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Chemotactic Response to Formate by *Campylobacter concisus* and Its Potential Role in Gingival Colonization

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Cells of *Campylobacter concisus* 288 were chemotactic toward formate, but not to any other compound tested. Chemicals that were not chemoattractants included 20 sugars, inorganic salts, amino acids and their derivatives, purines and pyrimidines, fatty acids, and natural mixtures such as saliva, serum, crevicular fluid, and mucin. Chemotaxis was measured quantitatively by a modification of the capillary method. Cells were suspended in 0.01 M Tris buffer, pH 7.5, supplemented with 5 mM KCl, 0.1% $Na_2S_2O_3$, and 0.1 mM dithiothreitol. Whole, parotid, and submandibular salivas were also suitable as chemotaxis buffers. Optimum response (0.4×10^6 to 1.5×10^6 cells per capillary) for chemotaxis occurred with 5×10^{-2} M formate at 30°C for 60 min. At 5 to 15°C, cells were motile, but chemotaxis was not detected. Glutamine, asparagine, homoserine, and methionine inhibited formate chemotaxis at threshold concentrations of 0.1 to 1.0 mM, whereas the threshold for all other amino acids tested was 50 mM. Sugars and fatty acids at concentrations up to 0.1 M did not inhibit formate chemotaxis. *C. concisus* 484, *Wolinella recta* 371, *W. curva* VPI 9584, and *W. succinogenes* 602 were also chemotactic to formate, but *C. fetus* subsp. *jejuni* VPI H641 and *C. fetus* subsp. *intestinalis* VPI 1176 were not. Motile bacteria harvested directly from subgingival plaque were also chemotactic to formate and, to a lesser extent, lactate. Selected sugars, other fatty acids, and amino acids did not serve as chemoattractants for these plaque bacteria.

Motility and chemotaxis have been shown to play an important role in bacterial colonization of several environments. Stanton and Savage (25) demonstrated that a nonmotile mutant of an isolate of *Roseburia cecicola* was unable to colonize the intestinal lining of conventional mice, whereas wild-type, motile cells readily colonized these linings. Similarly, Morooka and co-workers demonstrated that motility was an important factor in *Campylobacter jejuni* colonization of suckling mice (20). Other studies have shown that chemotaxis and motility were essential for virulence of *Vibrio cholerae* in mice and rabbits (5–8, 12). Mutants of *V. cholerae* defective in motility or chemotaxis were unable to penetrate or colonize the intestinal mucosa of these animals. Wild-type cells, however, colonized these linings and were shown to be chemotactic towards digests of intestinal mucosa.

Motile bacteria, including species of *Campylobacter*, *Wolinella*, *Selenomonas*, *Treponema*, and *Capnocytophaga*, are often present in high concentrations in the periodontal pockets of patients with rapidly progressing forms of periodontal disease (17, 23, 24, 27). However, relatively few motile organisms are found in saliva, on the buccal mucosa, or on the tongue dorsum (15, 16). Motile bacteria, therefore, appear to preferentially colonize subgingival sites. If such organisms were not able to locate the more protected areas of the mouth, salivary flow would remove them and they would be unable to persist (9). Nonmotile bacteria, on the other hand, persist by attaching to surfaces to prevent being washed away.

The purpose of this investigation was to characterize the motility and chemotactic properties of selected *Campylobacter* and *Wolinella* species isolated from subgingival plaque to better evaluate the role that these properties play in oral colonization.

(A preliminary report of these studies was previously presented at the Annual Meeting of the American Association for Dental Research [B. J. Paster and R. J. Gibbons, J. Dent. Res. **64**:372, 1985].)

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MATERIALS AND METHODS

Bacterial strains and culture conditions. The organisms used in this study were *Campylobacter concisus* 288, *C. concisus* 484, *C. fetus* subsp. *jejuni* VPI H641, *C. fetus* subsp. *intestinalis* VPI 1176, *Wolinella recta* 371, *W. curva* VPI 9584, and *W. succinogenes* 602. These strains were obtained from the collection of A. Tanner, Forsyth Dental Center.

