

Chemotaxis of *Escherichia coli* to Pyrimidines: a New Role for the Signal Transducer Tap[▽]

Xianxian Liu and Rebecca E. Parales*

Section of Microbiology, University of California, Davis, California 95616

Received 1 October 2007/Accepted 21 November 2007

Escherichia coli exhibits chemotactic responses to sugars, amino acids, and dipeptides, and the responses are mediated by methyl-accepting chemotaxis proteins (MCPs). Using capillary assays, we demonstrated that *Escherichia coli* RP437 is attracted to the pyrimidines thymine and uracil and the response was constitutively expressed under all tested growth conditions. All MCP mutants lacking the MCP Tap protein showed no response to pyrimidines, suggesting that Tap, which is known to mediate dipeptide chemotaxis, is required for pyrimidine chemotaxis. In order to confirm the role of Tap in pyrimidine chemotaxis, we constructed chimeric chemoreceptors (Tapsr and Tsr_{ap}), in which the periplasmic and cytoplasmic domains of Tap and Tsr were switched. When Tapsr and Tsr_{ap} were individually expressed in an *E. coli* strain lacking all four native MCPs, Tapsr mediated chemotaxis toward pyrimidines and dipeptides, but Tsr_{ap} did not complement the chemotaxis defect. The addition of the C-terminal 19 amino acids from Tsr to the C terminus of Tsr_{ap} resulted in a functional chemoreceptor that mediated chemotaxis to serine but not pyrimidines or dipeptides. These results indicate that the periplasmic domain of Tap is responsible for detecting pyrimidines and the Tsr signaling domain confers on Tapsr the ability to mediate efficient chemotaxis. A mutant lacking dipeptide binding protein (DBP) was wild type for pyrimidine taxis, indicating that DBP, which is the primary chemoreceptor for dipeptides, is not responsible for detecting pyrimidines. It is not yet known whether Tap detects pyrimidines directly or via an additional chemoreceptor protein.

Chemotaxis is the ability of motile bacteria to detect and respond to specific chemical ligands. Chemotactic responses of *Escherichia coli* and other motile bacteria are accomplished by signal transduction between transmembrane signal transducers, also known as methyl-accepting chemotaxis proteins (MCPs), and the flagellar motor. The four MCPs in *E. coli* are involved in directly or indirectly sensing chemical attractants, many of which are good carbon and energy sources for the organism. Tar mediates taxis toward aspartate and maltose (41), while the primary attractant detected by Tsr is serine (22). Taxis toward galactose and ribose is mediated by Trg (23), and Tap is required for taxis toward dipeptides (32).

Tsr, Tar, Trg, and Tap share significant sequence similarities and a common domain organization (45). Each protein has a periplasmic ligand-binding domain bracketed by two transmembrane (TM) domains, followed by a cytoplasmic signaling domain. MCP dimers form ternary complexes with the histidine kinase CheA and the adaptor protein CheW, both of which are located in the cytoplasm. Upon binding a chemoeffector, the MCP undergoes a conformational change, resulting in an altered rate of CheA autophosphorylation. Phosphorylated CheA is able to transfer phosphate to the response regulator CheY and the methylesterase CheB to modulate their activities. Phosphorylated CheY interacts with the flagellar motor complex and controls the direction of rotation. The methyltransferase CheR and the methylesterase CheB modulate the methylation level of MCPs and allow cell adaptation to

chemoeffectors. Details of this complex signal transduction mechanism can be found in recent reviews (7, 36, 40, 45).

No new attractants for *E. coli* have been identified in several years, but a recent study demonstrated that *E. coli* utilizes the pyrimidines thymine and uracil as the sole nitrogen sources at room temperature via a newly discovered pathway (29). To date, however, there have been no reports regarding chemotaxis to these compounds by *E. coli*. In this study, we investigated the chemotactic response of *E. coli* to pyrimidines. This work revealed a new role for the MCP Tap in chemotaxis, that of mediating the response to the pyrimidines thymine and uracil.

MATERIALS AND METHODS

Bacterial strains and plasmids. All bacterial strains used in this work are derivatives of *E. coli* strain RP437 (Table 1). The *dppA* deletion mutant was constructed using the method described by Datsenko and Wanner (12). Briefly, the Red helper plasmid pKD46 was introduced into RP437 to express λ Red recombinase (Table 1). A kanamycin resistance gene flanked by FRT (FLP recognition target) sites was PCR amplified with the primers dppAH1P4 and dppAH2P1, using pKD13 as the template (Table 1). The primers have 36 nucleotide extensions that are homologous to the flanking regions of *dppA*, in order to allow λ Red-mediated recombination to occur in the later step. The PCR product was introduced into RP437(pKD46) by electroporation, and kanamycin-resistant transformants were selected and purified. The $\Delta dppA::Km$ mutation in strain XL1 was confirmed by PCR and sequence analysis (data not shown).

A series of chimeric *tap*-*tsr* chemoreceptor genes was generated and cloned into the expression vector pRR48, which confers ampicillin resistance, carries the *lacI^q* gene, and allows the expression of genes from the *tac* promoter (42). The plasmids pXL2 and pXL5 were constructed by cloning the *AseI*-*HindIII* fragments of the PCR-amplified *tsr* and *tap* genes, respectively, into *NdeI*-*HindIII*-digested pRR48 (primers for the amplification of *tsr* were *tsr*NF and *tsr*CR; those for *tap* were *tap*NF and *tap*CR [Table 1]). To construct pXL3 and pXL4, the 5' half of *tsr* and the 5' half of *tap* were PCR amplified (primers for *tsr* were *tsr*NF and *tsr*NR, and primers for *tap* were *tap*NF and *tap*NR [Table 1]), gel purified, and cut with *AseI* and *NdeI*. They were ligated with *NdeI*-*HindIII* fragments

* Corresponding author. Mailing address: Section of Microbiology, 226 Briggs Hall, 1 Shields Ave., University of California, Davis, CA 95616. Phone: (530) 754-5233. Fax: (530) 752-9014. E-mail: reparales@ucdavis.edu.

[▽] Published ahead of print on 7 December 2007.

