB. Chemotactic responses are metabolism-independent, that is, non-metabolizable chemicals can serve as attractants and catabolic mutants remain attracted to the same compounds as wild-type strains. *E. coli* and many other bacteria also exhibit metabolism-dependent energy taxis responses (Alexandre and Zhulin, 2001; Taylor et al., 1999). These responses commonly involve sensing by homologs of the MCP-like protein Aer (Taylor, 2007), and signaling via the conserved CheA-CheY phosphorylation cascade (Taylor et al., 2007). Based on studies in a variety of bacteria and the analysis of numerous genome sequences, it appears that the fundamental characteristics of chemotaxis and energy taxis signal transduction systems are conserved among bacteria, although some variations are apparent (Armitage and Schmitt, 1997; Szurmant and Ordal, 2004; Zhulin, 2001).

Many hydrocarbon-degrading bacteria have sensory systems that allow cells to detect and respond behaviorally to hydrocarbons and various chemical derivatives. Several reports have suggested that chemotaxis may play a role in biodegradation by bringing cells into contact with the chemicals being degraded (Hazen, 1994; Hazen and Lopez-de-Victoria, 1994; Pandey and Jain, 2002; Parales and Harwood, 2002; Parales et al., 2008; Pieper et al., 1996). Chemotaxis could result in an increased bioavailability of hydrocarbons and may