Cells were grown routinely in mycoplasma base broth (BBL Microbiology Systems) supplemented with 0.2% ammonium formate, 0.3% disodium fumarate, and 5 μg of hemin per ml (26). The final pH of the medium was 7.2. For some experiments, formate and fumarate were omitted from the growth medium. Cultures were incubated at 37°C in Brewer jars evacuated and filled three times with 80% N_2–10% CO_2–10% H_2.

To ensure actively motile cells (1), bacteria were allowed to migrate on plates of the above medium with 0.7% agar and 0.04% formate. After 3 days of incubation, cells were picked from the edge of the migrating population. This enrichment was performed every 2 weeks.

Most *C. concisus* 288 cells possessed a single polar flagellum, as determined by electron microscopy with procedures previously described (22). A small percentage of cells had two polar flagella.

Preparation of cell suspensions. Cells were harvested from cultures in late stationary growth phase (final concentration, approximately 5×10^8 cells per ml) by centrifugation at 5,000 × *g* at 5°C. All subsequent centrifugations were performed at room temperature. Cells were washed two times with chemotaxis (C) buffer by centrifugation in a Beckman Microfuge 11 at 5,000 × *g* for 3 min. C buffer consisted of 0.01 M Tris buffer (pH 7.5), 5 mM KCl, 0.1% $Na_2S_2O_3$, and 0.1 mM dithiothreitol (DTT). DTT was added just prior to use.

