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Bacillus cereus Electron Transport and Proton Motive Force During Aerotaxis

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Aerotaxis (migration towards oxygen) of *Bacillus cereus* M63, a motile strain, was inhibited by potassium cyanide and 2-heptyl-4-hydroxyquinoline *N*-oxide, indicating a requirement for both the terminal oxidase (cytochrome aa_3) and the cytochrome *b* segment of the electron transport system. The concentration of oxygen that gave a half-maximal aerotactic response ($K_{0.5}$) was 0.31 μM, which was similar to the K_m for respiration (0.80 μM). The proton motive force increased from −135 to −177 mV when anaerobic cells were aerated, and it is proposed that the signal for aerotaxis is the increase in proton motive force that results from increased respiration. A strain of *B. cereus* T initially used in this study was immotile, grew as long chains of cells, and was deficient in autolytic enzyme. *B. cereus* M63 is a spontaneous derivative of *B. cereus* T that has normal motility.

Motile bacteria migrate to favorable conditions in response to chemical and physical stimuli such as nutrients, oxygen, pH, temperature, and illumination (for reviews, see references 6, 11, 15, 21, and 36). In flagellate bacteria, net migration is governed by the frequency of random direction changes (5, 22). A momentary reversal in the sense of flagellar rotation causes a bacterium to tumble, and it then swims in a new direction (17). The responses to various favorable stimuli are mediated by pathways that converge to affect the level of a tumble regulator, which modulates the probability of tumbling.

Three classes of chemotaxis pathways are known. Three type I pathways each involve a set of chemoeffectors and a corresponding methylatable transducing protein that spans the cytoplasmic membrane (14, 33). Each transducing protein transmits sensory signal to the signal-processing components that modulate the sense of flagellar rotation. Sensory adaptation for a set of effectors is mediated by a change in the steady-state level of methylation of the corresponding type I transducing protein (10). Thermotaxis and pH taxis may also be mediated by a type I pathway (13, 23, 28).

In type II pathways, the effectors are sugars, which are transported by the phosphotransferase system (26). Transport of the effector is essential for type II chemotactic pathways but not for type I pathways (2, 27).

In type III pathways, terminal oxido-reductases act as receptors for their respective terminal electron acceptors such as oxygen, nitrate, fumarate, and trimethylamine oxide (19, 34, 35). In *Salmonella typhimurium*, cytochrome *o*, nitrate reductase, fumarate reductase, and trimethylamine oxide reductase are receptors for type III chemotactic pathways. Evidence of the role of cytochrome *o* in aerotaxis (oxygen taxis) is shown by the inhibition of aerotaxis by cyanide, the oxygen dose-response relationship, and the fact that alternative electron acceptors inhibit the response to oxygen (19). Studies of the kinetics of respiration and aerotaxis of *S. typhimurium* have since confirmed the role of cytochrome *o* as the oxygen receptor (18). Taylor et al. (35) proposed that an increase in oxygen concentration causes an increase in electron flux in the respiratory chain, which results in an increase in the proton motive force and suppression of tumbling. Attempts to test this hypothesis with respiratory inhibitors in the gram-negative bacteria *S. typhimurium* and *Escherichia coli* have not succeeded. Therefore, to test the role of the electron transport system in the bacterial response to oxygen, we chose a gram-positive facultative anaerobe which is sensitive to respiratory inhibitors and membrane-active agents without special treatment. In this report, we discuss the oxygen receptor and the role of electron transport and proton motive force in *Bacillus cereus*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *B. cereus* T was obtained from J. S. Anderson (31). A motile strain, *B. cereus* M63, was isolated as a spontaneous revertant on tryptone semisolid agar swarm plates (1). Cells were grown at 30°C in a modified medium described by Anagnostopoulos and Spizizen (3) and supplemented with 0.1% (wt/vol) Casamino Acids (Difco Laboratories) and 0.6% glucose. For electron microscopy, exponential-phase cells were harvested and negatively stained as described by Fein (9).