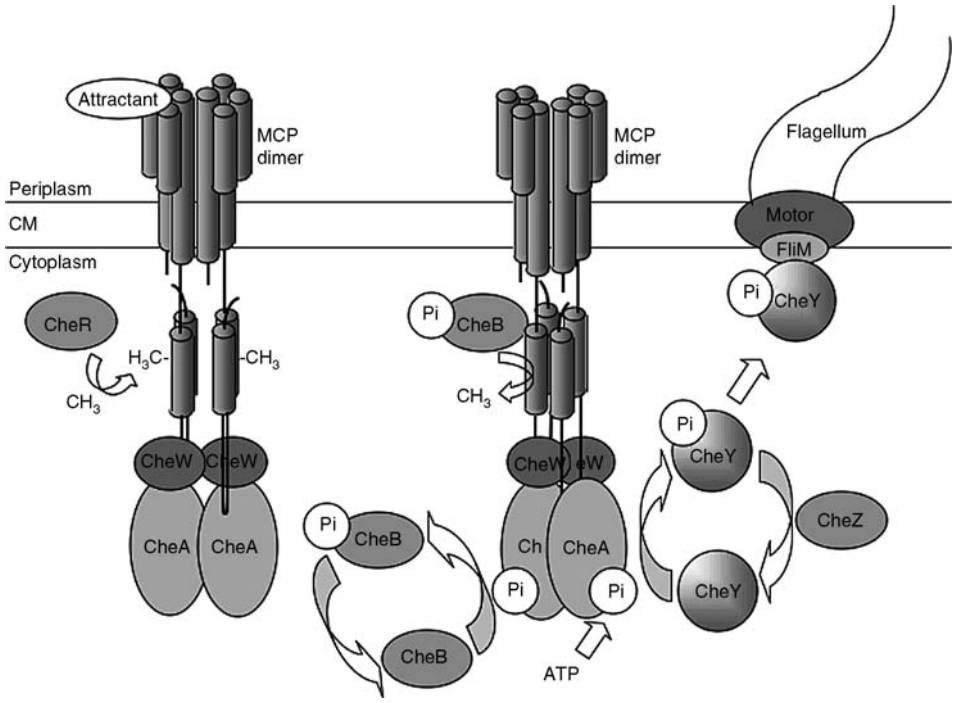

Figure 1

Schematic diagram of the chemosensory signaling system of enteric bacteria. MCP dimers with associated CheW and CheA proteins are shown in the presence (left) and absence of attractant (right). Cells responding to a gradient of attractant will sense the attractant bound to the periplasmic side of the cognate MCP and will continue swimming in the favorable direction due to the inability of CheA to autophosphorylate. In the absence of CheA-P, CheY remains in the inactive unphosphorylated state, and swimming behavior remains unchanged. Cells swimming down a gradient of attractant will sense the decrease in attractant concentration due to decreased occupancy of the MCPs. Under these conditions, the MCPs undergo a conformational change that is transmitted across the cytoplasmic membrane and stimulates CheA kinase activity. CheA-P phosphorylates CheY, which in its phosphorylated state binds to the FliM protein in the flagellar motor and causes a change in the direction of flagellar rotation allowing the cell to randomly reorient and swim off in a new direction. Dephosphorylation of CheY-P is is accelerated by the CheZ phosphatase. Under all conditions, the constitutive methyltransferase CheR methylates specific glutamyl residues on the cytoplasmic side of the MCP. Methylated MCPs stimulate CheA autophosphorylation, thus resetting the system such that further increases in attractant concentration can be detected. The methylesterase, CheB, becomes active when it is phosphorylated by CheA-P. CheB-P competes with CheR and removes methyl groups from the MCPs. CM, cytoplasmic membrane.

facilitate the transmission of catabolic plasmids in the environment (Harwood and Ornston, 1984). This chapter describes our current understanding of both positive and negative chemotactic responses to hydrocarbons and related compounds, as well as responses to metabolizable compounds via energy taxis.

2 Chemotaxis Assays

Bacteria sense and respond to chemical gradients. The result of this behavior is the accumulation of cells near the source of an attractant. Several qualitative and quantitative assays to measure bacterial chemotaxis are based on this fundamental characteristic of the chemotactic response. Some assays are particularly appropriate for the analysis of hydrocarbon taxis, while others are more difficult to use with volatile chemicals.

Chemotaxis to metabolizable compounds present in soft agar growth media can be visualized by using the soft agar swarm plate assay (Adler, 1973; Harwood et al., 1994). Cells are inoculated at the center of a Petri dish containing growth medium solidified with a low concentration of agar (typically 0.3%), and the bacteria generate a chemical concentration gradient as they degrade the attractant, which is typically a carbon and energy source for the organism. Chemotaxis is visualized as a ring of growth that moves toward the edge of the plate as cells swim through the agar following the self-generated gradient of attractant (*Fig. 2a*). Only metabolizable compounds can be tested as chemoattractants in this assay, and problems can arise when using volatile attractants because the chemicals can evaporate and redissolve in the medium, interfering with gradient formation.

Chemotaxis can also be visualized qualitatively with the agarose plug assay (Yu and Alam, 1997). In this assay, a drop of melted agarose containing the chemical to be tested is positioned between a microscope slide and a coverslip, and a suspension of motile cells surrounds the plug. The chemotactic response appears as cells that accumulate in a ring around the agarose plug, typically within a few minutes (*Fig. 2b*). This assay is useful for testing responses to volatile compounds such as hydrocarbons, because the response is rapid and the chamber is almost completely closed, thus limiting volatilization (Parales et al., 2000). The drop assay (Fahrner et al., 1994; Grimm and Harwood, 1997) has some similarities to the swarm plate and the agarose plug assays. In this case, cells are suspended in a viscous solution in a small Petri dish and the attractant is dropped into the center of the cell suspension. A gradient forms by diffusion and a ring of cells forms around the attractant within a short time (~15 min). Growth is not required for either the agarose plug or drop assay.

Chemotactic behavior can be measured either quantitatively or qualitatively using the capillary assay (Adler, 1973; Grimm and Harwood, 1997). In this assay, cells respond to a gradient of attractant diffusing out of a microcapillary tube into a suspension of motile bacteria. Chemotactic cells respond by swimming up the gradient and into the tube; the tube is then removed and the number of cells within is enumerated. In the qualitative capillary assay, a solidifying agent such as agarose is included in the capillary, preventing the cells from entering. This results in the accumulation of a cloud of chemotactic cells near the mouth of the capillary, which can be observed under low magnification (*Fig. 2c*). The qualitative capillary assay works well with poorly soluble, as well as with volatile compounds.

Temporal assays are used to monitor the behavior of cells in suspension in response to the addition of attractant. Quantitative assays can be carried out using computer-assisted motion analysis (Harwood et al., 1989, 1990), which requires dedicated software. Manual temporal assays that monitor adaptation of the bacteria to the attractants either directly or following videotaping of cells can also provide quantitative data (Parales, 2004; Shioi et al., 1987).