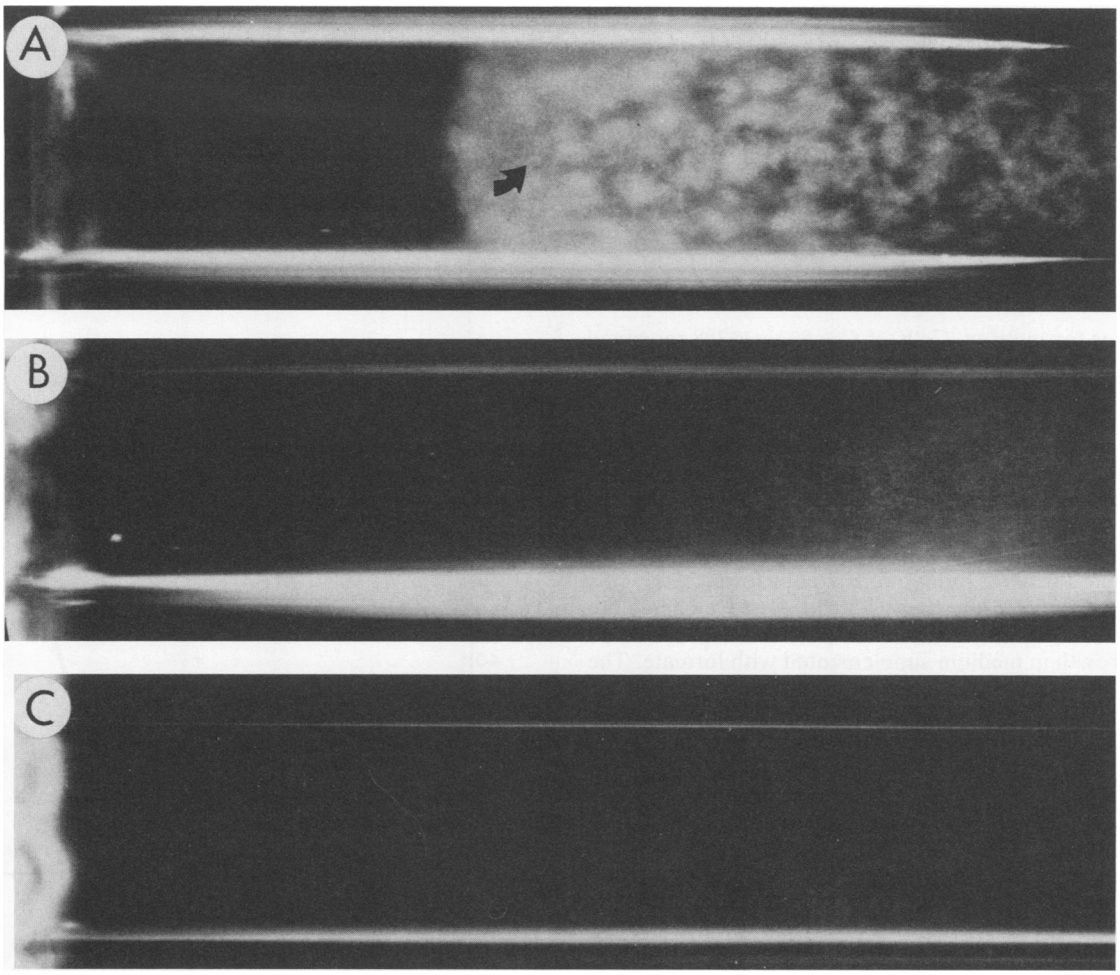

FIG. 1. Qualitative analysis of chemotaxis by *C. concisus* 288. Photographs of 1-μl capillary tubes after 60-min chemotaxis assay. (A) Capillary tube filled with 0.1 M formate. Note that the cells migrated into the tube to form a dense zone of bacteria (arrow). (B) Capillary tube filled with 0.01 M formate. Note that fewer cells migrated into the tube. (C) Capillary tube filled with C buffer alone. Cells (not visible) were randomly distributed near the mouth of the tube.

Similar concentrations of mercaptoethanol could replace DTT, but cysteine hydrochloride could not be substituted at any concentration for DTT. Final suspensions were adjusted to densities of 2×10^8 washed cells per ml (unless otherwise noted) in C buffer.

In some experiments, twice-washed cells were suspended in clarified whole saliva (heat inactivated at 60°C for 30 min), parotid saliva, or submandibular saliva supplemented with $Na_2S_2O_3$ and DTT. Salivas were collected as described previously (18).

Preparation of in vivo-grown subgingival motile bacteria. Bacterial plaque was removed from a periodontal pocket (depth of 5 mm) of a 32-year-old adult male with a sterile toothpick and suspended in 1 ml of C buffer. Plaque samples were taken at most every 2 days to ensure adequate build-up. Epithelial cells and debris were removed by centrifugation at $60 \times g$ for 1 min. The supernatant, containing primarily bacterial cells, was decanted and centrifuged for 3 min at $5,000 \times g$. The pellet was suspended in clarified whole saliva to yield a concentration of approximately 0.5×10^8 to 2.0×10^8 cells per ml. Between 25 and 50% of these cells were actively motile.

Chemotaxis assays. The techniques for measuring chemotaxis were based on a modification (13) of the procedures described by Adler (1). Capillary tubes (1 μl) filled with potential chemoattractants were placed into 0.15 ml of cell suspension in clean glass test tubes (10 by 75 mm) for 60 min unless otherwise noted. Attractants were dissolved in the buffer in which the cells were suspended (i.e., C buffer or saliva). The capillary tubes were held in place by surface tension. All experiments were performed in air. This modified technique allowed many samples to be handled at controlled temperatures in a water bath.

Compounds were screened qualitatively for chemotaxis by observing capillary tubes under dark-field illumination at 100× magnification (Fig. 1). For quantitative determinations, the number of cells in the capillary tubes was determined by viable-cell plate counts. Colonies were counted after 3 days of incubation. Chemotaxis was expressed as the ratio of the number of cells in the capillary with the attractant to the number of cells in a control capillary without attractant (R_{che}). Use of R_{che} normalizes the day-to-day variation in data (13, 21). Each experiment was run in duplicate or triplicate, and variations in samples usually ranged from 5 to 20%.