TABLE 1. Bacterial strains, plasmids, and primers used in this study

Strain, plasmid, or primer	Relevant characteristics or primer sequence ^a	Source or reference
<i>E. coli</i> strains		
RP437	Wild type reference strain for chemotaxis; F ⁻ <i>thr-1 leuB6 his-4 metF159 thi-1 ara-14 lacY1 mtl-1 xyl-5 rpsL136 tonA31 tsx-78 eda-50</i>	37
RP1131	RP437 <i>trg-2::Tn10</i>	J. S. Parkinson
RP2361	RP437 Δ <i>tar-386-2</i>	32, 39
RP3525	RP437 Δ <i>tap-365-4</i> Δ (<i>lac</i>) <i>U169 thr⁺leu⁺</i>	32, 39
RP5700	RP437 Δ <i>tsr-7028</i>	J. S. Parkinson
UU1250	RP437 Δ <i>aer-1 ygiG::Gm</i> Δ <i>tsr-7028</i> Δ (<i>tar-tap</i>) <i>5201 zbd::Tn5</i> Δ <i>trg-100 thr⁺met⁺</i>	9
UU1615	RP437 Δ <i>aer-1 ygiG::Gm</i> Δ (<i>tar-tap</i>) <i>5201 zbd::Tn5</i> Δ <i>trg-100 thr⁺met⁺</i>	4
UU1624	RP437 Δ <i>aer-1 ygiG::Gm</i> Δ <i>tsr-7028</i> Δ <i>tap-3654 zbd::Tn5</i> Δ <i>trg-100 thr⁺met⁺</i>	4
UU1625	RP437 Δ <i>aer-1 ygiG::Gm</i> Δ <i>tsr-7028 zbd::Tn5</i> Δ <i>trg-100</i>	J. S. Parkinson
XL1	RP437 Δ <i>dppA::Km</i>	This study
Plasmids		
pRR48	<i>lacI^q/p_{tac}</i> cloning vector, Ap ^r	42
pXL2	PCR fragment of <i>tsr</i> cloned into pRR48, Ap ^r	This study
pXL3	Chimeric fragment <i>tsrap</i> cloned into pRR48, Ap ^r	This study
pXL4	Chimeric fragment <i>tapsr</i> cloned into pRR48, Ap ^r	This study
pXL5	PCR fragment of <i>tap</i> cloned into pRR48, Ap ^r	This study
pXL8	Chimeric fragment <i>tsrap</i> plus the last 5 codons of <i>tsr</i> in pRR48, Ap ^r	This study
pXL9	Chimeric fragment <i>tsrap</i> plus the last 19 codons of <i>tsr</i> in pRR48, Ap ^r	This study
pKD13	Template plasmid, derivative of pANTS γ that contain an FRT-flanked kanamycin resistance gene	12
pKD46	Red helper plasmid, derivative of pINT-ts which contain <i>araC-paraB</i> and γ β exo	12
Primers		
tsrNF	5'-GCCGCGCATTAATGTTAAAACGTATCAAAATTGTGACCAGC-3'	This study
tsrNR	5'-CCACGCAAAGCCTGCTGCATATGGCGCAAACCTCTCTGCC-3'	This study
tsrCF	5'-GCCATTTTTGCCAGTCTGAAGCATATGCAGGGAGAGCTGATGC-3'	This study
tsrCR	5'-GCCGCGCCAAGCTTTAAAATGTTTCCCAGTTCCTCTCGC-3'	This study
tapNF	5'-CGCCGCATTAATGTTTAAATCGTATTCGAATTCGACCACGC-3'	This study
tapNR	5'-GCATCAGCTCTCCCTGCATATGCTTCAGACTGGCAAATAATGGC-3'	This study
tapCF	5'-GCAGAGAGTTTGCGCCATATGCAGCAGGCTTTGCGTGGGACGG-3'	This study
tapCR	5'-GCCGCGCCAAGCTTCAGGATACCACTGGCGCAATTTGTAAGTGC-3'	This study
tapCR5aa	5'-GCCGCGCCAAGCTTCAGGATACCACTGGCGCAATTTGTAAGTGC-3'	This study
tapCRp	5'-GGATACCACTGGCGCAATTTG-3'	This study
19aaFp	5'-CCAGCTGCGCGCGGTAATAATGG-3'	This study
19aaR	5'-GCCGCGCCAAGCTTTAAAATGTTTCCCAGTTC-3'	This study
dppAH1P4	5'-AACAACAAACATCACAAATGGAGCAGAATAATGCGTATTCGGGGATCCGTCGACC-3'	This study
dppAH2P1	5'-GCCATCAGTCTTGATGGCTTTTAAATTATTCGATAGGTGTAGGCTGGA GCTGCTTC-3'	This study

^a Underlined bases indicate the positions of restriction sites.

carrying the 3' half of *tap* (PCR amplified with the primers tapCF and tapCR [Table 1]) or the 3' half of *tsr* (PCR amplified with the primers tsrCF and tsrCR [Table 1]), respectively. The resulting chimeric genes *tsrap* and *tapsr* were inserted into the pRR48 plasmid under the control of the inducible *tac* promoter. *tsrap5* was constructed by PCR amplification of *tsrap* with the primers tsrNF and tapCR5aa (Table 1). *tsrap19* was constructed by the ligation of *tsrap* (PCR amplified with primers tsrNF and tapCRp [Table 1]) and the region encoding the 19 amino acids at the C terminus of *tsr* (amplified with primers 19aaFp and 19aaR [Table 1]). These two fragments were also inserted into pRR48, resulting in the plasmids pXL8 and pXL9. During the construction of pXL3 and pXL4, a single nucleotide change in codon 255 of *tap* was made to create an NdeI site, which did not appear to affect the receptor function of our new chimeric proteins or those of previously constructed Tar-Tap hybrids (43). All of the constructs were sequenced at the University of California, Davis, DNA sequence facility to verify the sequences of the inserted fragments.

Growth media and chemicals. Strains were maintained on Luria-Bertani (LB) agar (13). For capillary assays and growth studies, strains were grown in H1 minimal salts medium (2) containing 25 mM glycerol as the carbon source, 0.5 mM each of methionine, leucine, histidine, and threonine, and 1 μ g/ml thiamine to satisfy auxotrophic requirements. When pyrimidines were used as the nitrogen source, (NH₄)₂SO₄ was eliminated from the medium, and the pyrimidine was added to a final concentration of 1 mM. *E. coli* cultures were grown at 30°C for

chemotaxis assays. *E. coli* strains carrying pRR48 derivatives were grown in the presence of 100 μ g/ml ampicillin and were induced for 4 h with 200 μ M isopropyl- β -D-thiogalactopyranoside (IPTG).