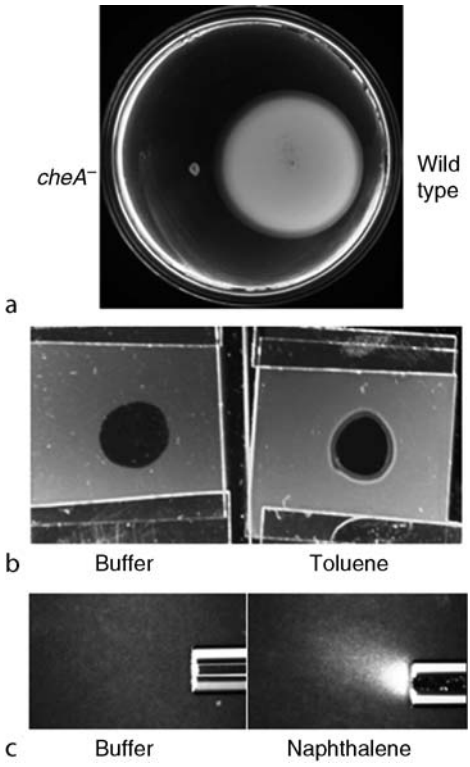

Figure 2

Examples of chemotaxis assay results. (a) Swarm plate assay. Wild type (right) and a chemotaxis mutant (CheA$^-$; left) were stabbed into semi-solid agar (0.3%) medium containing an attractant. This assay requires growth on the attractant(s) to generate the gradient. The chemotaxis mutant (left) grows at the point of inoculation but does not form a swarm ring. The wild-type strain (right) senses the gradient and follows it, forming a swarm ring. (b) Agarose plug assay. A solution of low melting temperature agarose containing the attractant is allowed to solidify between a slide and cover slip and motile bacterial cells suspended in buffer are introduced into the chamber. The attractant diffuses into the suspension and the cells respond to the gradient of attractant forming a ring of cells around the plug. Growth and metabolism are not required and a response is typically seen within 5 min. Left, buffer control; right, plug contains toluene. The response is seen as a white ring of cells accumulating at the optimum concentration of toluene. (c) Modified (qualitative) capillary assay. In this assay, attractant in a 1 μl capillary diffuses into a motile cell suspension. The cells sense the gradient of attractant and accumulate at the tip of the capillary. The response typically takes place within 5–30 min, and does not require attractant metabolism or cell growth. The response can be monitored under 20–40X magnification by dark field microscopy. Left, capillary contains buffer in agarose (negative control); right, capillary contains naphthalene crystals. The cells accumulating at the tip of the capillary in response to naphthalene appear as a white cloud.

3 Chemotaxis to Aromatic Hydrocarbons

The toluene-degrading strains *P. putida* F1, *Ralstonia pickettii* PKO1, and *Burkholderia vietnamiensis* (formerly *cepacia*) G4 showed toluene-inducible chemotactic responses to toluene (Parales et al., 2000). Benzene and ethylbenzene were also good chemoattractants for *P. putida* F1, which can utilize both substrates as sole sources of carbon and energy (Parales et al., 2000). *P. putida* F1 was also attracted to aromatic hydrocarbons that do not serve as growth substrates, including isopropylbenzene and naphthalene. Mutants of *P. putida* F1 that were unable to degrade toluene remained chemotactic to toluene, indicating that toluene was directly detected as the attractant. Toluene catabolism and chemotaxis are under the control of the same two-component regulatory system encoded by *todST* in *P. putida* F1. Strains with mutations in either *todS* or *todT* were unable to induce the *tod* catabolic operon (Lau et al., 1997) and did not respond to toluene in chemotaxis assays (Parales et al., 2000). These results indicate that toluene chemotaxis and catabolism are genetically linked in *P. putida* F1. However, the toluene chemoreceptor has not yet been identified.