Cell autolysis assay. Cell autolysis was assayed by the method of Fan and Beckman (8). The *B. cereus* cells were grown to mid-exponential phase, washed twice, and suspended in buffer containing 0.1 M Tris and 0.1 M KCl, pH 8.0. They were then incubated at 30°C, and their optical density at 600 nm was measured as a function of time with a Hitachi 100-20 spectrophotometer.

Kinetics of aerotaxis and respiration. The temporal oxygen gradient apparatus described by Laszlo and Taylor (19) was used for aerotaxis assays. Cells were observed with a microscope while a step gradient of oxygen was imposed, and the period of suppression of tumbling was timed. The response was analyzed as a function of oxygen concentration by using a double-reciprocal plot of the data.

The K_m for respiration was measured by the method of

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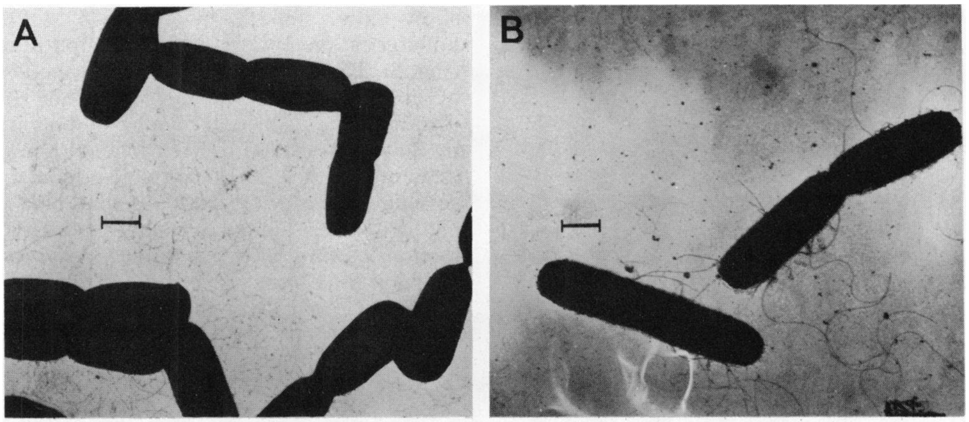

FIG. 1. Morphology of *B. cereus* T and motile revertant *B. cereus* M63 grown to exponential phase as described in the text. The cells were negatively stained with phosphotungstic acid and examined under an electron microscope. Bar, 0.75 μM. A, *B. cereus* T; B, *B. cereus* M63.

Longmuir (20, 30) as modified by Rice and Hempfling (29). The oxygen concentration of a cell suspension was measured as a function of time. The cell suspension was flushed with nitrogen until it was anaerobic, and a measured amount of air-saturated water was added. Data from a trace of rate of decrease in oxygen concentration due to metabolism over time were tabulated and then analyzed with a Texas Instruments TI FS990 computer, using a Basic program written by F. Lawler, Division of Perinatal Biology, Loma Linda University. The program calculated the K_m for respiration from a Lineweaver-Burk plot of the data.

Measurement of membrane potential. Membrane potential was estimated by two methods. Rapid changes were monitored by measuring the fluorescence of 3 ml of a cell suspension which contained 1 μl of a 1 mM ethanolic solution of the cyanine dye diS-C_3-(5) (19, 25, 32). Oxygen concentration was continuously monitored with a needle oxygen electrode (Transidyne General Corp.) and a silver-silver chloride reference electrode placed in the fluorometer cuvette.

The membrane potential was quantitated by measuring the partitioning of [*phenyl*-^{3}H]tetraphenyl phosphonium bromide by procedures described elsewhere (J. Shioi and B. L. Taylor, J. Biol. Chem., in press).