Chemotaxis assays. Bacterial cells were harvested at the early exponential phase (when the optical density at 660 nm [OD₆₆₀] was between 0.3 and 0.4) by centrifugation at 4,500 rpm for 5 min and washed once with chemotaxis buffer (10 mM potassium phosphate buffer [pH 7.0], 0.1 mM disodium EDTA) (37). Quantitative capillary assays were carried out as described previously, with slight modifications (2). One-microliter capillaries were filled with 0.63 μ l of chemotaxis buffer or an attractant dissolved in chemotaxis buffer under vacuum, as described previously (33). Washed cells were suspended in chemotaxis buffer to an OD₆₆₀ of approximately 0.1 (approximately 8×10^7 cells per ml). After the solutions were incubated for 30 min at 30°C, the capillary contents were collected and diluted, and cells were enumerated as CFU by plate counts on LB plates. Chemotactic responses were also observed directly with the modified capillary assay at room temperature (18). For this assay, microcapillaries containing chemicals dissolved in chemotaxis buffer in 2% low-melting-temperature agarose were introduced into suspensions of motile cells in the same buffer at an OD₆₆₀ of approximately 0.1, and the accumulation of cells at the capillary tip was visualized and photographed by microscopy at a magnification of $\times 40$.

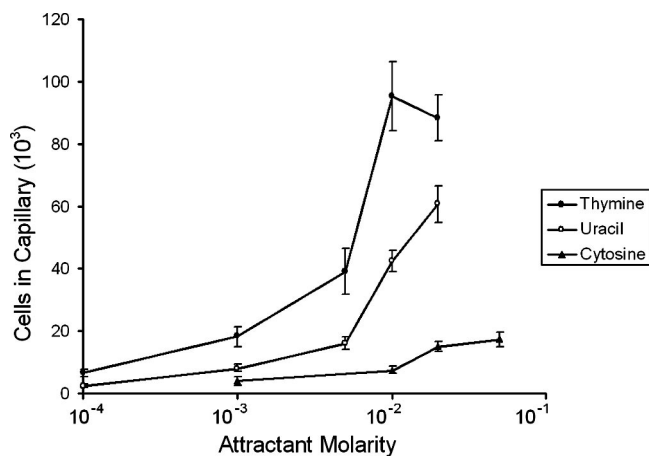


FIG. 1. Concentration response curves for chemotactic responses to pyrimidines by *E. coli* RP437 (wild type). Cells were grown at 30°C in H1 minimal salts medium containing 25 mM glycerol, the required amino acids, and thiamine. Assays were performed at 30°C with each pyrimidine up to its limit of solubility. Results are the averages of at least 10 capillaries from at least three independent experiments; error bars indicate standard errors. Data are not corrected for background accumulation in capillaries containing buffer only ($\sim 5 \times 10^3$ cells).

RESULTS

Wild-type *E. coli* responds chemotactically to pyrimidines.

Quantitative capillary assays were used to measure the chemotactic responses of *E. coli* RP437 to various concentrations of pyrimidines at 30°C. Each compound was tested at concentrations up to the limit of solubility. In all experiments, negative controls (chemotaxis buffer) and positive controls (1 mM aspartate) were included. *E. coli* RP437, which is wild type for chemotaxis, responded to thymine and uracil (Fig. 1). No response was observed for 20 mM cytosine in a modified capillary assay (data not shown), and only very weak responses were detected to relatively high concentrations of cytosine (20 mM and 50 mM) in quantitative capillary assays (Fig. 1). Of the three pyrimidines tested, thymine was the strongest attractant and had the lowest threshold concentration. The chemotactic responses of *E. coli* RP437 to 20 mM thymine (Fig. 2) and to 20 mM uracil (data not shown) were visualized directly by microscopy of modified capillary assays at room temperature. Because the wild-type response to cytosine was very weak, subsequent studies focused on the responses to thymine and uracil.

E. coli RP437 was able to utilize each of the pyrimidines as the sole nitrogen source. When cells were grown in minimal medium at room temperature (21°C) with 25 mM glycerol as the sole carbon source and 1 mM pyrimidine as the sole nitrogen source, the doubling times were 9.3 h, 11.8 h, and 11.2 h for cytosine, thymine, and uracil, respectively. However, when cells were grown at 37°C, only cytosine was used as the sole nitrogen source by *E. coli* RP437. The chemotactic response of cells grown with pyrimidines at room temperature was not improved compared to that of cells grown with ammonium sulfate as the sole nitrogen source (data not shown). Glutamine is typically used as the sole nitrogen source for nitrogen limitation studies (24). Therefore, cells were grown with 5 mM glutamine as the sole nitrogen source and were assayed for

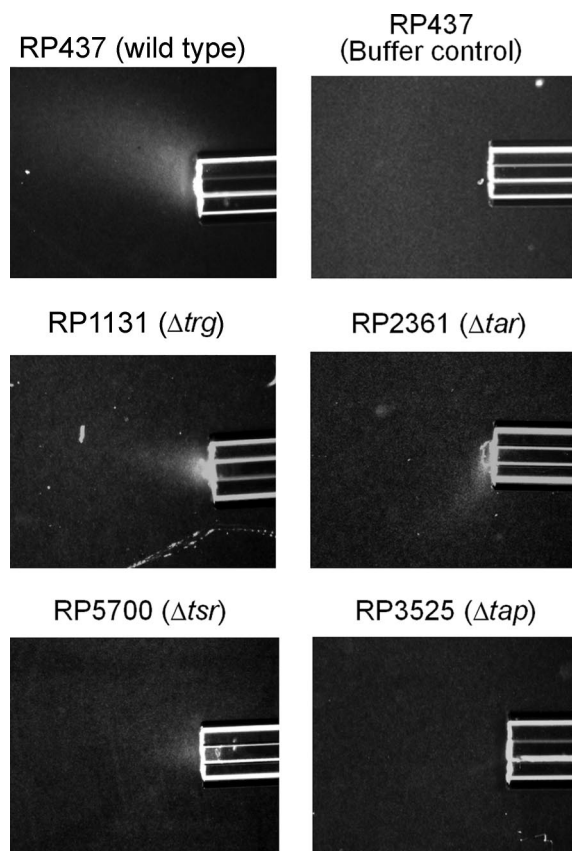


FIG. 2. Chemotactic responses of wild-type and mutant *E. coli* strains to thymine in modified capillary assays. Cells were grown as described in the legend to Fig. 1. All capillaries contained 20 mM thymine in chemotaxis buffer solidified with 2% low-melting-temperature agarose, except for the top right capillary, which contained only buffer solidified with agarose to show the absence of any response by the wild type to buffer. Assays were carried out at room temperature for 20 min as described in Materials and Methods.

their chemotactic responses to pyrimidines. The responses observed with qualitative capillary assays were similar to those for cells grown with ammonium sulfate as the sole nitrogen source (data not shown). Together, these results indicate that the chemotactic response to pyrimidines was constitutively expressed under all tested growth conditions.

