JOURNAL OF BACTERIOLOGY, June 2002, p. 3130–3134 Vol. 184, No. 11
0021-9193/02/$04.00+0 DOI: 10.1128/JB.184.11.3130–3134.2002

Construction and Characterization of a *cheA* Mutant of *Treponema denticola*

Renate Lux, Jee-Hyun Sim, Jon P. Tsai, and Wenyuan Shi*

School of Dentistry and Molecular Biology Institute, University of California, Los Angeles, Los Angeles, California 90095-1668

Received 2 October 2001/Accepted 11 March 2002

The *Treponema denticola cheA* gene, encoding the central kinase of the general chemotaxis pathway, was analyzed for its role in chemotaxis and tissue penetration. The *cheA* gene was interrupted by insertion of an *ermF-ermAM* gene cassette. Reverse transcription-PCR confirmed that the other downstream chemotaxis genes within the same operon (*cheW*, *cheX*, and *cheY*) were still expressed in the *cheA* mutant strain. Lack of *cheA* resulted in decreased swarming on soft-agar swarm plates and failure to respond chemotactically to a mixture of nutrients. Behavioral analyses using video microscopy revealed that the *cheA* mutant exhibited coordinated cell movement. The cellular reversal frequency, however, was severely reduced, indicating that CheA in *T. denticola* mainly controls cellular reversal and that active chemotaxis signaling input is not required for coordination of flagellar rotation at both cell poles.

Chemotaxis is the ability of motile organisms to navigate through their environment in response to gradients of chemicals. This feature allows bacteria to migrate towards favorable environments and avoid harmful situations and has also been implicated as an important virulence factor for a variety of pathogens (8, 10–12, 24, 30, 42). Previous findings suggest the requirement of a functional chemotaxis pathway for tissue penetration by *T. denticola* (36).

The biochemistry and genetics of bacterial chemotaxis have been well characterized in two closely related enteric bacteria, *Escherichia coli* and *Salmonella enterica* serovar Typhimurium (1, 38). Chemotactic stimuli are detected by the membrane-spanning (or soluble) methyl-accepting chemotaxis proteins (MCPs) and subsequently transformed into an appropriate motor response by the two-component system CheA/CheY. The histidine kinase CheA forms a membrane-associated complex with the MCPs and CheW and undergoes ATP-dependent autophosphorylation in compliance with the stimulus bound to the respective MCP. The nature of the stimulus is then communicated to the motor via phosphotransfer reactions from CheA to the response regulator CheY, which controls the direction of flagellar motor rotation according to its phosphorylation level. Repellents increase autophosphorylation of CheA, whereas attractants decrease this activity.

Chemotaxis has been demonstrated in various spirochetes, and a number of chemotactic stimuli have been identified. *Borrelia burgdorferi* recognizes serum as an attractant and H_2O_2, nonphysiological pH (>8.5 or <6.8), KCl, $CaCl_2$, and small alcohols as repellents (47). *Brachyspira hyodysenteriae* responds to mucin, fucose, galactose, lactose, serine, cysteine, and blood (29, 39), while *Leptospira interrogans* responds to hemoglobin (25, 51). *Spirochaeta aurantia* performs chemotaxis towards glucose, xylose, and various other carbohydrates (21), whereas for *Treponema denticola* glucose, rabbit serum, albumin, and growth medium are all attractants (32, 48).

Homologues of many of the known chemotaxis genes are present in spirochetes (22, 28, 33; www.tigr.org/tdb/mdb/mdbinprogress.html). Therefore, the general strategy of transforming an environmental stimulus into a motor response is presumably very similar to that in other bacteria (37). However, there are some very distinctive features of the motility and chemotaxis of spirochetes that do not occur in other bacteria (31). The flagella are inserted in a subpolar location near both ends of the bacterium and rotate within the periplasm. Models that have been developed for translation of *Leptospira illini* and *B. burgdorferi* suggest that the periplasmic flagella rotate counterclockwise at the anterior end and in the opposite direction at the posterior end of the cell, promoting clockwise movement of the cell body (3, 4, 14–16, 31). If both motors spin in the same direction, the movement becomes noncoordinated and results in a jerky movement called flexing (21). However, little is known about how the direction of flagellar rotation at both ends is coordinated and modulated by the chemotaxis proteins.

