

Biphasic Excitation by Leucine in *Escherichia coli* Chemotaxis

Shahid Khan^{1*} and David R. Trentham²

Molecular Biology Consortium, Chicago, Illinois 60612,¹ and National Institute for Medical Research, London NW7 1AA, United Kingdom²

Received 29 July 2003/Accepted 10 October 2003

Leucine concentration jumps (applied by photolysis of inert derivatives) triggered swim or tumble responses in *Escherichia coli* mutants lacking Tsr or Tar, respectively. Wild-type *E. coli* bacteria were attracted in spatial assays when the initial leucine concentration difference was 5 to 120 μ M but were repulsed when it was over 0.5 mM. Their responses to concentration jumps confirmed earlier deductions regarding biphasic excitation.

Among several hydrophobic amino acids that repel *Escherichia coli*, L- (or D-) leucine is the most potent. A microfluidic assay developed by Mao et al. (11) showed that at low concentrations L-leucine was also an attractant. We reached a similar conclusion upon analysis, using the photorelease assay, (7) of *E. coli* chemotactic excitation behavior and have used this finding to characterize biphasic excitation (8). Photolabile derivatives of leucine, *O*-2,6-dinitrobenzyl-L-leucine (DNB-Leu), and *N*-1-(2-nitrophenyl)ethoxycarbonyl-L-leucine (NPEC-Leu) were synthesized for rapid photogeneration of leucine (Fig. 1). The methyl-accepting chemotaxis protein (MCP) Tsr mediates repulsion from leucine (15). Tsr was the predominant receptor in the Δtar strain RP2361 lacking the other major MCP, Tar. The rate of change of direction (RCD) (a measure of the population angular speed) (7) of this strain increased upon leucine photorelease, as expected. A saturation response was obtained upon photorelease of 0.5 mM leucine (Fig. 2A). No repellent response was seen in the Δtsr strain RP5700. Furthermore, as anticipated on the basis of the results of a previous study (11), a swim response (i.e., decreased RCD) was obtained (Fig. 2B). A jump in concentration from 0 to 5 μ M elicited a saturation smooth-swim response; a jump in concentration from 0 to 50 nM elicited a detectable response. Thus, the attractant excitation response to leucine is comparable in strength to that seen upon serine photorelease (7).

The Δtsr strain responded by swimming smoothly when either DNB-Leu or NPEC-Leu was used. Photorelease of protons is known to elicit smooth swim responses in Δtsr strains (8). This was ruled out as a potential cause of the response seen upon leucine photorelease as follows. First, increasing the buffer concentration of morpholineethanesulfonic acid (MES) from 10 to 100 mM did not alter the response. Second, although DNB-Leu photolysis liberates protons, NPEC-Leu photolysis results in net hydroxide ion release during the 2-s observation time following photolysis (Fig. 1). The Δtar Δtsr mutant RP3851 did not respond (Fig. 2C). Thus, Tar was the major determinant for the swim response. The $\Delta cheRcheB$ strain RP2859 has normal wild-type bias but greatly reduced response to aspartate (9, 14). This strain also did not respond.

Leucine response sensitivity must therefore involve a role for the MCP methylesterase CheB and/or methyltransferase CheR, as seen for aspartate.

Spatial assays were conducted (as described previously) (1, 15) to explore the consequences of dual-signal generation for chemotactic migration. The half-maximal doses ($L_{1/2}$) for repulsion from plugs containing leucine were 10 and 3.6 mM for wild-type and Δtar strains, respectively. The higher $L_{1/2}$ observed for the wild type (relative to that observed for Δtar *E. coli*) may be due to attenuation of the Tsr repellent response by Tar-mediated attraction. The Δtsr strain did not respond (Fig. 3A). Capillary, rather than plug, assays provided a better test for attraction. The Δtar strain was repelled, but wild-type and Δtsr *E. coli* bacteria accumulated in the capillary when the initial concentration difference between it and the pond was 0 to 120 μ M or lower (Fig. 3B). Accumulation would decrease at higher concentrations, since the concentration gradient centered on the $L_{1/2}$ would move further away from the capillary mouth by the end of the assay (6). However, the observed decrease was more severe than expected on this basis. It was similar to that observed for competition of the attractant, aspartate, with the repellent, valine (2). The fact that the response declined for Δtsr as well as wild-type strains in the spatial assay (Fig. 3B) suggests that repulsion from leucine is not mediated solely by Tsr. The wild-type response to aspartate (Fig. 3B inset) was an order of magnitude stronger and was maintained over a larger concentration range than the leucine attractant response (consistent with attenuation of the Tar-mediated swim signal by the Tsr tumble signal). Our spatial assay data broadly agree with previously reported leucine plug and aspartate capillary assay results (2, 15) and with the findings of Mao et al. (11), who recorded attractant responses with concentrations of leucine down to 1 μ M.