Tap mediates chemotaxis toward pyrimidines. To determine which MCP was responsible for pyrimidine chemotaxis, qualitative capillary assays were used to screen single and multiple MCP knockout mutants (kindly provided by J. S. Parkinson) for chemotactic response to 20 mM thymine or 20 mM uracil. RP3525, a Δtap mutant, showed no response to either pyrimidine, while RP1131 ($trg::Tn10$), RP2361 (Δtar), and RP5700 (Δtsr) retained the ability to respond (Fig. 2; Table 2). Among the multiple MCP knockout mutants tested, UU1250 ($\Delta[tar-tap]$ Δtsr Δtrg), UU1615 ($\Delta[tar-tap]$ Δtrg), and UU1624 (Δtsr Δtrg Δtap) were not attracted to pyrimidines (Table 2). UU1625, a strain carrying only Tap and Tar, responded to pyrimidines (Table 2). In summary, all tested strains lacking *tap* did not show chemotaxis toward thymine or uracil, while all tested strains with an intact *tap* gene and at least one high-abundance MCP (Tar or Tsr) showed taxis toward thymine and

TABLE 2. Qualitative capillary assay results with *E. coli* wild type and MCP deletion mutants

Chemical stimulus (concn)	RP437 (wild-type) response	Mutant (deletion) chemotaxis response							
		RP1131 (Δ trg)	RP2361 (Δ tar)	RP3525 (Δ tap)	RP5700 (Δ tsr)	UU1250 (Δ [tar-tap] Δ tsr Δ trg)	UU1615 (Δ [tar-tap] Δ trg)	UU1624 (Δ tsr Δ trg Δ tap)	UU1625 (Δ tsr Δ trg)
None (buffer only)	–	–	–	–	–	–	–	–	–
CAA (2%) ^a	+	+	+	+	+	+	+	+	+
Aspartate (10^{-3} M)	+	+	–	+	+	–	–	+	+
Thymine (2×10^{-2} M)	+	+	+	–	+	–	–	–	+
Uracil (2×10^{-2} M)	+	+	+	–	+	–	–	–	+

^a CAA, Casamino Acids was used as the positive control in these assays.

uracil. These results indicate that Tap, which is known to be involved in dipeptide chemotaxis (32), plays an essential role in chemotaxis to pyrimidines.

Using quantitative capillary assays, the responses of the Δ tap mutant strain RP3525 to thymine and uracil were determined. The peak attractant concentrations determined with the wild-type strain RP437 (Fig. 1) were used for each pyrimidine. RP3525 cells did not respond to either pyrimidine but were still attracted to aspartate (Fig. 3). These results further confirm that Tap is required for pyrimidine chemotaxis.

Chimeric MCP Tapsr is able to mediate pyrimidine chemotaxis. Chimeric MCPs were constructed by genetically switching the periplasmic sensing domains and the cytoplasmic signaling domains of Tap and Tsr, effectively fusing the N- and C-terminal domains of the two proteins at a site located 42 amino acids downstream of the second TM domain (Fig. 4A). This site corresponds to a conserved NdeI site present in *tsr* and *tar*, which has been used to construct Tap-Tar and Trg-Tsr hybrids (15, 43). The chimeric protein containing the periplasmic domain and both TM segments of Tap fused to the cytoplasmic portion of Tsr was designated Tapsr; the reciprocal fusion protein was designated Tsrp. Tsrp5 and Tsrp19 are derivatives of Tsrp, with the last 5 and 19 amino acids from

Tsr, respectively, added to the C-terminal end to provide the essential C-terminal pentapeptide required for efficient signaling (Fig. 4A).

Wild-type and chimeric MCP genes were expressed individually from the multicopy vector pRR48 in UU1250, an *E. coli* strain lacking all four of the native MCPs (Table 1). Chemotactic responses were measured after induction with IPTG, using quantitative capillary assays (Fig. 4B). As expected, UU1250(pXL2) (Tsr⁺) was attracted to serine but not to pyrimidines or the dipeptide L-prolyl-L-leucine (Pro-Leu). UU1250(pXL5), expressing wild-type Tap, did not respond to any of the tested compounds. The strain expressing Tapsr (UU1250[pXL4]) responded to thymine, uracil, and Pro-Leu but not to serine (Fig. 4B). Cells of UU1250(pXL3), expressing the chimeric MCP Tsrp, did not accumulate above background levels (approximately 4×10^3 cells/capillary) in any attractant-containing capillary. UU1250(pXL9), expressing Tsrp19, was attracted to serine but not to thymine, uracil, or Pro-Leu (Fig. 4B). We also generated a chimeric version of Tsrp with the pentapeptide NWETF at the C terminus (Tsrp5) (Table 1 and Fig. 4A) and found that this MCP did not confer UU1250 with the ability to respond to serine in qualitative capillary assays (data not shown), indicating that more than the C-terminal pentapeptide is required to confer signaling. None of the strains responded to any of the chemoattractants when they were grown in the absence of IPTG (data not shown). The results indicate that the periplasmic domain of Tap is responsible for detecting thymine and uracil and that the Tsr signaling domain confers on Tapsr the ability to mediate efficient chemotaxis.

Periplasmic DBP is not required for pyrimidine chemotaxis. In *E. coli*, chemotaxis to dipeptides is mediated by Tap, but the primary chemoreceptor is the periplasmic binding protein for the transport of dipeptides, which is encoded by *dppA* (32). A Δ dppA mutant was constructed and tested with qualitative capillary assays to determine whether it was involved in the response to pyrimidines. The mutant showed wild-type responses to thymine and uracil as well as the expected defect in taxis to Pro-Leu (Fig. 5).

DISCUSSION

Very little is known about chemotactic response to pyrimidines, and to the best of our knowledge, this is the first quantitative analysis of bacterial chemotaxis to pyrimidine bases. The nucleosides cytidine and uridine were tested as chemoattractants for *E. coli* B14 in capillary assays, but no response was

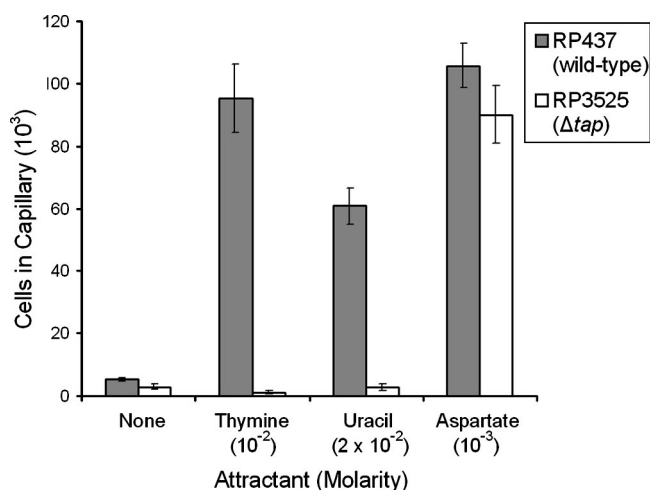


FIG. 3. Chemotactic responses of *E. coli* RP437 (wild type) and RP3525 (Δ tap) to thymine and uracil. Cells were grown as described in the legend to Fig. 1. Results are the averages of at least 10 capillaries from at least three independent experiments; error bars indicate standard errors. "None" indicates that capillaries contained chemotaxis buffer only.