The cell body of spirochetes is particularly long: the cell poles are about 10 to 15 μm apart in *T. denticola* and even further in the larger spirochetes, such as some members of the genera *Cristispira* (30 to 180 μm) and *Spirochaeta* (5 to 250 μm) (26). Therefore, the typical strategy of signal transduction via simple diffusion to the motors is calculated to be too slow to achieve the observed transmission of chemosensory information between the motors located at the distant poles of the cell. A limited signaling range of only a few micrometers for diffusible internal signals, such as phosphorylated CheY, has been demonstrated in filamentous *E. coli* (27, 46). Consequently, the change in membrane potential that has been observed in *S. aurantia* upon addition of chemotactic stimuli has been suggested to be involved in chemotactic signal transduction of spirochetes (17–20). These unusual features of spirochete motility and chemotaxis raise the interesting question of how chemotactic signal processing and coordination between the flagellar motors occur.

* Corresponding author. Mailing address: School of Dentistry and Molecular Biology Institute, University of California, Los Angeles, CA 90095-1668. Phone: (310) 825-8356. Fax: (310) 794-7109. E-mail: wenyuan@ucla.edu.

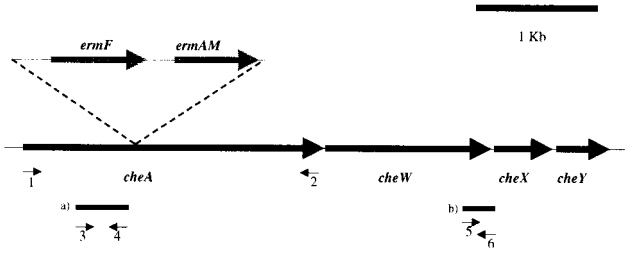

FIG. 1. Schematic of the general chemotaxis operon of *T. denticola* containing *cheA*, *cheW*, *cheX*, and *cheY*. The insertion site of the *ermF-ermAM* gene cassette is shown as well as the primer pairs that were used for amplification of the entire *cheA* gene (1, bamf; 2, bamr) and for RT-PCR (3, BamCheAF; 4, LSCheA; 5, BamHIF; 6, X-BglR). The resulting RT-PCR products upstream (350 bp) (a) and downstream (260 bp) (b) of the *ermF-ermAM* insertion site are indicated.

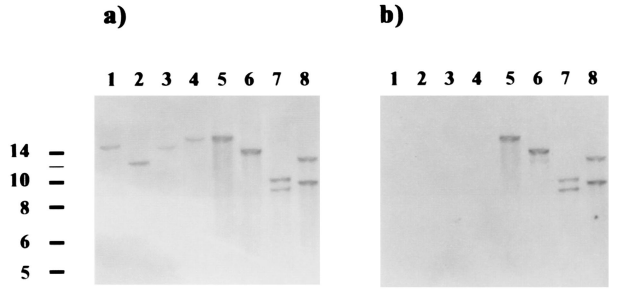

FIG. 2. Southern blot analysis of *cheA* gene inactivation mutants. Lanes: 1 to 4, wild-type strain ATCC 35405; 5 to 8, Erm^r *cheA* gene inactivation mutant; 1 and 5, *Eag*I-digested DNA; 2 and 6, *Sac*II-digested DNA; 3 and 7, *Sac*I-digested DNA; 4 and 8, *Bst*XI-digested DNA. The probes were a 2.4-kb fragment containing the entire *cheA* gene, derived by *Bam*HI digestion of pJT9 (a), and a 2.1-kb *Nhe*I fragment containing *ermF-ermAM* from pJT10 (b). Molecular sizes (kilobases) are on the left.

In this study we constructed a *cheA* gene inactivation mutant of *T. denticola* ATCC 35405 to address some of the above questions by interrupting the general chemotaxis pathway and its interaction with the flagellar motor. This mutant allows examination of the unstimulated direction of motor rotation and supports the idea that *cheA* is essential for chemotaxis in *T. denticola* as in other bacterial species (1).

Construction of a *cheA* mutant of *T. denticola*. CheA is the central kinase of the general chemotaxis pathway, and it has been shown for other bacteria that its inactivation abolishes all chemotaxis responses (7, 8, 13, 23, 44, 45, 50). Some bacterial species, including the pathogenic spirochete *B. burgdorferi*, contain multiple copies of the general chemotaxis genes, such as *cheA* (9). Genome and Southern blot analysis of the *T. denticola* chromosome revealed the presence of only one copy of *cheA* in this oral spirochete (22, 32; www.tigr.org/tdb/mdb/mdbinprogress.html). In *T. denticola*, *cheA* is organized in an operon with the other general chemotaxis genes, *cheW* and *cheY*, as well as with *cheX*, a putative spirochete chemotaxis gene of unknown function (Fig. 1). In this study, a *cheA* mutant was constructed by disrupting the gene through introduction of an *ermF-ermAM* gene cassette that confers resistance to erythromycin. This approach has previously been used to successfully create a variety of gene inactivation mutants of *T. denticola* (28, 33–35).