In experiments with bacteria harvested from subgingival plaque, cell numbers were determined by direct microscopic count. Briefly, cells within the capillary tubes were expelled

into 9.5 μl of C buffer. This suspension was spread over a known area (1 cm^2) on a clean microscope slide. After air drying, heat fixation, and simple staining, the slide was observed under an oil immersion lens. Strip counts were performed, and the total cell number was calculated by multiplying the average cell number per strip by the number of strips per square centimeter. Microscopic counts performed in this manner correlated well with those obtained with a Petroff-Hausser counting chamber.

RESULTS

Conditions for motility. Cells of *C. concisus* 288 remained motile in C buffer and saliva for more than 2 h under the conditions used. Cells moved rapidly in straight lines with occasional tumbling. Without DTT in the buffers, cells became sluggish and motility ceased after 1 h. Similar results were obtained with the other bacterial strains tested as well as with in vivo-grown bacteria, although the latter were more actively motile in clarified whole saliva than in C buffer.

Chemoattractants of *C. concisus* 288. Formate, in the form of ammonium formate, sodium formate, or formic acid, was the only compound tested that elicited a positive chemotactic response. Formate chemotaxis was not dependent on previous growth in medium supplemented with formate. The peak response occurred with 5×10^{-2} M formate (Fig. 2). The lowest concentration that elicited a positive response (threshold level) was calculated to be approximately 10^{-4} M by extrapolating a double-log plot of the data shown in Fig. 2 (19). When the assay was performed with clarified whole saliva, nearly identical results were obtained (Fig. 2). Cells suspended in parotid or submandibular saliva gave results similar to those obtained with whole saliva (data not shown). When formate was present at the same concentration in both the capillary tube and the bacterial suspension, only low background numbers of cells migrated into the capillary tube. This control demonstrated that the response of the cells was chemotactic rather than chemokinetic (i.e., their motility was not stimulated by a chemical).

A wide variety of compounds did not serve as chemoattractants for *C. concisus* 288 (Table 1). Compounds tested over a range of concentrations between 10^{-6} and 10^{-1} M included 20 sugars, inorganic salts, amino acids, purines and pyrimidines, fatty acids, and natural mixtures (Table 1). A positive control with 0.1 M formate as the attractant was included in all experiments.

Conditions for optimal chemotaxis. Conditions for the optimal chemotactic response of *C. concisus* 288 to 5×10^{-2} M formate in C buffer were established as follows. The cell concentration was 2×10^8 per ml. The time of incubation was 60 min; further incubation did not result in additional migration of cells into the capillary tubes (Fig. 3). The pH optimum was between 6.7 and 8.0, and no chemotaxis was detected below pH 5.2 or above pH 8.5. The optimum temperature was 30°C, but significant chemotactic responses were observed at temperatures up to 37°C (Fig. 4). Cells were actively motile at 5 to 15°C, but they did not respond to 0.1 M formate at these temperatures (Fig. 4).

In C buffer without KCl, chemotaxis of *C. concisus* 288 to 0.1 M formate was not detected. The concentration of KCl was tested over the range from 0.1 to 100 mM, and 5 mM proved to be optimal. With $Na_2S_2O_3$ deleted from the C buffer, formate chemotaxis was not consistent. When optimal concentrations of $Na_2S_2O_3$ or other compounds known to be electron acceptors (Table 2) were included in the C buffer, consistent positive chemotaxis to formate was obtained. Formate chemotaxis in C buffer with KNO_3 as the electron acceptor was dependent on previous growth of the cells in a KNO_3-supplemented medium.