Reagents. The cyanine dye diS-C_3-(5) was a gift from A. S. Waggoner, Amherst College. [*phenyl*-^{3}H]tetraphenyl phosphonium bromide was purchased from Nuclear Research Center, Negev, Israel. Unlabeled tetraphenyl phosphonium bromide was obtained from Aldrich Chemical Co.

RESULTS

Morphology and motility of *B. cereus*. *B. cereus* is a gram-positive facultative anaerobe known to exhibit aerotaxis (4). The strain obtained from the departmental strain collection was immotile and grew as chains. Cells grown from spore stock of *B. cereus* T obtained from J. S. Anderson (University of Minnesota, St. Paul, where *B. cereus* T was originally isolated) lacked flagella and grew as chains (Fig. 1A). A motile derivative (strain M63) of *B. cereus* T, selected on tryptone semisolid agar, did not form chains and was flagellate (Fig. 1B). Fein (9) has shown that *Bacillus subtilis* strains deficient in the autolytic enzyme lack flagella and grow as chains. We determined that the original strain of *B. cereus* T was defective in autolysis and that autolysis was restored in strain M63 (Fig. 2).

Kinetics of aerotaxis. In a series of temporal assays, *B. cereus* M63 was kept anaerobic for 1 min in growth medium and then exposed to a predetermined concentration of oxygen. When anaerobiosis was first imposed, the cells tumbled, became immotile, and then resumed random motility. When the bacteria were exposed to oxygen, their tumbling was temporarily suppressed and they showed a smooth swimming response. At least five determinations of the duration of the response were made at each oxygen concentration and averaged. The $K_{0.5}$ was determined by least-squares linear regression analysis of the data in reciprocal form.

The $K_{0.5}$ for aerotaxis in *B. cereus* M63 was 0.31 μM (Fig. 3). The $K_{0.5}$ is a reasonable approximation of the dissociation constant for a receptor, provided that receptor binding is close to equilibrium. If the oxygen receptor were the terminal oxidase, the $K_{0.5}$ should be similar to the K_m for respiration. Aerotaxis should also be inhibited by potassium cyanide, and this was confirmed.

Kinetics of respiration. The rate of respiration was determined as a function of oxygen concentration by computer analysis of the variation in the amount of oxygen consumed over time by *B. cereus* M63 in a closed vessel (29). The K_m for respiration was 0.80 μM (Fig. 4). This value was similar to the $K_{0.5}$ value for the behavioral response and indicated that cytochrome aa_3, the terminal oxidase in *B. cereus* (7,

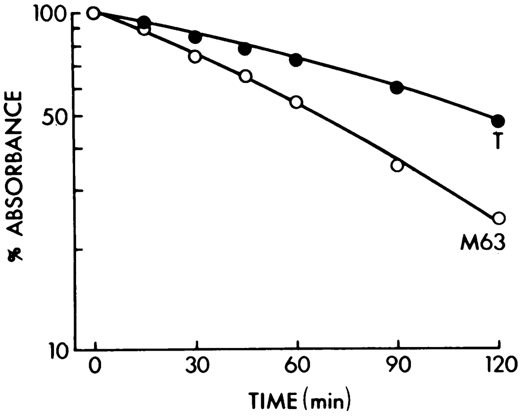

FIG. 2. Autolysis of *B. cereus* T and *B. cereus* M63 cells. The cells were washed and suspended as described in the text. The decrease in absorbance at 600 nm was measured, and the initial absorbance was designated as 100%.

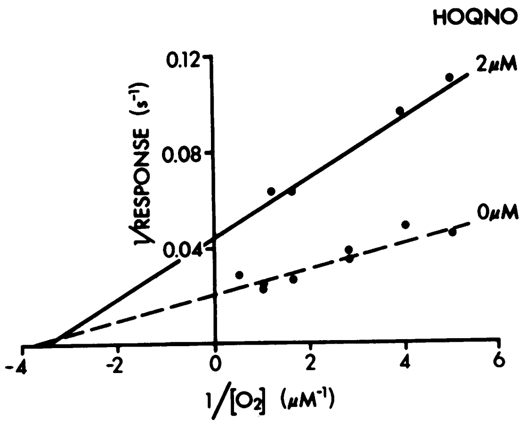

FIG. 3. Double-reciprocal plot of the dependence of aerotaxis of *B. cereus* M63 on oxygen in the absence and presence of HOQNO. Each point is the mean of at least five determinations. HOQNO concentrations: - - -, 0; ——, 2.0 µM.