Cultures of *T. denticola* ATCC 35405 (5) were grown anaerobically (85% N_2, 10% H_2, 5% CO_2) at 35°C in TYGVS broth (41), and chromosomal DNA was isolated as described previously (40). This chromosomal DNA served as a template for amplification of *T. denticola cheA* using the following primers, which were designed according to published sequences (22; www.tigr.org/tdb/mdb/mdbinprogress.html) and introduce a *Bam*HI recognition site (underlined) at either end of the gene: 5′ CGGGATCCATGAGTGATTATCTTGATATC 3′ (bamf) and 5′ CGGGATCCCTACCAAATTGAAGCCTCG 3′ (bamr) (Fig. 1). The resulting PCR product was digested with *Bam*HI and cloned into the *Bam*HI site that is present in the multiple cloning site of the pUC18 vector (49) to yield pJT9. A fragment encoding *ermF-ermAM* including the respective promoter regions was equally PCR amplified from plasmid pKMR4PE (6) using the following primer pair, which introduces *Nhe*I recognition sites (underlined) at both ends of the fragment: 5′ CTAGCTAGCCCGATAGCTTCCGCTATT 3′ and 5′ CTAGCTAGCGAAGCTGTCAGTAGTATAC 3′. This fragment was *Nhe*I digested and cloned into a naturally occurring *Nhe*I site located at position 885 of the approximately 2.4-kb *cheA*. A plasmid with insertion of *ermF-ermAM* in the direction of *cheA* expression was chosen and called pJT10. pJT10 was linearized with *Aat*II, an enzyme that cuts only once in the pUC18 backbone. Different amounts (1, 2, and 5 μg) of the linearized plasmid were electroporated into *T. denticola* ATCC 35405 as described previously (34) with the modifications developed by Limberger and coworkers (35). The gene inactivation mutants were selected on TYGVS plates (0.5% agar) containing erythromycin (25 μg/ml). About 1,000 Erm^r colonies/μg of DNA were obtained. Similar high yields for gene inactivation mutants were observed by other investigators (35). These results, however, vary from experiment to experiment (J. Izard, personal communication). Ten Erm^r colonies were chosen, and insertion of the Erm^r cassette into *cheA* was confirmed by colony PCR. Two of these mutants were selected for further studies.

Confirmation of the insertion of the *ermF-ermAM* cassette into *cheA*. We used Southern blotting to verify the correct insertion of *ermF-ermAM* into the *cheA* gene. Chromosomal DNA of ATCC 35405 and the two putative *cheA* mutants was isolated, digested, and separated on a 1% agarose gel. The DNA was transferred onto a nylon membrane (Bio-Rad) and hybridized with DNA probes (described below) for Southern blot analysis. The respective signals were detected by using the DNA labeling and detection kit from Boehringer (Mannheim, Germany). The Southern blot results confirmed the correct integration of the Erm^r cassette into the mutant strains. The blot was first hybridized with a 2.4-kb *cheA*-specific probe that reacted with specific fragments of the wild-type DNA and with different-size fragments in the mutant strains as expected (Fig. 2a). Since the mutant strains yielded identical results, only one is shown. The fragments generated by *Eag*I or *Sac*II digestion in the mutant run higher than those in the wild type, as these enzymes do not cut within the *ermF-ermAM* genes. *Sac*I and *Bst*XI both have recognition sites within these genes, resulting in two fragments for the mutant strain. The probe was then stripped off the blot according to the instructions for the DNA