Biphasic excitation was first observed for protons (8) (sensed by Tar as attractant and by Tsr as repellent). Leucine seems to behave similarly (Fig. 4A). As argued previously (8), the smooth-swim phase of biphasic excitation is due to generation of a second signal and not to adaptation of the initial repellent signal, since the tumble response in Δtar strains lasted for many seconds. Since the Tar $L_{1/2}$ was lower than the Tsr $L_{1/2}$, we expected that a small (0 to 5 μ M leucine) jump would predominantly elicit swimming and that a prestimulus background leucine concentration between the Tar $L_{1/2}$ and Tsr $L_{1/2}$ concentrations would greatly reduce the proportion of the swim

* Corresponding author. Mailing address: Molecular Biology Consortium, Chicago Technology Park, 2201 W. Campbell Park Dr., Chicago, IL 60612. Phone: (312) 942-9017. Fax: (312) 829-4069. E-mail: kh01@tigger.uic.edu.

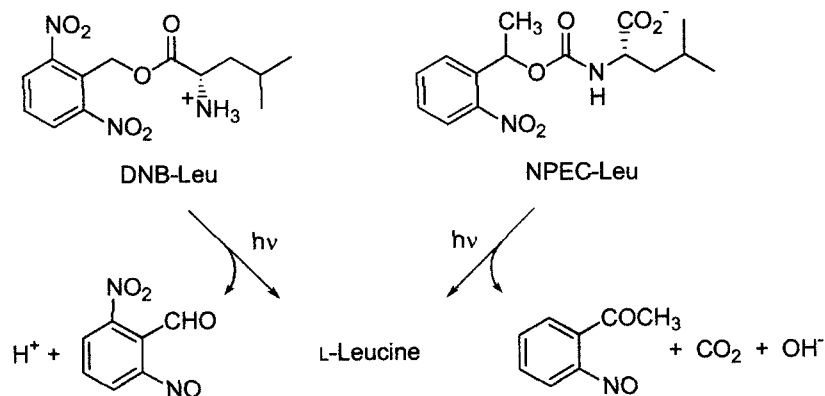


FIG. 1. Caged leucines and their photolysis products. CO_2 generated on NPEC-Leu photolysis hydrates to HCO_3^- over a time period much longer than the observation times of the experiments described below (see reference 7). Details of the synthesis of DNB-Leu and NPEC-Leu will be reported later and are available upon request.