P. putida G7, *Pseudomonas* sp. strain NCIB 9816-4, *P. putida* RKJ1, and *Ralstonia* sp. strain U2 utilize naphthalene as a sole source of carbon and energy, and all four strains have been reported to be chemotactic to naphthalene (Grimm and Harwood, 1997; Samanta and Jain, 2000; Wood et al., 2006). The response to naphthalene by these strains requires the presence of the resident naphthalene catabolic plasmid (Grimm and Harwood, 1997; Samanta and Jain, 2000). Naphthalene chemotaxis in strains G7 and NCIB 9816-4 is induced during growth with naphthalene (Grimm and Harwood, 1997). NahY, the chemoreceptor for naphthalene in *P. putida* G7 is a MCP that is encoded downstream of the naphthalene catabolic genes on the NAH7 catabolic plasmid. Inactivation of *nahY* resulted in the loss of the chemotactic response to naphthalene (Grimm and Harwood, 1999). The chemotactic response of *P. putida* G7 to naphthalene was quantified (Marx and Aitken, 1999), and mathematical models describing chemotaxis to naphthalene were developed based on this data (Marx and Aitken, 1999, 2000a). Experiments using wild-type *P. putida* G7, a nonmotile mutant, and a non-chemotactic variant lacking *nahY* demonstrated that chemotaxis enhances biodegradation in a heterogeneous system (Marx and Aitken, 2000b). The same set of strains was also used to show that chemotactic bacteria were more efficient at degradation of naphthalene dissolved in a nonaqueous-phase liquid (Law and Aitken, 2003). An additional study demonstrated bacterial chemotaxis in water-saturated porous media by evaluating chemotaxis of *P. putida* G7 to naphthalene in an environment of packed glass beads (Pedit et al., 2002). These studies provide evidence that chemotactic bacteria may be more effective for bioremediation applications, especially at sites where contaminants are unevenly distributed or adsorbed to soil particles.

Chemotactic responses to naphthalene and larger polycyclic aromatic hydrocarbons (PAHs) were investigated with three *Pseudomonas* isolates obtained from coal-tar contaminated sites (Ortega-Calvo et al., 2003). All three strains grew on naphthalene; one (*P. putida* 10D) also grew on phenanthrene and pyrene. All were chemotactic to naphthalene; *P. putida* 10D was also chemotactic to phenanthrene but not to pyrene. This strain also showed a repellent response to anthracene, a three-ring PAH that did not serve as a growth substrate.

Chemotaxis to biphenyl has been reported for biphenyl-degrading strains such as *Pseudomonas* sp. strain B4 (Chávez et al., 2006; Gordillo et al., 2007). This strain was chemotactic to biphenyl and monochlorobiphenyls, which serve as growth substrates (Gordillo et al., 2007). The response to biphenyl and chlorobiphenyls did not require induction. *Pseudomonas* sp.

strain B4 also responded to benzoate, a growth substrate and intermediate in biphenyl degradation, but not 2- and 3-chlorobenzoates, which accumulate following growth on 2- and 3-chlorobiphenyls. 3-Chlorobenzoate was toxic to strain B4 and actually appeared to result in a slight repellent response. Benzoate- or biphenyl-grown cells responded weakly to 4-chlorobenzoate, in contrast to glucose-grown cells, which did not respond. Chemotaxis to biphenyl was also reported for two other biphenyl-degrading strains, *P. putida* P106 and *Rhodococcus erythropolis* NY05 (Wu et al., 2003). Biphenyl is also an attractant for naphthalene-grown *P. putida* G7, although biphenyl is not a growth substrate for this strain. It is likely that biphenyl is sensed by the NAH7 plasmid-encoded naphthalene chemoreceptor NahY in *P. putida* G7 (Grimm and Harwood, 1997, 1999).