16, 24), was the oxygen receptor in the aerotactic pathway.

Role of the electron transport system in aerotaxis. Taylor and co-workers (35) proposed that the electron transport system and not just the terminal oxidase was a part of the sensory transduction pathway for aerotaxis. The respiratory inhibitor, 2-heptyl-4-hydroxyquinoline *N*-oxide (HOQNO) was used to test this hypothesis. The noncompetitive pattern of inhibition of respiration (Fig. 4) is explained by the fact that HOQNO acts at the *b* cytochromes (24; unpublished data). When aerotaxis was assayed in the presence of 2 µM HOQNO, a noncompetitive pattern was obtained (Fig. 3). This result is consistent with HOQNO inhibition at cytochrome *b* and supports the hypothesis that aerotaxis is mediated by the whole electron transport system and not just by the terminal oxidase.

In anaerobic medium, ferricyanide can replace oxygen as the electron acceptor for respiration in *B. cereus*. Ferricyanide is also an attractant for this species. In a temporal assay, the mean response time to 2 mM potassium ferricyanide by *B. cereus* M63 was 8.0 ± 1.5 s.

Role of proton motive force in aerotaxis. In *S. typhimurium*, anaerobiosis causes membrane depolarization, and the cells show slow, smooth swimming (12, 19). *B. cereus* also undergoes membrane depolarization during anaerobiosis (Fig. 5). This was reflected in a large increase in fluorescence of diS-C_3-(5) after the cells used all the oxygen in the medium. The change in membrane potential was quantified by flow dialysis and [^{3}H]tetraphenyl phosphonium bromide (data not shown). The membrane potential was −177 mV for aerobic cells and −135 mV for anaerobic cells. The anaerobic cells were initially nonmotile (Fig. 5) but regained random motility after ca. 2 min. However, unlike *S. typhimurium*, *B. cereus* showed gradual membrane repolarization during anaerobiosis, which correlated with the recovery of motility. A stepwise increase in oxygen concentration was accompanied by a rapid return of the membrane potential to the base-line level. This result is consistent with the hypothesis of Taylor et al. (35) that an electron transport-induced change in the proton motive force is the signal that regulates tumbling frequency in aerotaxis.

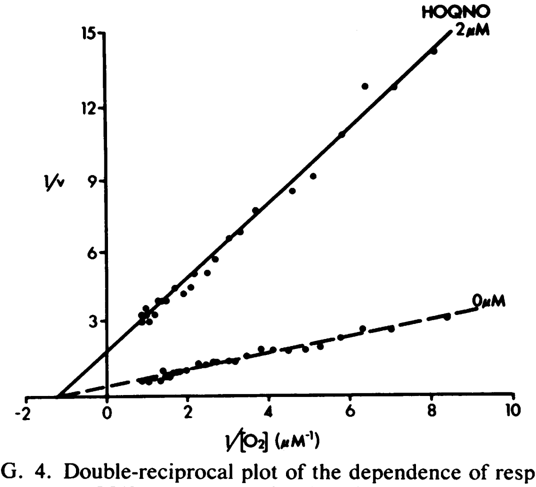

FIG. 4. Double-reciprocal plot of the dependence of respiration in *B. cereus* M63 on oxygen in the absence and presence of HOQNO. HOQNO concentrations: - - -, 0; ——, 2.0 µM.