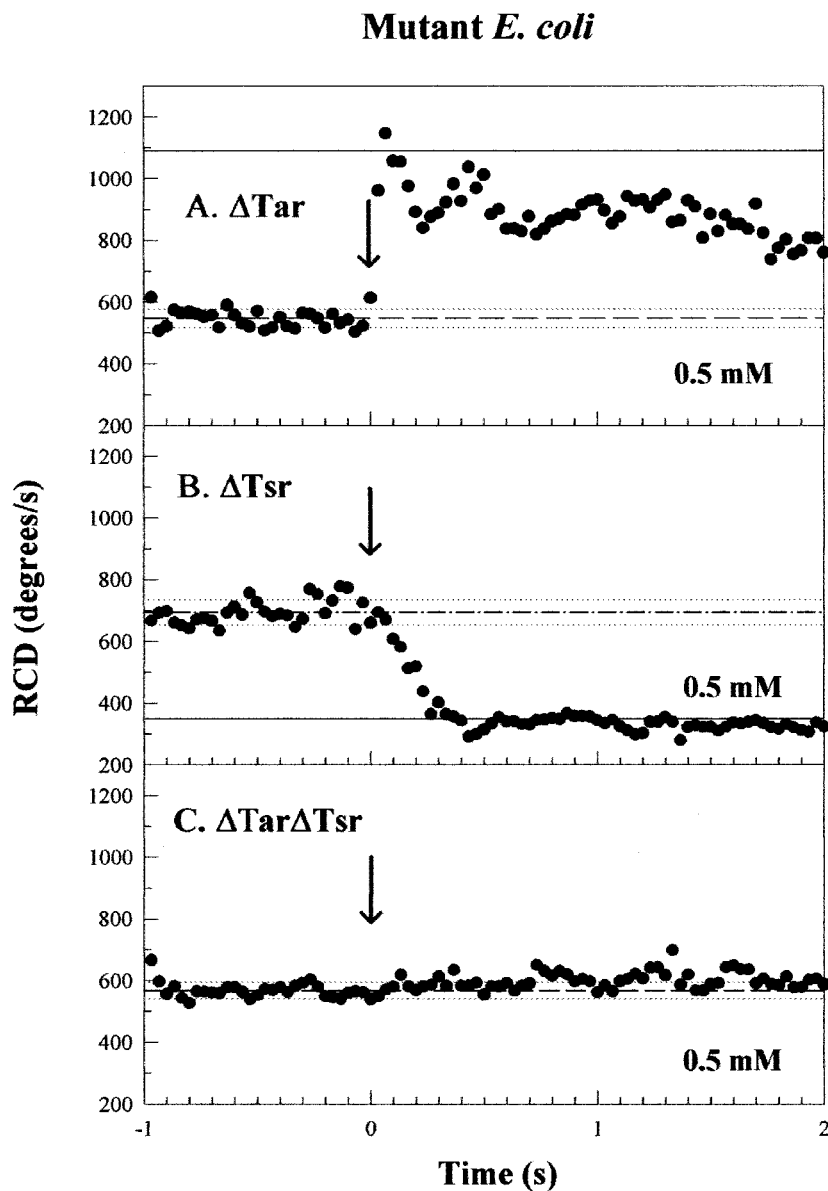


FIG. 2. Excitation responses of mutant *E. coli* upon a jump in leucine concentration from 0 to 500 μM applied by flash photolysis of NPEC-Leu. (A) RP2361 (Δtar); (B) RP5700 (Δtsr); (C) RP3851 ($\Delta tar \Delta tsr$). Arrows denote photolyzing flashes. Dashed and dotted lines denote the prestimulus RCD and its frame-to-frame standard deviation, respectively. Solid lines indicate RCD (rate of change of direction) values for smooth-swimming (low RCD) and tumbly (high RCD) mutant populations, as determined by Khan et al. (7).