4 Chemotaxis to Linear Alkanes

A *Flavimonas oryzihabitans* isolate that was obtained from soil contaminated with gas oil (a complex mixture of linear and aromatic hydrocarbons) was shown to be chemotactic to gas oil and hexadecane (Lanfranconi et al., 2003). The isolate grew on tetradecane, pentadecane, hexadecane, and 2,6,10,14-tetramethyl pentadecane as sole carbon sources. Responses to gas oil and hexadecane were demonstrated using qualitative capillary assays and agarose plug assays, but the chemoreceptor has not been identified. Unpublished data reported by Smits et al. indicated that *P. aeruginosa* PAO1 is chemotactic to hexadecane (Smits et al., 2003). A gene designated *tlpS*, which is located downstream of the alkane hydroxylase gene *alkB1* on the *P. aeruginosa* PAO1 genome, has been predicted to encode a MCP that may play a role in alkane chemotaxis (Smits et al., 2003), but no experimental evidence has been reported. Similarly, the *alkN* gene appears to encode a MCP that could be involved in alkane chemotaxis in *P. putida* GPo1. The *alkN* gene is located in a cluster of genes for alkane degradation on the OCT plasmid (van Beilen et al., 2001). Unfortunately, *P. putida* GPo1 was not motile enough for chemotaxis assays (van Beilen et al., 2001).

5 Chemotaxis to Nitroaromatic Compounds and Explosives

Nitroaromatic compounds are used as solvents and in the production of pesticides, herbicides, dyes, explosives, and polymers (Spain et al., 2000). *Ralstonia* sp. SJ98 was isolated from pesticide-contaminated agricultural soil for its ability to grow on 4-nitrophenol (Samanta et al., 2000). The strain also grew on 4-nitrocatechol, 3-methyl-4-nitrophenol and 2- and 4-nitrobenzoate, and was chemotactic to these nitroaromatic growth substrates. It was also chemotactic to other structurally similar nitroaromatic compounds that did not serve as growth substrates (Bhushan et al., 2000; Pandey et al., 2002; Samanta et al., 2000). In bench-scale experiments, *Ralstonia* sp. SJ98 was capable of generating and following a gradient of 4-nitrophenol in soil and degrading it in the process, suggesting that this strain could be useful for bioremediation of nitroaromatic contaminants in the field (Paul et al., 2006). The identity of the chemoreceptor(s) responsible for the detection of nitroaromatic compounds in *Ralstonia* sp. SJ98 has not been reported. However, NbaY was shown to be required for chemotaxis to 2-nitrobenzoate in the 2-nitrobenzoate-degrading strain *Pseudomonas fluorescens* KU-7 (Iwaki et al., 2007). NbaY is 29% identical in sequence to NahY, the naphthalene chemoreceptor from *P. putida* G7.

Chemotaxis to mononitrotoluenes, dinitrotoluenes, and 2,4,6-trinitrotoluene (TNT) by the 2,4-dinitrotoluene degrading strains *B. cepacia* R34 and *Burkholderia* sp. strain DNT was reported (Leungsakul et al., 2005). However, the response to nitroaromatics took up to three days to visualize, whereas most chemotactic responses are observable in well under an hour with the assay used. No nitroarene chemoreceptors were identified.

The 4-nitrotoluene (4NT) degrading strains *P. putida* TW3 (Rhys-Williams et al., 1993) and *Pseudomonas* sp. strain 4NT (Haigler and Spain, 1993) have similar chemotaxis systems for the detection of the 4NT degradation intermediate 4-nitrobenzoate (Parales, 2004). The chemotactic response is induced by β-ketoadipate, and it appears that a broad specificity chemoreceptor (possibly like the 4-hydroxybenzoate receptor PcaK from *P. putida* PRS2000 (Harwood et al., 1994)) recognizes a wide range of substituted benzoates, including nitrobenzoates and aminobenzoates.

Clostridium sp. strain EDB2 is an obligate anaerobe that is chemotactic to the cyclic nitramine explosives hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and 2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaazaisowurtzitane (CL-20) (Bhushan et al., 2004). The strain transformed RDX, HMX, and CL-20 to nitrite, nitrous oxide, formaldehyde, and formate. Chemotactic responses to RDX, HMX, and CL-20 were due to the detection of released nitrite, rather than the explosives themselves. Previous studies demonstrated nitrate and/or nitrite chemotaxis by bacteria capable of anaerobic respiration (Lee et al., 2002; Taylor et al., 1979). In most cases the response appears to be due to energy taxis (Taylor and Zhulin, 1999; Taylor et al., 1999) and not a specific interaction of a chemoreceptor with the nitrite. *Clostridium* sp. are generally not capable of anaerobic respiration and therefore it is not expected that strain EDB1 can use nitrite as an electron acceptor. At this time the receptor for the detection of nitrite released from cyclic nitramine explosives is not known.