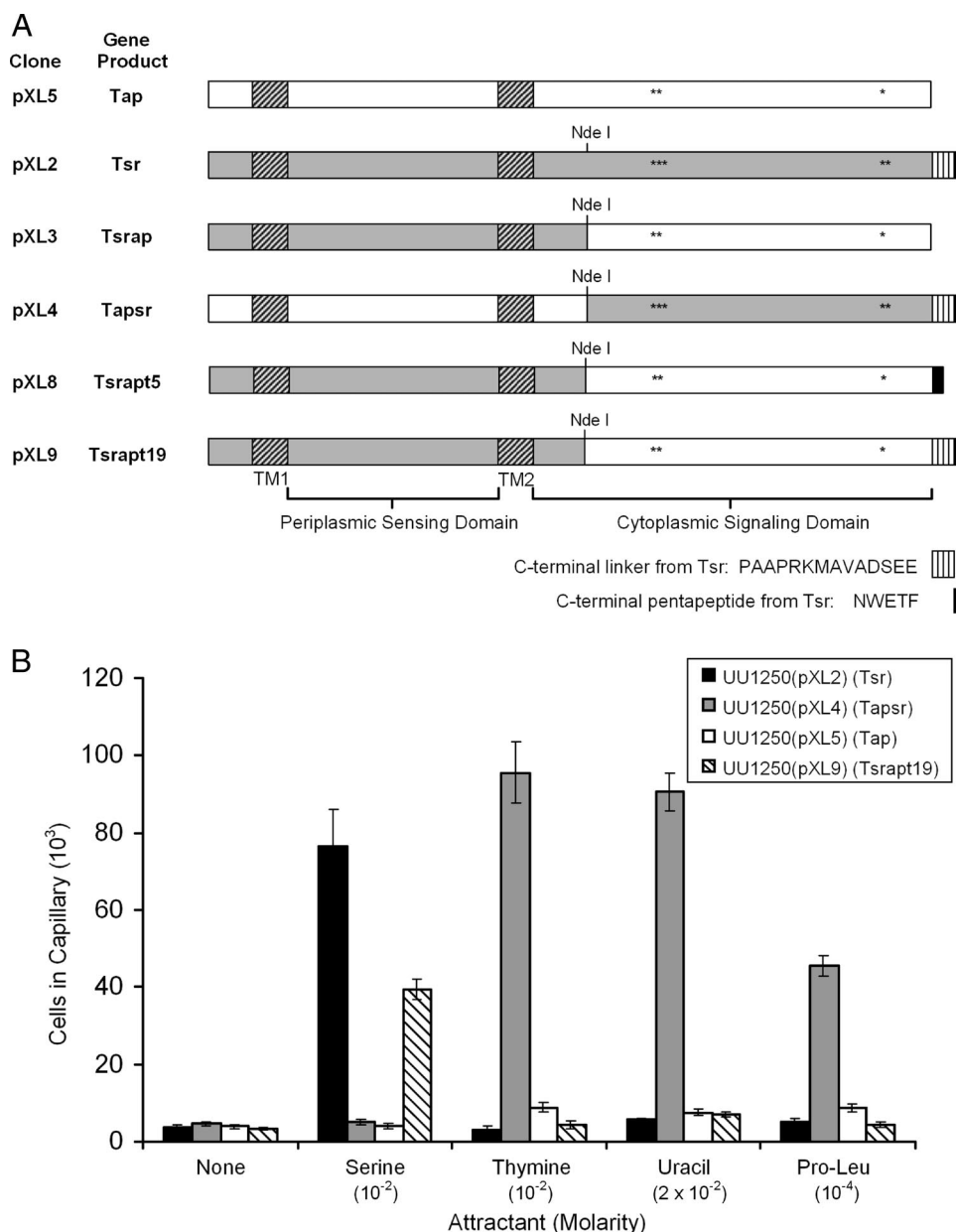


FIG. 4. Chemotaxis of *E. coli* strains expressing wild-type or chimeric MCPs. (A) Diagram of wild-type and chimeric chemoreceptors expressed from the pXL2, pXL3, pXL4, pXL5, pXL8, and pXL9 derivatives of pRR48. Regions originating from Tap are in white; those from Tsr are in gray. Transmembrane regions (TM1 and TM2) are indicated by diagonally striped rectangles. The C-terminal linker region of Tsr, which is required for clustering at cell poles (30), is indicated by vertical stripes. The C-terminal pentapeptide NWTEF (from Tsr), which is required for binding CheR and CheB (8, 44), is indicated in black. Known or predicted methylation sites in Tsr and Tap are indicated by asterisks. (B) Quantitative chemotactic responses of UU1250(pXL2) (Tsr), UU1250(pXL4) (Tapsr), UU1250(pXL5) (Tap), and UU1250(pXL9) (Tsrapt19) to serine, thymine, uracil, and Pro-Leu. No responses to any of the tested attractants were detected with UU1250(pXL3) (Tsr_{ap}) or UU1250(pXL8) (Tsrapt5). Cells were grown at 30°C in H1 minimal salts medium containing 25 mM glycerol, the required amino acids, thiamine, and ampicillin and were induced with IPTG. Results are the averages of at least 10 capillaries from at least three independent experiments; error bars indicate standard errors. "None" indicates that capillaries contained chemotaxis buffer only.

detected; pyrimidines were apparently not tested in this study (3). Another study tested the chemotactic responses of *Vibrio fischeri*, a luminescent marine bacterium that forms a symbiotic association with the Hawaiian squid *Euprymna scolopes*. Using swarm plate assays to monitor the perturbation of serine swarm rings, DeLoney-Marino et al. demonstrated that *V. fischeri* responded to the pyrimidine nucleosides thymidine,

cytidine, and uridine (14). In addition, *V. fischeri* clearly responded to deoxynucleoside triphosphates. The organism also appeared to respond very weakly to relatively high concentrations (≥ 66 mM) of thymine and uracil and therefore demonstrated a preference for nucleosides and nucleotides over the pyrimidine bases. The authors proposed that the response to components of DNA and RNA could play a role in the ability