DISCUSSION

Previous studies of the mechanism of aerotaxis in the gram-negative *S. typhimurium* established that the sensory oxygen receptor is cytochrome *o*, the terminal oxidase of the electron transport system (18, 19, 35). Although data consistent with mediation of aerotaxis by the flux of electrons through the electron transport system were presented, there was no direct evidence that components of the electron transport system, other than cytochrome *o*, are involved in aerotaxis. The present studies of aerotaxis of the gram-positive organism *B. cereus* show that the cytochrome *b* segment of the electron transport system is required for aerotaxis in addition to the terminal oxidase, cytochrome

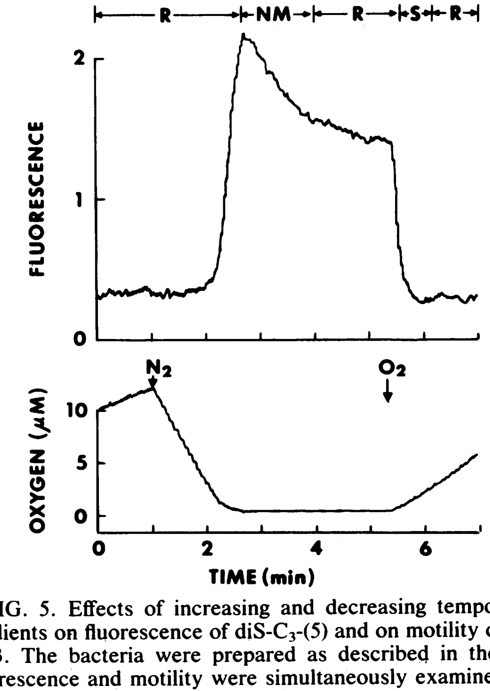

FIG. 5. Effects of increasing and decreasing temporal oxygen gradients on fluorescence of diS-C_3-(5) and on motility of *B. cereus* M63. The bacteria were prepared as described in the text. The fluorescence and motility were simultaneously examined in a fluorometer and in a flow cell under a microscope. The oxygen concentration in the fluorometer cuvette was altered by flushing the air space at the top of the cuvette with either nitrogen or air. The composition of the gas ventilating the flow cell was regulated manually to correspond to the reading of the oxygen electrode in the fluorometer cuvette. R, Random swimming pattern; S, smooth swimming pattern; NM, nonmotile.

aa$_3$. The receptor was identified on the basis of inhibition of aerotaxis by potassium cyanide, comparison of the $K_{0.5}$ for aerotaxis (0.31 μM) and the K_m (0.80 μM) for respiration, and confirmation that cytochrome *aa*$_3$ is the only cytochrome oxidase in *B. cereus* M63 from early exponential phase (unpublished data). The requirement for the cytochrome *b* segment of the electron transport system was shown by inhibition of aerotaxis and respiration by HOQNO. Inhibition was noncompetitive with respect to oxygen. The simplest explanation of the need for the electron transport system in aerotaxis to have two components is that aerotaxis is mediated by the flux of electrons through the electron transport system. Attempts to study the effect of HOQNO on respiration and aerotaxis in *S. typhimurium* and *E. coli* were unsuccessful, presumably because HOQNO did not penetrate the outer membrane.

When anaerobic *B. cereus* M63 cells at pH 7.5 were exposed to air, the proton motive force increased from −135 to −177 mV. This correlated with the excitation phase of the aerotactic response of smooth swimming. The decrease in proton motive force after aerobic cells were deprived of oxygen corresponded to the initiation of a tumbling response. These results are similar to those obtained with *S. typhimurium* (19) and suggest that the signal for aerotaxis is the increase in proton motive force that results from increased respiration when the concentration of oxygen is increased. Responses to manipulation of the proton motive force in both *S. typhimurium* and *B. cereus* are consistent with this hypothesis. The motility pattern of *B. cereus* during the aerotaxis temporal assay was qualitatively similar to the response of *S. typhimurium*. Unlike *S. typhimurium*, *B. cereus* underwent a nonmotile period when anaerobiosis began. The smooth-swimming *B. cereus* response to an oxygen temporal stimulus reached a maximum of ca. 30 s, which is approximately twice the maximum response for *S. typhimurium*, and this facilitated precise determination of the $K_{0.5}$ for the oxygen receptor.