6 Chemotaxis to Chlorinated Hydrocarbons

As already mentioned, the biphenyl-degrading strain *Pseudomonas* sp. strain B4 is chemotactic to 2-, 3- and 4-chlorobiphenyl and also to 2,3-dichlorobiphenyl (Gordillo et al., 2007), but chemotaxis to polychlorinated biphenyls (PCBs) has not been reported. Chlorobenzoates are intermediates in the degradation of PCBs and other chlorinated aromatic compounds. Although *P. putida* PRS2000 cannot grow on chlorobenzoates, benzoate- or 4-hydroxybenzoate-grown cells were attracted to 3- and 4-chlorobenzoate (Harwood, 1989; Harwood et al., 1990). Detection of these compounds appears to be due to recognition of structurally related chlorobenzoates by a benzoate or a 4-hydroxybenzoate chemoreceptor.

Chlorinated alkenes such as trichloroethylene (TCE), dichloroethylene (DCE), and perchloroethylene (PCE) are used as solvents, degreasing agents, and cleaning agents in dry cleaning, and they are common groundwater pollutants. Toluene-grown *P. putida* F1 is attracted to TCE, *cis*-1,2-DCE, and PCE (Parales et al., 2000). *P. putida* F1 is unable to grow on these compounds, but toluene dioxygenase, the enzyme that catalyzes the first step in toluene degradation, is capable of oxidizing and detoxifying TCE (Li and Wackett, 1992; Wackett and Gibson, 1988). Analysis of the chemotactic response of *P. putida* F1 to TCE in a packed column demonstrated bacterial chemotaxis in porous media and provided evidence that chemotaxis is relevant in a variety of environmental conditions and does not just occur in the aqueous phase (Olson et al., 2004).

Similarly, *o*-xylene-grown *P. stutzeri* OX1 was shown to respond to these compounds, as well as *trans*-1,2-DCE, 1,1-DCE, and vinyl chloride. Toluene-grown *P. putida* F1 and *B. vietnamiensis* G4 were also attracted to additional chlorinated ethenes (Vardar et al., 2005). Chlorinated alkenes do not serve as carbon and energy sources for these bacteria, but toluene *o*-monooxygenase from *B. vietnamiensis* G4 is capable of oxidizing TCE and DCE isomers (Shields and Francesconi, 1996), and the corresponding enzyme from *P. stutzeri* OX1 oxidizes TCE, PCE, and DCE isomers, as well as chloroform (Chauhan et al., 1998; Ryoo et al., 2000). The genes encoding the *P. putida* F1 toluene dioxygenase and *B. vietnamiensis* G4 toluene monooxygenase are induced by TCE (Leahy et al., 1996). Together, these data suggest that if an appropriate carbon and energy source were available, such chemotactic strains could follow and detoxify a moving plume of chlorinated alkene-contaminated groundwater.

Pseudomonas stutzeri KC transforms and detoxifies carbon tetrachloride (CT) under anoxic conditions using nitrate as a terminal electron acceptor (Criddle et al., 1990; Dybas et al., 1995; Lewis et al., 2001). Motility and chemotaxis of *P. stutzeri* KC to nitrate were shown to enhance bioremediation of CT by allowing cells to follow the self-generated gradient of nitrate in laboratory-scale groundwater-saturated aquifer columns containing CT and nitrate (Witt et al., 1999). The authors concluded that the cells' ability to move toward higher concentrations of nitrate increased CT degradation because the organism requires denitrifying conditions in order to transform CT. The results of this study and a recent report demonstrating nitrate and acetate chemotaxis by *P. stutzeri* KC in porous media support the proposal that chemotaxis enhances biodegradation in the environment (Roush et al., 2006).