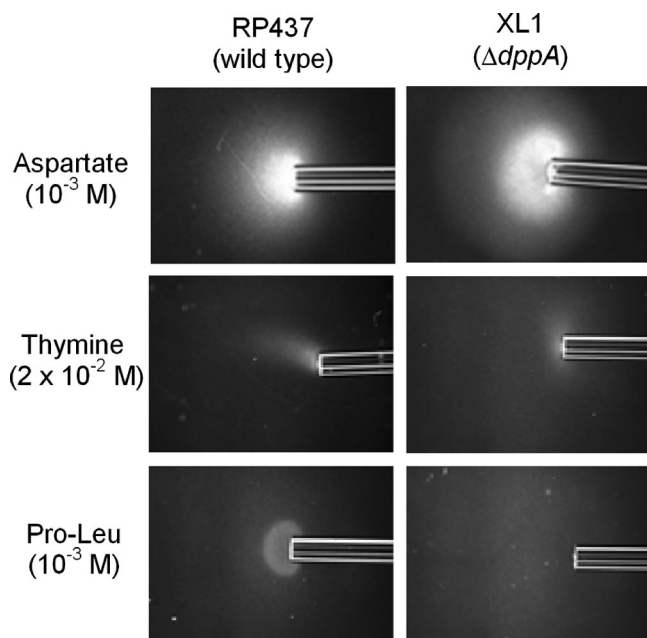


FIG. 5. Chemotactic phenotype of the *E. coli* $\Delta dppA$ mutant in modified capillary assays. Cells were grown as described in the legend to Fig. 1. Capillaries contained aspartate, thymine, or Pro-Leu at the indicated concentrations. Assays were carried out at room temperature for 20 min as described in Materials and Methods.

of *V. fischeri* to colonize its host, particularly in light of the fact that apoptosis of cells in the light organ of *E. scolopes* occurs during colonization (17). The chemoreceptor(s) involved in these responses was not identified.

Until recently, *E. coli* K-12 was not known to grow with thymine or uracil as the nitrogen source, but a study published in 2006 reported that the K-12 derivative NCM3876 utilizes both of the pyrimidines as the sole nitrogen sources via a new pathway at room temperature but not at 37°C (29). We found a similar pattern of temperature-sensitive growth with thymine and uracil for *E. coli* RP437, the standard strain used for studying chemotaxis, and demonstrated that RP437 is chemotactic to thymine and uracil at room temperature (Fig. 2) and at 30°C (Fig. 1). Although the genes required for pyrimidine utilization (*rutABCDEFGF*) were shown to be expressed at higher levels under nitrogen-limiting conditions (29), the chemotactic response to pyrimidines appeared to be constitutive in strain RP437. Pyrimidine utilization at relatively low temperatures suggests that these compounds are used as nitrogen sources during that portion of the *E. coli* life cycle which occurs outside the animal host. The chemotactic responses observed within the same temperature range are consistent with the possibility that *E. coli* uses its pyrimidine chemotaxis system to locate alternative nitrogen sources when it is in the free-living state. Interestingly, most uropathogenic isolates of *E. coli* that were tested lacked both the *trg* and the *tap* genes, suggesting a difference in their selective environments (25). The thresholds for detection of thymine and uracil are relatively high, and it is not clear at this time whether the chemotactic response is physiologically relevant. Pyrimidines have been detected in soil samples, but concentrations were not reported (38); however,

one could image the presence of relatively high concentrations of pyrimidines, for example, in rotting vegetation, where cells are lysing, and RNA and DNA are being released and degraded.

Slocum and Parkinson (39) reported the characterization of *E. coli* strains with *tap* deletions but were unable to identify a mutant phenotype. They did, however, make the insightful suggestion that Tap might be responsible for the detection of untested types of compounds such as peptides, vitamins, or nucleotides. A role for Tap was reported a year later when Manson et al. (32) demonstrated that Tap functions as the signal transducer for chemotactic responses to dipeptides. The current report reveals that a second set of chemoattractants, the pyrimidines thymine and uracil, requires the participation of Tap.

MCPs form clusters at cell poles (31) and signal collaboratively (5). The two high-abundance MCPs Tar and Tsr are present at cellular levels approximately 10-fold higher than those of the low-abundance MCPs Trg and Tap (21, 27). The pentapeptide NWETF, present at the C terminus of high-abundance MCPs, has been found to be the binding site for both CheR and CheB (8, 44) and is required for effective adaptation (35). Low-abundance MCPs lack this pentapeptide and are methylated and demethylated inefficiently in the absence of high-abundance MCPs. For this reason, cells containing only low-abundance receptors have strong counterclockwise flagellar rotational biases and do not respond well to gradients (15, 16, 43). Tap is a low-abundance MCP lacking the C-terminal ~20 amino acid residues that are required for efficient signaling and as a result, strains expressing Tap as the sole MCP do not show chemotactic responses (Fig. 4B) (43). To confirm that Tap was responsible for mediating pyrimidine chemotaxis and to test whether pyrimidines are sensed through the periplasmic domain of Tap, we constructed hybrid Tap-Tsr MCPs. Functional Tap-Tar and Trg-Tsr hybrids have been constructed and analyzed previously (15, 16, 43). In each case, a hybrid MCP with the N-terminal sensing domain of a low-abundance MCP (Tap or Trg) fused to the C-terminal signaling domain of a high-abundance MCP (Tar or Tsr) was able to sense the compound(s) detected by the low-abundance MCP. For example, the Tap-Tar hybrid (designated Tap_r) detected dipeptides but not aspartate (the attractant sensed by Tar) (43), and Trs_r (the Trg-Tsr hybrid) responded to ribose and galactose (the attractants sensed by Trg) but did not sense serine (15, 16). All of our results (Fig. 4B) indicate that the periplasmic domain of Tap is responsible for sensing thymine and uracil and that the Tsr signaling domain confers on Tap_r the ability to mediate efficient chemotaxis. Interestingly, the addition of the C-terminal 23 amino acids from Tar onto Tap (Tap_l) was insufficient to confer signaling ability on this chimeric MCP (43). In contrast, we found that the C-terminal 19 amino acids of Tsr were sufficient to allow signaling by the chimeric Tsr_{ap}19. Whether the differences in signaling capacities between Tap_l and Tsr_{ap}19 are due to differences in the C-terminal sequences of Tar and Tsr or the fact that our chimeric protein contained both the N terminus and the extreme C terminus of Tsr is not clear at this time. However, we did find that the addition of only the terminal pentapeptide NWETF onto Tsr_{ap} resulted in a nonfunctional MCP, suggesting that the C-terminal linker upstream of the pentapeptide is

essential. These results are consistent with reports demonstrating that the C-terminal ~20 amino acids of high-abundance chemoreceptors are required for chemoreceptor clustering (30) and that the C-terminal linker upstream of the pentapeptide is important for adaptational modification (28).