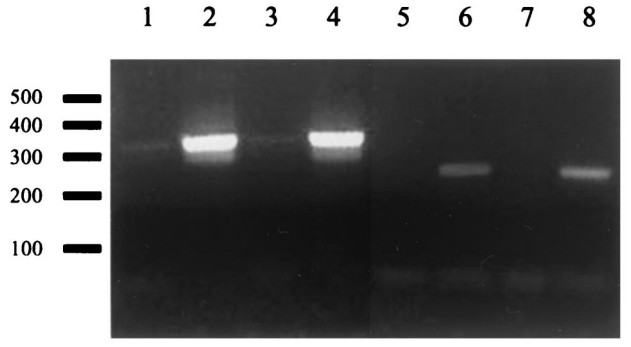

FIG. 3. RT-PCR analysis of RL101. Lanes 1 to 4, amplification of a ~350-bp fragment upstream of the Erm^r insertion site using primer pair BamCheAF and JSCheA; lanes 5 to 8, amplification of an ~260-bp fragment downstream of the Erm^r insertion site using primer pair BamHIF and X-BglR. Lanes 1, 2, 5, and 6, wild-type samples; lanes 3, 4, 7, and 8, *cheA* mutant RNA. Samples in lanes 2, 4, 6, and 8 were treated with reverse transcriptase, whereas samples in lanes 1, 3, 5, and 7 were not.

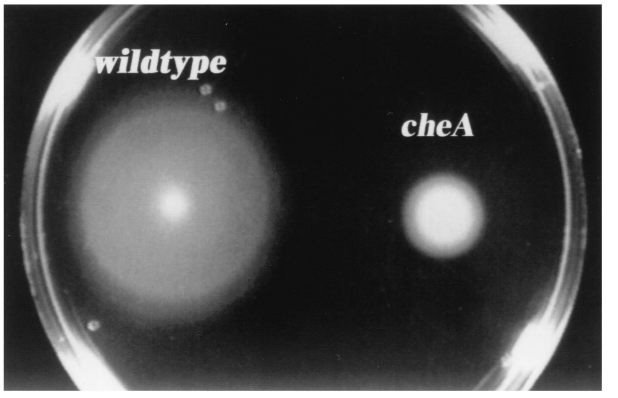

FIG. 4. Wild-type and *cheA* mutant behavior on TYGVS swarm plates containing 0.4% agarose. Shown are typical swarms formed by an inoculum of about 5×10^7 motile bacteria after 3 days of anaerobic incubation at 35°C.

labeling and detection kit and rehybridized with an *ermF-ermAM*-specific probe (Fig. 2b). This probe did not bind to the wild-type DNA and reacted in the mutant strains with the same fragments that were detected earlier with the *cheA* probe. One of the *cheA* mutant strains was named RL101 and used throughout this study for further analysis.

Expression of downstream genes in the *cheA* gene inactivation mutant. As shown in Fig. 1, *cheA* is the first gene in an operon that encodes other central functions of the chemotaxis pathway. Accordingly, there was concern regarding possible polar effects due to *ermF-ermAM* insertion. It has been reported that insertion of the *ermF-ermAM* gene cassette does not have significant polar effects on the expression of downstream genes in an operon (35). To examine if this was also the case in the *cheA* mutant strain, transcription of the genes located downstream of *cheA* was analyzed by reverse transcription-PCR (RT-PCR) (Fig. 3). RNA was extracted by using the RNeasy kit (Qiagen) according to the manufacturer's instructions and treated with RNase-free DNase for 1 h at 37°C. About 1 μg of RNA was incubated with reverse transcriptase and random primers to generate cDNA of wild-type *T. denticola* ATCC 35405 and the mutant RL101. Similar quantities of these cDNAs and the respective control reaction mixtures without addition of reverse transcriptase were used as templates for PCRs with primer pairs that specifically amplify regions upstream and downstream of the *ermF-ermAM* insertion site (Fig. 1). The primer pair BamCheAF (5′ TCCCGA GGATCGTAACTCCAT 3′) and LSCheAR (5′ GGAGAAA CTGATGCAGGAATA 3′) is complementary to bp 111 to 460 of *cheA* and amplifies a 350-bp fragment upstream of the *ermF-ermAM* insertion site. Another primer pair, BamHIF (5′ TTGAGTACCATGATTGTACT 3′) and X-BglR (5′ TTATT TCTCCTTATCCTTTTA 3′), was used to examine relative transcription levels downstream of the resistance cassette insertion site. This primer pair amplifies a 256-bp fragment located at bp 1102 of *cheW* and bp 1 of *cheX*. No significant difference between the wild type and the mutant strain was apparent (Fig. 3), indicating that significant expression of downstream genes still occurs in the *cheA* mutant strain. Therefore, chemotaxis and motility phenotypes of RL101 should be a result of lack of *cheA* only.