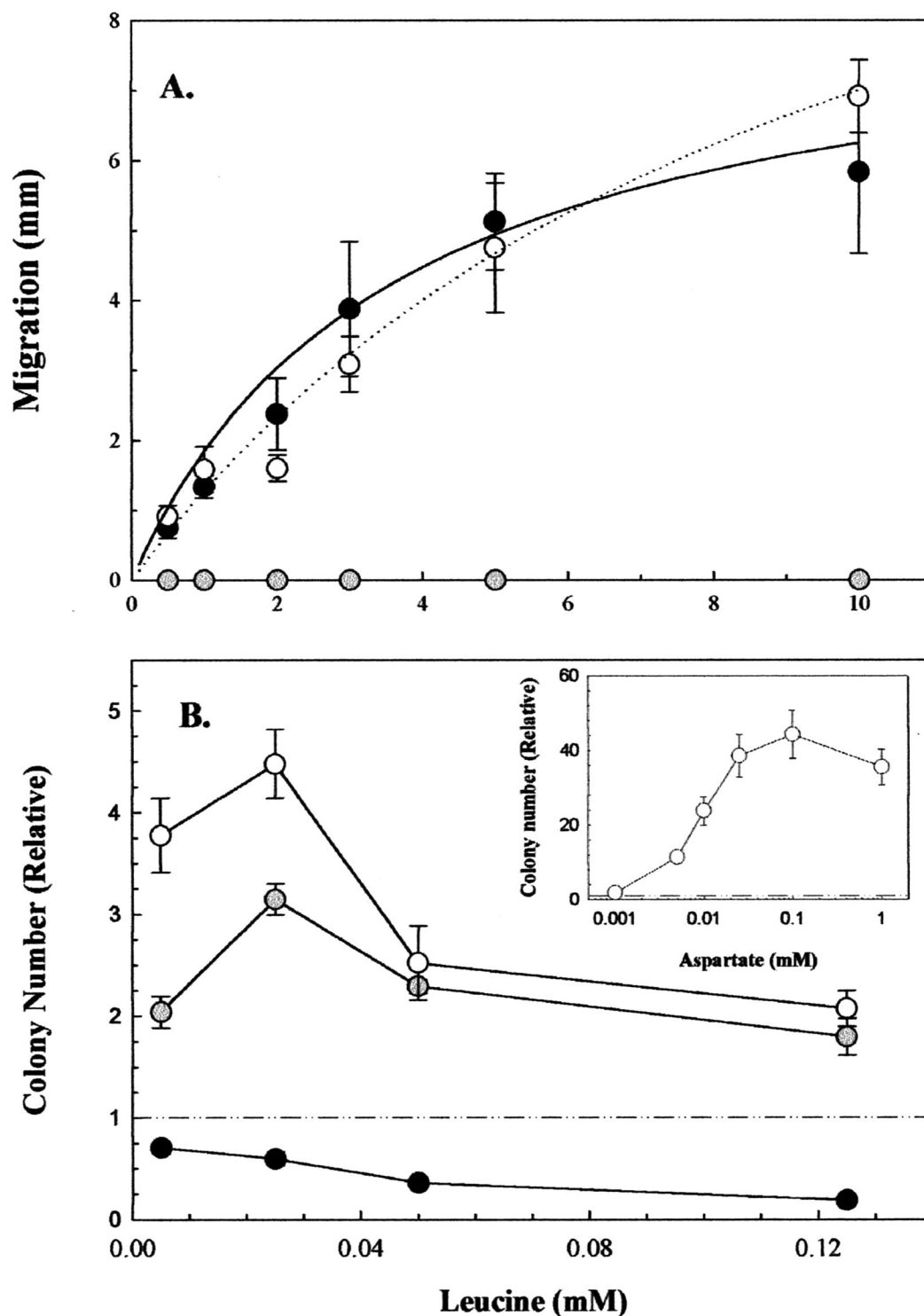


FIG. 3. Migration of wild-type RP437 (open circles), Δtar RP2361 (filled circles), and Δtsr RP5700 (circles shaded in gray) *E. coli* bacteria in spatial assays. Each value denotes the mean (\pm standard error) of triplicate measurements. The assay duration was 20 min in all cases. (A) Plug assay: solid and dotted lines denote weighted least squares best fits to Michaelis-Menten saturation curves, with maximum migration values of 13.9 mm for wild-type and 8 mm for Δtar strains. (B) Capillary assay.

signal in comparison to the tumble signal. These expectations were met (Fig. 4B and C). In the case of protons, it was not feasible to adjust prestimulus pH over a wide range to separate the two signals; effects of the pH jumps on motor operation

and/or flagellar bundle formation were a concern. Thus, this study extends our earlier work to establish biphasic excitation as a consequence of antagonistic signal generation.