The similarity of the mechanism for aerotaxis of *S. typhimurium*, *E. coli* (D. J. Laszlo, Ph.D. thesis, Loma Linda University, Loma Linda, Calif., 1981), and *B. cereus* cells suggests that the mechanism may be universal in aerobic and facultative bacteria (34).

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LITERATURE CITED

1. **Adler, J.** 1966. Chemotaxis in bacteria. Science **153**:708–716.
2. **Adler, J., and W. Epstein.** 1974. Phosphotransferase-system enzymes as chemoreceptors for certain sugars in *Escherichia coli* chemotaxis. Proc. Natl. Acad. Sci. U.S.A. **71**:2895–2899.
3. **Anagnostopoulos, C., and J. Spizizen.** 1961. Requirements for transformation in *Bacillus subtilis*. J. Bacteriol. **81**:741–746.
4. **Baracchini, O., and J. C. Sherris.** 1959. The chemotactic effect of oxygen on bacteria. J. Pathol. Bacteriol. **77**:565–574.
5. **Berg, H. C., and D. A. Brown.** 1972. Chemotaxis in *Escherichia coli* analyzed by three-dimensional tracking. Nature (London) **239**:500–504.
6. **Boyd, A., and M. Simon.** 1982. Bacterial chemotaxis. Annu. Rev. Physiol. **44**:501–517.
7. **Doi, R. H., and H. Halvorson.** 1961. Comparison of electron transport systems in vegetative cells and spores of *Bacillus cereus*. J. Bacteriol. **81**:51–58.
8. **Fan, D. P., and M. M. Beckman.** 1973. Mutant of *Bacillus subtilis* with a temperature-sensitive autolytic amidase. J. Bacteriol. **114**:798–803.
9. **Fein, J. E.** 1979. Possible involvement of bacterial autolytic enzymes in flagellar morphogenesis. J. Bacteriol. **137**:933–946.
10. **Goy, M. F., M. S. Springer, and J. Adler.** 1977. Sensory transduction in *Escherichia coli*: role of a protein methylation reaction in sensory adaptation. Proc. Natl. Acad. Sci. U.S.A. **74**:4964–4968.
11. **Hazelbauer, G. L., and S. Harayama.** 1983. Sensory transduction in bacterial chemotaxis. Int. Rev. Cytol. **81**:33–70.
12. **Khan, S., and R. M. Macnab.** 1980. The steady-state counterclockwise/clockwise ratio of bacterial flagellar motors is regulated by protonmotive force. J. Mol. Biol. **138**:563–597.
13. **Kihara, M., and R. M. Macnab.** 1981. Cytoplasmic pH mediates pH taxis and weak-acid repellent taxis of bacteria. J. Bacteriol. **145**:1209–1221.
14. **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. U.S.A. **76**:260–264.
15. **Koshland, D. E., Jr.** 1981. Biochemistry of sensing and adaptation in a simple bacterial system. Annu. Rev. Biochem. **50**:765–782.
16. **Lang, D. R., J. Felix, and D. G. Lundgren.** 1972. Development of a membrane-bound respiratory system prior to and during sporulation in *Bacillus cereus* and its relationship to membrane structure. J. Bacteriol. **110**:968–977.
17. **Larsen, S. H., J. Adler, J. J. Gargus, and R. W. Hogg.** 1974. Chemomechanical coupling without ATP: the source of energy for motility and chemotaxis in bacteria. Proc. Natl. Acad. Sci. U.S.A. **71**:1239–1243.
18. **Laszlo, D. J., B. L. Fandrich, A. Siraram, B. Chance, and B. L. Taylor.** 1984. Cytochrome *o* as a terminal oxidase and receptor for aerotaxis is *Salmonella typhimurium*. J. Bacteriol. **159**:663–667.