The *cheA* mutant is defective in chemotaxis. Swarming on soft agar is a useful assay for examining chemotaxis, since chemotaxis mutants exhibit a poor swarming phenotype (2). To compare the swarming behavior of RL101 to that of its parent, ATCC 35045, about 5×10^8 exponentially growing motile cells were harvested by low-speed centrifugation ($1{,}000 \times g$) for 6 min and resuspended in 20 μl of TYGVS. This bacterial suspension (2 μl; about 5×10^7 bacteria) was placed in a TYGVS swarm plate (0.4% agarose). The plates were incubated anaerobically at 35°C for 3 days, and swarming diameters of 20 swarms for each strain were measured. Wild-type swarms were about three times larger than the ones produced by the mutant strain (Fig. 4), even though the mutant and its wild-type parent contained the same number of motile cells (>95%) as determined by dark-field microscopy (magnification, ×400; Nikon) (data not shown). To ensure that growth impairment of the mutant strain was not the reason for this difference in swarming, the amount of cells that had been placed on the swarm plates was inoculated into 5 ml of TYGVS medium. Growth curves of wild-type and mutant strains were practically identical (data not shown).

The chemotactic properties of the *cheA* mutant strain were further analyzed in capillary assays. Capillary assays were performed as described previously for *B. burgdorferi* (47) but with prereduced reagents under anaerobic conditions. Capillaries were filled with the growth medium TYGVS as an attractant or with chemotaxis buffer as a negative control and prereduced by anaerobic incubation for at least 24 h prior to the experiment. As shown in Fig. 5, the wild type exhibited a strong response to the growth medium whereas the *cheA* mutant was unable to accumulate in the attractant-filled capillaries in numbers exceeding the negative control.

Video microscopy was used to observe the motility bias of the wild-type and *cheA* mutant *T. denticola* strains. Motile cells were grown to mid-log phase in TYGVS, and 2 to 3 μl of this culture was placed on a slide and covered with an 18- by 18-mm cover slide. The cover slide was sealed with petroleum jelly to prevent dehydration. Without addition of viscous substances

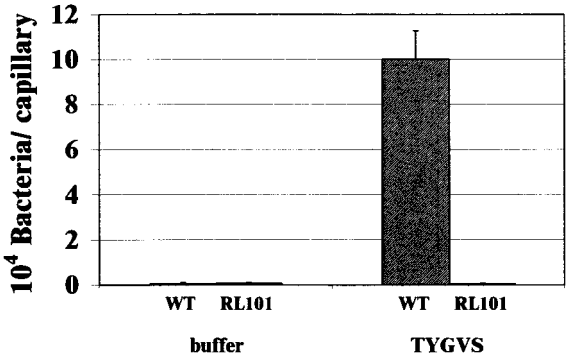

FIG. 5. Capillary assay of wild-type ATCC 35405 and *cheA* mutant strain RL101. Chemotaxis responses towards the spirochete growth medium TYGVS are shown. Capillaries filled with chemotaxis buffer were used as a negative control. Data are averages from two independent experiments that were performed with triplicate capillaries for the different solutions tested.

such as methylcellulose or gelatin, rotational movement in the growth medium alone results in very little or no translational displacement. Under these conditions the bacteria rotate "on the spot," facilitating long-term observation of bacteria that exhibit a motility bias that greatly favors one direction of movement and consequently rapidly leave the field of view. The bacteria were observed at room temperature through a 60× objective lens using phase-contrast microscopy (Nikon) and recorded with a video camera (HyperHAD/CCD-IRIS/RGB; Sony). The videotapes were analyzed by slow-motion replay. Five independent wild-type and *cheA* mutant cultures were each observed over a 5-min period, and about 50 individual bacteria were examined. Wild-type cells reversed direction of rotation every 1 to 9 s, with an average of 3.6 ± 1.6 s. In contrast, the *cheA* mutants failed to change direction of rotation during the 5-min observation period. In *B. burgdorferi*, inactivation of its similarly organized *cheA2* gene also results in a nonreversing phenotype (N. Charon, personal communication).