The action of the repellent amino acids may be due to

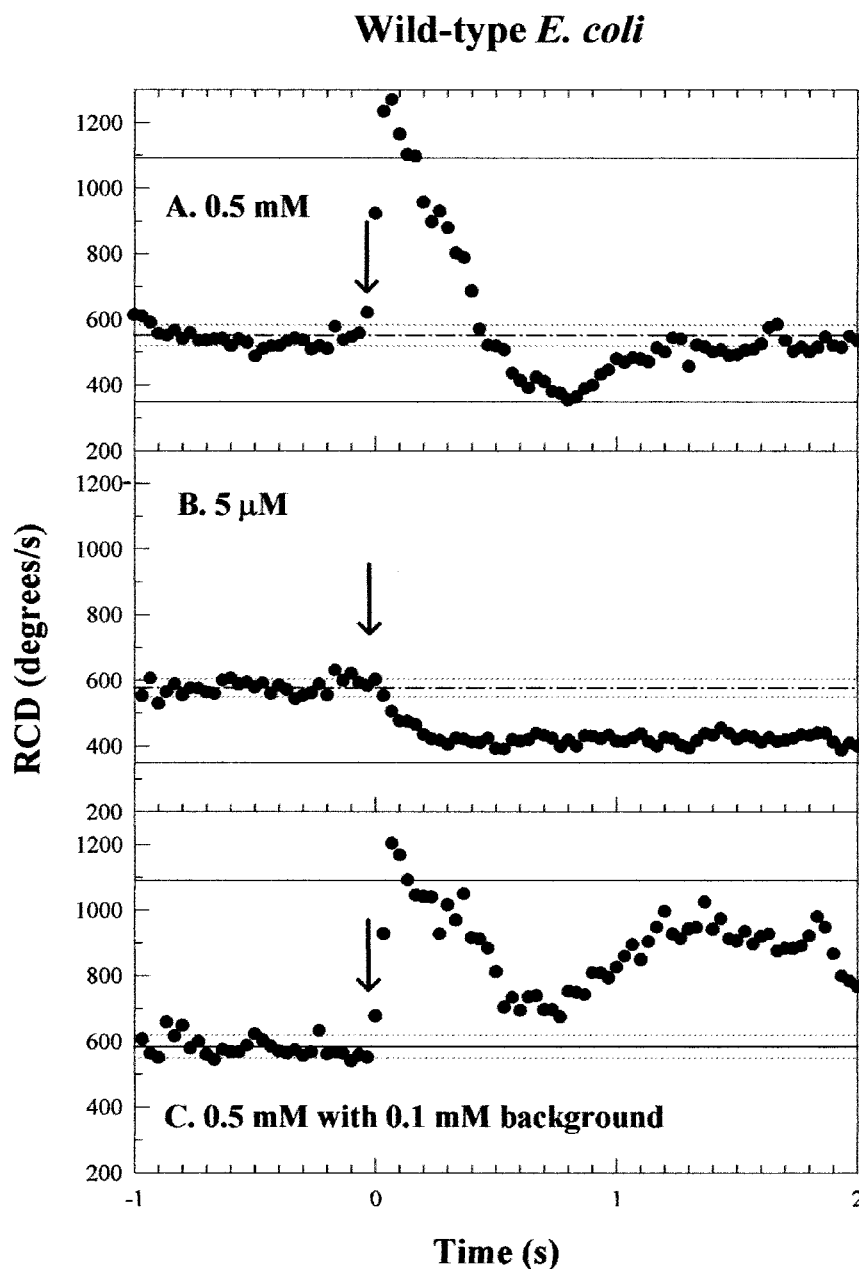


FIG. 4. Excitation responses of wild-type *E. coli* RP437. (A) Leucine concentration jump from 0 to 500 μM . (B) Leucine concentration jump from 0 to 5 μM . (C) Leucine concentration jump from 100 to 600 μM . Arrows and lines have the same connotations as described for Fig. 2.

nonspecific effects on membrane properties, since their $L_{1/2}$ values are two orders of magnitude larger than those for amino acid attractants. However, Eisenbach et al. (5) have argued against this explanation for leucine. In addition, leucine, while most potent, is less hydrophobic than some other amino acid repellents (15). Repulsion at high leucine concentrations, together with strong attraction at low concentrations, may keep expression of the leucine/Lrp regulon, a key control element in amino acid biosynthesis (4), in an optimal range (analogous to those of pH and temperature taxis). Leucine may be added to

the list of effectors that trigger antagonistic signals from different MCPs (10, 13, 16, 17).