In *E. coli*, amino acid attractants bind directly to MCPs, whereas sugars and dipeptides form complexes with specific periplasmic binding proteins, which then interact with the appropriate MCPs. The periplasmic DBP component of the dipeptide permease, which transports a variety of di- and tripeptides (1), was shown to be required for dipeptide chemotaxis (32). DBP functions as a high-affinity binding protein that interacts with Tap when bound to dipeptide substrates, and it is the only known periplasmic chemoreceptor for nonsugar substrates (32). Although we considered it unlikely that DBP would be involved in taxis to pyrimidines, we decided it was worth testing because prior to the current study, dipeptides were the only chemoattractants identified that were sensed through Tap. Using a $\Delta dppA$ mutant, we showed that DBP is not required for pyrimidine chemotaxis because the mutant still responded to thymine and uracil (Fig. 5). At this time, we do not know whether Tap serves as the primary chemoreceptor for pyrimidines, but if a periplasmic binding protein is not involved, pyrimidines would be the first attractants that interact directly with Tap. Periplasmic binding proteins that are known to be involved in chemotaxis are typically components of ABC transporters. Although there are currently no ABC transporters identified for pyrimidines in *E. coli*, there are several annotated ABC transporter gene clusters in the *E. coli* genome for which substrates remain unidentified. The known pyrimidine transporters in *E. coli* include the membrane-bound cytosine and uracil transporters CodB and UraA (6, 11), both of which have 12 predicted membrane-spanning segments (6, 10). In addition, RutG, which is encoded in the pyrimidine utilization gene cluster, has recently been predicted to be a uracil permease with 11 membrane-spanning segments (29). There are currently two examples of membrane-bound major facilitator superfamily transporters that are also required for chemotaxis to their substrates. These include PcaK, the transporter/receptor for 4-hydroxybenzoate in *Pseudomonas putida* PRS2000 (19, 34), and TfdK, the transporter/receptor for 2,4-dichlorophenoxyacetate in *Ralstonia eutropha* JMP134 (20, 26). The specific roles of these two proteins in chemotaxis are not completely clear, but each is absolutely required for the chemotactic response. Future work will be required to address the question of whether Tap binds pyrimidines directly or via an additional chemoreceptor protein.

ACKNOWLEDGMENTS

We thank Sandy Parkinson for providing strains, numerous valuable suggestions, and constructive comments on the manuscript. We thank Valley Stewart and Li-Ling Chen for providing strains and for helpful advice on the construction of the $\Delta dppA$ mutant; Carrie Harwood, Eric Kofoid, Sydney Kustu, Jack Meeks, and Michele Igo for helpful discussions; and several attendees of the BLAST meeting and two anonymous reviewers for their useful suggestions.

This work was supported by a University of California Toxic Substances Research and Teaching Program Investigator grant.

REFERENCES

- Abouhamad, W. N., M. Manson, M. M. Gibson, and C. F. Higgins. 1991. Peptide transport and chemotaxis in *Escherichia coli* and *Salmonella typhimurium*: characterization of the dipeptide permease (Dpp) and the dipeptide-binding protein. *Mol. Microbiol.* **5**:1035–1047.
- 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.
- Adler, J., G. L. Hazelbauer, and M. M. Dahl. 1973. Chemotaxis toward sugars in *Escherichia coli*. *J. Bacteriol.* **115**:824–847.
- Ames, P., and J. S. Parkinson. 2006. Conformational suppression of inter-receptor signaling defects. *Proc. Natl. Acad. Sci. USA* **103**:9292–9297.
- Ames, P., C. A. Studdert, R. H. Reiser, and J. S. Parkinson. 2002. Collaborative signaling by mixed chemoreceptor teams in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **99**:7060–7065.
- Anderssen, P. S., D. Frees, R. Fast, and B. Mygind. 1995. Uracil uptake in *Escherichia coli* K-12: isolation of *uraA* mutants and cloning the gene. *J. Bacteriol.* **177**:2008–2013.
- Baker, M. D., P. M. Wolanin, and J. B. Stock. 2006. Signal transduction in bacterial chemotaxis. *Bioessays* **28**:9–22.
- Barnakov, A. N., L. A. Barnakova, and G. L. Hazelbauer. 1999. Efficient adaptational demethylation of chemoreceptors requires the same enzyme-docking site as efficient methylation. *Proc. Natl. Acad. Sci. USA* **96**:10667–10672.
- Bibikov, S. I., A. C. Miller, K. K. Gosink, and J. S. Parkinson. 2004. Methylation-independent aerotaxis mediated by the *Escherichia coli* Aer protein. *J. Bacteriol.* **186**:3730–3737.
- Danielsen, S., D. Boyd, and J. Neuhard. 1995. Membrane topology analysis of the *Escherichia coli* cytosine permease. *Microbiology* **141**:2905–2913.
- Danielsen, S., M. Kilstrup, K. Barilla, B. Jochimsen, and J. Neuhard. 1992. Characterization of the *Escherichia coli* *codBA* operon encoding cytosine permease and cytosine deaminase. *Mol. Microbiol.* **6**:1335–1344.
- Datsenko, K. A., and B. L. Wanner. 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. USA* **97**:6640–6645.
- Davis, R. W., D. Botstein, and J. R. Roth. 1980. *Advanced bacterial genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- DeLoney-Marino, C. R., A. J. Wolfe, and K. L. Visick. 2003. Chemoattraction of *Vibrio fischeri* to serine, nucleosides, and *N*-acetylneuraminic acid, a component of squid light-organ mucus. *Appl. Environ. Microbiol.* **69**:7527–7530.
- Feng, X., J. W. Baumgartner, and G. L. Hazelbauer. 1997. High- and low-abundance chemoreceptors in *Escherichia coli*: differential activities associated with closely related cytoplasmic domains. *J. Bacteriol.* **179**:6714–6720.
- Feng, X., A. A. Lilly, and G. L. Hazelbauer. 1999. Enhanced function conferred on low-abundance chemoreceptor Trg by a methyltransferase-docking site. *J. Bacteriol.* **181**:3164–3171.
- Foster, J. S., and M. J. McFall-Ngai. 1998. Induction of apoptosis by cooperative bacteria in the morphogenesis of host epithelial tissues. *Dev. Genes Evol.* **208**:295–303.
- Grimm, A. C., and C. S. Harwood. 1997. Chemotaxis of *Pseudomonas putida* to the polyaromatic hydrocarbon naphthalene. *Appl. Environ. Microbiol.* **63**:4111–4115.
- Harwood, C. S., N. N. Nichols, M.-K. Kim, J. L. Ditty, and R. E. Parales. 1994. Identification of the *pcaRKF* gene cluster from *Pseudomonas putida*: involvement in chemotaxis, biodegradation, and transport of 4-hydroxybenzoate. *J. Bacteriol.* **176**:6479–6488.
- Hawkins, A. C., and C. S. Harwood. 2002. Chemotaxis of *Ralstonia eutropha* JMP134(pJP4) to the herbicide 2,4-dichlorophenoxyacetate. *Appl. Environ. Microbiol.* **68**:968–972.
- Hazelbauer, G. L., P. Engstrom, and S. Harayama. 1981. Methyl-accepting chemotaxis protein III and transducer gene *trg*. *J. Bacteriol.* **145**:43–49.
- Hedblom, M. L., and J. Adler. 1980. Genetic and biochemical properties of *Escherichia coli* mutants with defects in serine chemotaxis. *J. Bacteriol.* **144**:1048–1060.
- Kondoh, H., C. B. Ball, and J. Adler. 1979. Identification of a methyl-accepting chemotaxis protein for the ribose and galactose chemoreceptors of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **76**:260–264.
- Kustu, S., D. Burton, E. Garcia, L. McCarter, and N. McFarland. 1979. Nitrogen control in *Salmonella*: regulation by the *glnR* and *glnF* gene products. *Proc. Natl. Acad. Sci. USA* **76**:4576–4580.
- Lane, M. C., A. L. Lloyd, T. A. Markyvech, E. C. Hagan, and H. L. Mobley. 2006. Uropathogenic *Escherichia coli* strains generally lack functional Trg and Tap chemoreceptors found in the majority of *E. coli* strains strictly residing in the gut. *J. Bacteriol.* **188**:5618–5625.
- Leveau, J. H., A. J. Zehnder, and J. R. van der Meer. 1998. The *tfdK* gene product facilitates uptake of 2,4-dichlorophenoxyacetate by *Ralstonia eutropha* JMP134(pJP4). *J. Bacteriol.* **180**:2237–2243.
- Li, M., and G. L. Hazelbauer. 2004. Cellular stoichiometry of the components of the chemotaxis signaling complex. *J. Bacteriol.* **186**:3687–3694.
- Li, M., and G. L. Hazelbauer. 2006. The carboxyl-terminal linker is important for chemoreceptor function. *Mol. Microbiol.* **60**:469–479.
- Loh, K. D., P. Gyaneshwar, E. Papadimitriou, R. Fong, K.-S. Kim, R. E. Parales, Z. Zhou, W. Inwood, and S. Kustu. 2006. A previously undescribed pathway for pyrimidine catabolism. *Proc. Natl. Acad. Sci. USA* **103**:5114–5119.