In summary, we constructed and characterized a *cheA* mutant strain of *T. denticola*. Unlike previous *T. denticola* chemotaxis mutants, such as *dmcA* and *dmcB* mutants (28, 33), the *cheA* mutant is totally defective in chemotaxis. The only copy of *cheA* in *T. denticola* was inactivated in this mutant by insertion of an Erm^r gene. Expression of the downstream genes *cheW*, *cheX*, and *cheY* was confirmed to occur in the mutant. This facilitates examination of the *cheA* phenotype specifically, rather than a general deficiency in the chemotaxis operon. Consistent with genome data, there is only one chemotaxis system in *T. denticola*. Inactivation of the *cheA* gene abolishes all chemotaxis responses tested. The *cheA* mutant strain RL101 was unable to exhibit a measurable chemotactic response in capillary assays and showed a diminished swarm size on swarm plates, a phenotype that is found in *cheA* mutants of other bacteria, such as the spirochete *B. burgdorferi* (Charon, personal communication). Lack of CheA in flagellated bacteria causes the motor to turn exclusively in one direction, resulting in a constantly running phenotype in most species (1, 43) or a tumbly phenotype, as described for *Bacillus subtilis* (13). In spirochetes the situation is more complex, as current models for motility suggest that the motors at both ends have to rotate in opposite directions to promote translational movement of the cell. Interestingly, the *T. denticola cheA* mutant constructed in this study and also the *cheA2* mutant of *B. burgdorferi* exhibit a constantly running phenotype, implying that the default state of the motor at both ends may be rotation in opposite directions and that no active input from the chemotaxis proteins is required for coordination. CheA appears to be involved only in regulating the cellular reversal frequency, so either coordination of motor rotation at either cell end is physical or there is a different system that regulates coordination of flagellar rotation.

With genome information available and the recent development of genetic tools such as gene inactivation, we are now in a position to further our understanding of the special features of spirochete chemotaxis and their implication in virulence.

We thank Howard Kuramitsu, Nyles Charon, and James Miller for their support of our work.

This work was partially supported by NIH grants DE12532 and GM54666 to W.S.

REFERENCES

1. **Armitage, J. P.** 1999. Bacterial tactic responses. Adv. Microb. Physiol. **41:** 229–289.
2. **Armstrong, J. B., J. Adler, and M. M. Dahl.** 1967. Nonchemotactic mutants of *Escherichia coli*. J. Bacteriol. **93:**390–398.
3. **Berg, H. C.** 1976. How spirochetes may swim. J. Theor. Biol. **56:**269–273.
4. **Charon, N. W., G. R. Daughtry, R. S. McCuskey, and G. N. Franz.** 1984. Microcinematographic analysis of tethered *Leptospira illini*. J. Bacteriol. **160:** 1067–1073.
5. **Cheng, S. L., R. Siboo, T. C. Quee, J. L. Johnson, W. R. Mayberry, and E. C. Chan.** 1985. Comparative study of six random oral spirochete isolates. Serological heterogeneity of *Treponema denticola*. J. Periodontal Res. **20:**602–612.
6. **Chi, B., S. Chauhan, and H. Kuramitsu.** 1999. Development of a system for expressing heterologous genes in the oral spirochete *Treponema denticola* and its use in expression of the *Treponema pallidum flaA* gene. Infect. Immun. **67:**3653–3656.
7. **Flanary, P. L., R. D. Allen, L. Dons, and S. Kathariou.** 1999. Insertional inactivation of the *Listeria monocytogenes cheYA* operon abolishes response to oxygen gradients and reduces the number of flagella. Can. J. Microbiol. **45:**646–652.
8. **Foynes, S., N. Dorrell, S. J. Ward, R. A. Stabler, A. A. McColm, A. N. Rycroft, and B. W. Wren.** 2000. *Helicobacter pylori* possesses two CheY response regulators and a histidine kinase sensor, CheA, which are essential for chemotaxis and colonization of the gastric mucosa. Infect. Immun. **68:**2016–2023.
9. **Fraser, C. M., S. Casjens, W. M. Huang, G. G. Sutton, R. Clayton, R. Lathigra, O. White, K. A. Ketchum, R. Dodson, E. K. Hickey, M. Gwinn, B. Dougherty, J. F. Tomb, R. D. Fleischmann, D. Richardson, J. Peterson, A. R. Kerlavage, J. Quackenbush, S. Salzberg, M. Hanson, R. van Vugt, N. Palmer, M. D. Adams, J. Gocayne, J. C. Venter, et al.** 1997. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. Nature **390:** 580–586.
10. **Freter, R., and P. C. O'Brien.** 1981. Role of chemotaxis in the association of motile bacteria with intestinal mucosa: fitness and virulence of nonchemotactic *Vibrio cholerae* mutants in infant mice. Infect. Immun. **34:**222–233.
11. **Freter, R., P. C. O'Brien, and M. S. Macsai.** 1979. Effect of chemotaxis on the interaction of cholera vibrios with intestinal mucosa. Am. J Clin. Nutr. **32:**128–132.
12. **Freter, R., P. C. O'Brien, and M. S. Macsai.** 1981. Role of chemotaxis in the association of motile bacteria with intestinal mucosa: in vivo studies. Infect. Immun. **34:**234–240.
13. **Fuhrer, D. K., and G. W. Ordal.** 1991. *Bacillus subtilis* CheN, a homolog of CheA, the central regulator of chemotaxis in *Escherichia coli*. J. Bacteriol. **173:**7443–7448.
14. **Goldstein, S. F., K. F. Buttle, and N. W. Charon.** 1996. Structural analysis of the *Leptospiraceae* and *Borrelia burgdorferi* by high-voltage electron microscopy. J. Bacteriol. **178:**6539–6545.
15. **Goldstein, S. F., and N. W. Charon.** 1988. Motility of the spirochete Leptospira. Cell Motil. Cytoskelet. **9:**101–110.
16. **Goldstein, S. F., N. W. Charon, and J. A. Kreiling.** 1994. *Borrelia burgdorferi* swims with a planar waveform similar to that of eukaryotic flagella. Proc. Natl. Acad. Sci. USA **91:**3433–3437.
17. **Goulbourne, E. A., Jr., and E. P. Greenberg.** 1981. Chemotaxis of *Spirochaeta*