Biphasic excitation reveals that integration of signals from different MCPs is not complete at the receptor level. This observation raises interesting issues regarding receptor-receptor interactions if, as believed, different MCPs are part of the same receptor cluster activating a common kinase (3, 12). Quantitative analysis of biphasic excitation (made possible by availability of the caged leucines) should be valuable for deciphering these interactions. In addition, biphasic excitation may

now serve as a rapid diagnostic for dual-signal generation. The Tsr receptor also mediates responses towards the other repellent amino acids (15). It would be of interest to determine whether these compounds also attract, utilizing other MCP family members to do so.

We thank Meghan Gleason for assistance with behavioral assays and Gordon Reid for synthesis of the caged leucines.

This work was supported by grant R01-GM49319 from the National Institutes of Health.

REFERENCES

1. Adler, J. 1973. A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by *Escherichia coli*. *J. Gen. Microbiol.* **74**:77–91.
2. Adler, J., and W. W. Tso. 1974. "Decision"-making in bacteria: chemotactic response of *Escherichia coli* to conflicting stimuli. *Science* **184**:1292–1294.
3. 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.
4. D'Ari, R., R. T. Lin, and E. B. Newman. 1993. The leucine-responsive regulatory protein: more than a regulator? *Trends Biochem. Sci.* **18**:260–263.
5. Eisenbach, M., C. Constantinou, H. Aloni, and M. Shinitzky. 1990. Repellents for *Escherichia coli* operate neither by changing membrane fluidity nor by being sensed by periplasmic receptors during chemotaxis. *J. Bacteriol.* **172**:5218–5224.
6. Futrelle, R. P., and H. C. Berg. 1972. Specification of gradients used for studies of chemotaxis. *Nature* **239**:517–518.
7. Khan, S., F. Castellano, J. L. Spudich, J. A. McCray, R. S. Goody, G. P. Reid, and D. R. Trentham. 1993. Excitatory signaling in bacteria probed by caged chemoeffectors. *Biophys. J.* **65**:2368–2382.
8. Khan, S., J. L. Spudich, J. A. McCray, and D. R. Trentham. 1995. Chemotactic signal integration in bacteria. *Proc. Natl. Acad. Sci. USA* **92**:9757–9761.
9. Kim, C., M. Jackson, R. Lux, and S. Khan. 2001. Determinants of chemotactic signal amplification in *Escherichia coli*. *J. Mol. Biol.* **307**:119–135.
10. Krikos, A., M. P. Conley, A. Boyd, H. C. Berg, and M. I. Simon. 1985. Chimeric chemosensory transducers of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **82**:1326–1330.
11. Mao, H., P. S. Cremer, and M. D. Manson. 2003. A sensitive, versatile microfluidic assay for bacterial chemotaxis. *Proc. Natl. Acad. Sci. USA* **100**:5449–5454.
12. Mello, B. A., and Y. Tu. 2003. Quantitative modeling of sensitivity in bacterial chemotaxis: the role of coupling among different chemoreceptor species. *Proc. Natl. Acad. Sci. USA* **100**:8223–8228.
13. Nara, T., L. Lee, and Y. Imae. 1991. Thermosensing ability of Trg and Tap chemoreceptors in *Escherichia coli*. *J. Bacteriol.* **173**:1120–1124.
14. Sourjik, V., and H. C. Berg. 2002. Receptor sensitivity in bacterial chemotaxis. *Proc. Natl. Acad. Sci. USA* **99**:123–127.
15. Tso, W. W., and J. Adler. 1974. Negative chemotaxis in *Escherichia coli*. *J. Bacteriol.* **118**:560–576.
16. Yamamoto, K., R. M. Macnab, and Y. Imae. 1990. Repellent response functions of the Trg and Tap chemoreceptors of *Escherichia coli*. *J. Bacteriol.* **172**:383–388.
17. Yamamoto, K., and Y. Imae. 1993. Cloning and characterization of the *Salmonella typhimurium*-specific chemoreceptor Tcp for taxis to citrate and from phenol. *Proc. Natl. Acad. Sci. USA* **90**:217–221.