30. **Lybarger, S. R., U. Nair, A. A. Lilly, G. L. Hazelbauer, and J. R. Maddock.** 2005. Clustering requires modified methyl-accepting sites in low-abundance but not high-abundance chemoreceptors of *Escherichia coli*. *Mol. Microbiol.* **56**:1078–1086.
31. **Maddock, J. R., and L. Shapiro.** 1993. Polar location of the chemoreceptor complex in the *Escherichia coli* cell. *Science* **259**:1717–1723.
32. **Manson, M. D., V. Blank, G. Brade, and C. F. Higgins.** 1986. Peptide chemotaxis in *E. coli* involves the Tap signal transducer and the dipeptide permease. *Nature* **321**:253–256.
33. **Meyer, G., T. Schneider-Merck, S. Bohme, and W. Sand.** 2002. A simple method for investigations on the chemotaxis of *Acidithiobacillus ferrooxidans* and *Desulfovibrio vulgaris*. *Acta Biotechnol.* **22**:391–399.
34. **Nichols, N. N., and C. S. Harwood.** 1997. PcaK, a high-affinity permease for the aromatic compounds 4-hydroxybenzoate and protocatechuate from *Pseudomonas putida*. *J. Bacteriol.* **179**:5056–5061.
35. **Okumura, H., S. Nishiyama, A. Sasaki, M. Homma, and I. Kawagishi.** 1998. Chemotactic adaptation is altered by changes in the carboxy-terminal sequence conserved among the major methyl-accepting chemoreceptors. *J. Bacteriol.* **180**:1862–1868.
36. **Parkinson, J. S., P. Ames, and C. A. Studdert.** 2005. Collaborative signaling by bacterial chemoreceptors. *Curr. Opin. Microbiol.* **8**:116–121.
37. **Parkinson, J. S., and S. E. Houts.** 1982. Isolation and behavior of *Escherichia coli* deletion mutants lacking chemotaxis functions. *J. Bacteriol.* **151**:106–113.
38. **Schreiner, O., and E. C. Shorey.** 1910. Pyrimidine derivatives and purine bases in soils. *J. Biol. Chem.* **8**:385–393.
39. **Slocum, M. K., and J. S. Parkinson.** 1985. Genetics of methyl-accepting chemotaxis proteins in *Escherichia coli*: null phenotypes of the *tar* and *tap* genes. *J. Bacteriol.* **163**:586–594.
40. **Sourjik, V.** 2004. Receptor clustering and signal processing in *E. coli* chemotaxis. *Trends Microbiol.* **12**:569–576.
41. **Springer, M. S., M. F. Goy, and J. Adler.** 1977. Sensory transduction in *Escherichia coli*: two complementary pathways of information processing that involve methylated proteins. *Proc. Natl. Acad. Sci. USA* **74**:3312–3316.
42. **Studdert, C. A., and J. S. Parkinson.** 2005. Insights into the organization and dynamics of bacterial chemoreceptor clusters through in vivo crosslinking studies. *Proc. Natl. Acad. Sci. USA* **102**:15623–15628.
43. **Weerasuriya, S., B. M. Schneider, and M. D. Manson.** 1998. Chimeric chemoreceptors in *Escherichia coli*: signaling properties of Tar-Tap and Tap-Tar hybrids. *J. Bacteriol.* **180**:914–920.
44. **Wu, J., J. Li, G. Li, D. G. Long, and R. M. Weis.** 1996. The receptor binding site for the methyltransferase of bacterial chemotaxis is distinct from the sites of methylation. *Biochemistry* **35**:4984–4993.
45. **Zhulin, I. B.** 2001. The superfamily of chemotaxis transducers: from physiology to genomics and back. *Adv. Microbial Phys.* **45**:157–198.