aurantia: involvement of membrane potential in chemosensory signal transduction. J. Bacteriol. **148:**837–844.
18. **Goulbourne, E. A., Jr., and E. P. Greenberg.** 1983. Inhibition of *Spirochaeta aurantia* chemotaxis by neurotoxins. J. Bacteriol. **155:**1443–1445.
19. **Goulbourne, E. A., Jr., and E. P. Greenberg.** 1980. Relationship between proton motive force and motility in *Spirochaeta aurantia*. J. Bacteriol. **143:** 1450–1457.
20. **Goulbourne, E. A., Jr., and E. P. Greenberg.** 1983. A voltage clamp inhibits chemotaxis of *Spirochaeta aurantia*. J. Bacteriol. **153:**916–920.
21. **Greenberg, E. P., and E. Canale-Parola.** 1977. Chemotaxis in *Spirochaeta aurantia*. J. Bacteriol. **130:**485–494.
22. **Greene, S. R., and L. V. Stamm.** 1999. Molecular characterization of a chemotaxis operon in the oral spirochete, *Treponema denticola*. Gene **232:** 59–68.
23. **Hamblin, P. A., B. A. Maguire, R. N. Grishanin, and J. P. Armitage.** 1997. Evidence for two chemosensory pathways in *Rhodobacter sphaeroides*. Mol. Microbiol. **26:**1083–1096.
24. **Hawes, M. C., and L. Y. Smith.** 1989. Requirement for chemotaxis in pathogenicity of *Agrobacterium tumefaciens* on roots of soil-grown pea plants. J. Bacteriol. **171:**5668–5671.
25. **Hiramune, T., C. Shiraiwa, N. Kikuchi, and R. Yanagawa.** 1990. A basic study of chemotaxis of leptospiras. Zentralbl. Vetmed. B **37:**749–752.
26. **Holt, J. G. (ed.).** 1994. Bergey's manual of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
27. **Ishihara, A., J. E. Segall, S. M. Block, and H. C. Berg.** 1983. Coordination of flagella on filamentous cells of *Escherichia coli*. J. Bacteriol. **155:**228–237.
28. **Kataoka, M., H. Li, S. Arakawa, and H. Kuramitsu.** 1997. Characterization of a methyl-accepting chemotaxis protein gene, *dmcA*, from the oral spirochete *Treponema denticola*. Infect. Immun. **65:**4011–4016.
29. **Kennedy, M. J., and R. J. Yancey, Jr.** 1996. Motility and chemotaxis in *Serpulina hyodysenteriae*. Vet. Microbiol. **49:**21–30.
30. **Lee, S. H., S. M. Butler, and A. Camilli.** 2001. Selection for in vivo regulators of bacterial virulence. Proc. Natl. Acad. Sci. USA **98:**6889–6894.
31. **Li, C., A. Motaleb, M. Sal, S. F. Goldstein, and N. W. Charon.** 2000. Spirochete periplasmic flagella and motility. J. Mol. Microbiol Biotechnol. **2:**345–354.
32. **Li, C. Y., J. P. Tsai, Y. W. Han, Z. Yang, L. E. Wolinsky, H. Kuramitsu, and W. Shi.** 1998. Chemotaxis and the *cheA* mutant of *Treponema denticola*. J. Dent. Res. **77:**228.
33. **Li, H., S. Arakawa, Q. D. Deng, and H. Kuramitsu.** 1999. Characterization of a novel methyl-accepting chemotaxis gene, *dmcB*, from the oral spirochete *Treponema denticola*. Infect. Immun. **67:**694–699.
34. **Li, H., J. Ruby, N. Charon, and H. Kuramitsu.** 1996. Gene inactivation in the oral spirochete *Treponema denticola*: construction of an *flgE* mutant. J. Bacteriol. **178:**3664–3667.