19. **Laszlo, D. J., and B. L. Taylor.** 1981. Aerotaxis in *Salmonella typhimurium*: role of electron transport. J. Bacteriol. **145**:990–1001.
20. **Longmuir, I. S.** 1954. Respiration rate of bacteria as a function of oxygen concentration. Biochem. J. **57**:81–87.
21. **Macnab, R. M.** 1982. Sensory reception in bacteria. Symp. Soc. Exp. Biol. **35**:77–104.
22. **Macnab, R. M., and D. E. Koshland, Jr.** 1972. The gradient-sensing mechanism in bacterial chemotaxis. Proc. Natl. Acad. Sci. U.S.A. **69**:2509–2512.
23. **Maeda, K., and Y. Imae.** 1979. Thermosensory transduction in *Escherichia coli*: inhibition of the thermoresponse by L-serine. Proc. Natl. Acad. Sci. U.S.A. **76**:91–95.
24. **McFeters, G. A., D. F. Wilson, and G. A. Strobel.** 1970. Cytochromes in a cyanide-resistant strain of *Bacillus cereus*. Can. J. Microbiol. **16**:1221–1226.
25. **Miller, J. B., and D. E. Koshland, Jr.** 1977. Sensory electrophysiology of bacteria: relationship of the membrane potential to motility and chemotaxis in *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S.A. **74**:4752–4756.
26. **Niwano, M., and B. L. Taylor.** 1982. Novel sensory adaptation mechanism in bacterial chemotaxis to oxygen and phosphotransferase substrates. Proc. Natl. Acad. Sci. U.S.A. **79**:11–15.
27. **Pecher, A., I. Renner, and J. W. Lengeler.** 1983. The phosphoenolpyruvate-dependent carbohydrate: phosphotransferase system enzymes II, a new class of chemosensors in bacterial chemotaxis, p. 517–529. *In* S. Horst and C. Veeger (ed.), Symposium on Mobility and Recognition in Cell Biology. Walter de Gruyter & Co., Berlin.
28. **Repaske, D. R., and J. Adler.** 1981. Change in intracellular pH of *Escherichia coli* mediates the chemotactic response to certain attractants and repellents. J. Bacteriol. **145**:1196–1208.
29. **Rice, C. W., and W. P. Hempfling.** 1978. Oxygen-limited continuous culture and respiratory energy conservation in *Escherichia coli*. J. Bacteriol. **134**:115–124.
30. **Schindler, F. J.** 1967. Determination of oxygen affinity. Methods Enzymol. **10**:629–634.

31. **Schrader, W. P., and J. S. Anderson.** 1978. Membrane-bound nucleotidase of *Bacillus cereus*. J. Bacteriol. **133**:576–583.
32. **Sims, P. J., A. S. Waggoner, C.-H. Wang, and J. F. Hoffman.** 1974. Studies on the mechanism by which cyanine dyes measure membrane potential in red blood cells and phosphatidylcholine vesicles. Biochemistry **13**:3315–3330.
33. **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. U.S.A. **74**:3312–3316.
34. **Taylor, B. L.** 1983. Role of proton motive force in sensory transduction in bacteria. Annu. Rev. Microbiol. **37**:551–573.
35. **Taylor, B. L., J. B. Miller, H. M. Warrick, and D. E. Koshland, Jr.** 1979. Electron acceptor taxis and blue light effect on bacterial chemotaxis. J. Bacteriol. **140**:567–573.
36. **Taylor, B. L., and S. M. Panasenko.** 1984. Biochemistry of chemosensory behavior in prokaryotes and unicellular eukaryotes, p. 11–112. *In* G. Colombetti and F. Lenci (ed.), Membranes and sensory transduction. Plenum Publishing Corp., London.