7 Chemotaxis to (Methyl)Phenols

A recent study reported that *P. putida* harboring the (methyl)phenol (*dmp*) degradation pathway encoded on pVI150 exhibited metabolism-dependent taxis to phenolic compounds (Sarand et al., 2008). The response was shown to be mediated by the energy taxis transducer Aer2, a membrane-anchored MCP-like protein that carries a PAS domain. Inactivation of *aer2* eliminated both aerotaxis and metabolism-dependent taxis to metabolizable phenols, succinate, and glucose. This is the first report to demonstrate a direct involvement of an energy taxis receptor in a tactic response to aromatic compounds.

8 Repellent Responses

Hydrophobic hydrocarbons and related chemicals can accumulate in cellular membranes, causing loss of membrane integrity and dissipation of membrane potential (Sikkema et al., 1995). Negative chemotactic responses (repellent responses) allow motile bacteria to avoid environments with toxic concentrations of these chemicals. In fact, it appears that some bacteria may be capable of exhibiting both positive and negative responses to potentially toxic chemicals depending on the concentration. For example, although *P. putida* F1 exhibits an inducible positive response to toluene, a constitutive repellent response to high concentrations of toluene was observed (Parales et al., 2000).

Negative chemotaxis has been reported for several marine pseudomonads in response to chloroform, toluene, and benzene (Young and Mitchell, 1973). In addition, *P. aeruginosa* displayed repellent responses to TCE, PCE, 1,1,1-trichloroethane (TCA), chloroform, and dichloromethane (Shitashiro et al., 2003). The repellent response to TCE and chloroform required the Cluster I chemotaxis genes *cheYZABW* as well as *cheR*, and the three MCP-encoding genes *pctA*, *B*, and *C* (Shitashiro et al., 2005). PctA, PctB, and PctC also mediate positive responses to amino acids (Taguchi et al., 1997).

9 Conclusions and Research Needs

Many bacteria have chemotactic responses that allow them to detect and respond behaviorally to hydrocarbons. As recent studies have demonstrated, chemotaxis may help to overcome mass transfer limitations by bringing the biodegradative organisms to sorbed hydrocarbons. The ability of specific bacteria to actively sense and respond to hydrocarbon substrates provides a competitive advantage when concentrations of these compounds are limiting due to low bioavailability. In addition, the ability of an organism to sense and follow a gradient of a particular chemical or class of chemicals that it is *unable* to degrade could bring it into close contact with organisms carrying relevant transmissible catabolic plasmids for the degradation of these chemicals. In this way, chemotaxis could stimulate the dissemination of catabolic plasmids among environmental bacteria, and hence could enhance the biodegradative capacity of the population.

Although many bacteria that utilize hydrocarbons are also capable of detecting these chemicals, surprisingly few receptors have been identified to date. Of those that have been identified, some are encoded on catabolic plasmids and others on the chromosome. In addition, receptor genes are frequently located near genes that code for the degradation of the molecule of interest, which is different from *E. coli*, where the receptor genes are located in or near operons devoted to chemotaxis and motility functions. The genetic context of receptor genes on catabolic plasmids or in operons with the catabolic genes suggests that chemotaxis may play an important role in biodegradation. In most cases, chemotactic responses to hydrocarbons are inducible, and in at least one case the energy taxis receptor Aer2 is responsible for the response. These data indicate that chemotaxis to hydrocarbons is linked genetically, physiologically, or bioenergetically to metabolism. Regardless of the mechanism, either through sensing the energy state or through the presence of a hydrocarbon molecule itself in the environment, the end result is beneficial to the organism by bringing them into contact with useful sources of carbon and energy.

Because of the inherent toxicity of these chemicals, there must be a relatively narrow range of hydrocarbon concentrations that can be tolerated by most microorganisms. Because hydrocarbons are known to alter membrane structure and dissipate the pH gradient across the membrane, cells must carefully control the level of hydrocarbons to which they are exposed. Therefore, it seems plausible that some bacteria may have both attractant and repellent responses to hydrocarbons in order to access optimal concentrations for growth and limit exposure to damaging concentrations.

While many hydrocarbon substrates have been identified as attractants for a number of bacterial species, more work will be required to elucidate the molecular basis for these physiological responses.

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