35. **Limberger, R. J., L. L. Slivienski, J. Izard, and W. A. Samsonoff.** 1999. Insertional inactivation of *Treponema denticola tap1* results in a nonmotile mutant with elongated flagellar hooks. J. Bacteriol. **181:**3743–3750.
36. **Lux, R., J. N. Miller, N. H. Park, and W. Shi.** 2001. Motility and chemotaxis in tissue penetration of oral epithelial cell layers by *Treponema denticola*. Infect. Immun. **69:**6276–6283.
37. **Lux, R., A. Moter, and W. Shi.** 2000. Chemotaxis in pathogenic spirochetes: directed movement toward targeting tissues? J. Mol. Microbiol Biotechnol. **2:**355–364.
38. **Manson, M. D., J. P. Armitage, J. A. Hoch, and R. M. Macnab.** 1998. Bacterial locomotion and signal transduction. J. Bacteriol. **180:**1009–1022.
39. **Milner, J. A., and R. Sellwood.** 1994. Chemotactic response to mucin by *Serpulina hyodysenteriae* and other porcine spirochetes: potential role in intestinal colonization. Infect. Immun. **62:**4095–4099.
40. **Moore, D.** 1999. Preparation and analysis of DNA, p. 2.0.1–2.12.7. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology. John Wiley & Sons, New York, N.Y.
41. **Ohta, K., K. K. Makinen, and W. J. Loesche.** 1986. Purification and characterization of an enzyme produced by *Treponema denticola* capable of hydrolyzing synthetic trypsin substrates. Infect. Immun. **53:**213–220.
42. **O'Toole, R., D. L. Milton, and H. Wolf-Watz.** 1996. Chemotactic motility is required for invasion of the host by the fish pathogen *Vibrio anguillarum*. Mol. Microbiol. **19:**625–637.
43. **Parkinson, J. S.** 1977. Behavioral genetics in bacteria. Annu. Rev. Genet. **11:**397–414.
44. **Parkinson, J. S.** 1976. *cheA*, *cheB*, and *cheC* genes of *Escherichia coli* and their role in chemotaxis. J. Bacteriol. **126:**758–770.
45. **Rudolph, J., and D. Oesterhelt.** 1996. Deletion analysis of the *che* operon in the archaeon *Halobacterium salinarium*. J. Mol. Biol. **258:**548–554.
46. **Segall, J. E., A. Ishihara, and H. C. Berg.** 1985. Chemotactic signaling in filamentous cells of *Escherichia coli*. J. Bacteriol. **161:**51–59.
47. **Shi, W., Z. Yang, Y. Geng, L. E. Wolinsky, and M. A. Lovett.** 1998. Chemotaxis in *Borrelia burgdorferi*. J. Bacteriol. **180:**231–235.
48. **Umemoto, T., T. Jinno, Y. Taiji, and T. Ogawa.** 2001. Chemotaxis of oral treponemes toward sera and albumin of rabbit. Microbiol. Immunol. **45:**571–577.
49. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19:**259–268.
50. **Ward, M. J., A. W. Bell, P. A. Hamblin, H. L. Packer, and J. P. Armitage.** 1995. Identification of a chemotaxis operon with two *cheY* genes in *Rhodobacter sphaeroides*. Mol. Microbiol. **17:**357–366.
51. **Yuri, K., Y. Takamoto, M. Okada, T. Hiramune, N. Kikuchi, and R. Yanagawa.** 1993. Chemotaxis of leptospires to hemoglobin in relation to virulence. Infect. Immun. **61:**2270–2272.