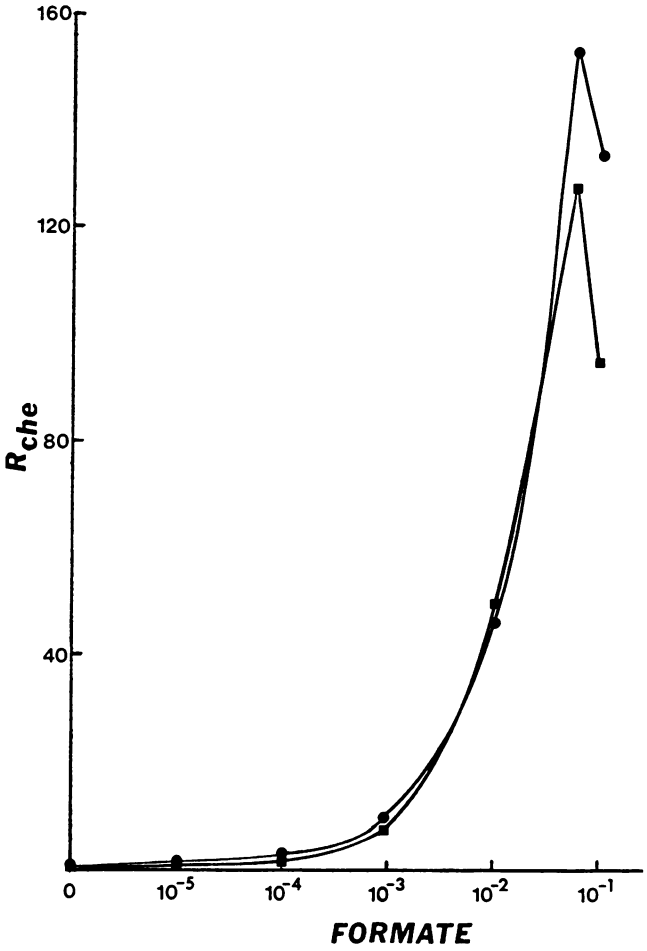

FIG. 2. Concentration-response curves for formate chemotaxis by *C. concisus* 288. For cells suspended in C buffer (■), the maximum number of cells at peak response was 4×10^5 per capillary; the background number was 3×10^3 cells per capillary. For cells suspended in whole saliva (●), the maximum number of cells at peak response was 1.5×10^6 per capillary; the background number was 9×10^3 cells per capillary. The incubation time for both was 60 min.

Formate chemotaxis by other bacterial strains. Similar chemotactic responses to formate were observed qualitatively with *C. concisus* 484, two oral *Wolinella* species, and a bovine rumen species, *W. succinogenes* (Table 3 and Fig. 1). Two intestinal species of *Campylobacter* were not observed to be chemotactic to any concentration of formate tested. It is not known whether the above strains are chemotactic to other compounds.

Inhibition studies. When present with 0.1 M formate, all amino acids tested (Table 1) were found to inhibit formate chemotaxis but not the motility of *C. concisus* 288. Glutamine, asparagine, homoserine, and methionine inhibited formate chemotaxis at threshold concentrations of 0.1 to 1.0 mM (Fig. 5), whereas the threshold for all other amino acids tested was 50 mM. If the assays were performed with cell suspensions in clarified whole saliva, inhibition of formate chemotaxis was restricted and cells were protected from the inhibitory effect of the amino acids (Fig. 5). Sugars and other

TABLE 1. Nonattractants of *C. concisus* 288[a]

C. concisus-nonattractant compounds		
Sugars/derivatives	Amino acids/derivatives	Fatty acids
Arabinose	20 common amino acids	Acetate
Ribose		Butyrate
Xylose	δ-NH_4 *n*-valeric acid	Isobutyrate
Arabinol	Citrulline	Valerate
Xylitol	Homocysteine	Isovalerate
	Homoserine	
Galactose	Putrescine	Citrate
Glucose	Spermidine PO_4	Fumarate
Fructose	Spermine	Lactate
Fucose	Triglycine	Succinate
Mannose	Glycyl-asparagine	
Rhamnose	Urea	Others
Sorbose		Ethanol
Mannitol	Purines/pyrimidines	Glycerol
Sorbitol	Adenine	Formamide
Cellobiose	Cytosine	Formaldehyde
Lactose	Guanine	Methanol
Maltose	Thymine	
Melibiose	Uracil	
Sucrose		
Raffinose	"Natural" compounds	
N-Acetylglucosamine	Bile	
	Bovine serum albumin	
	Mucin	
Inorganic compounds	Saliva (whole, parotid, submandibular)	
KNO_3	Serum, human	
$Na_2S_2O_3$	Crevicular fluid	
NH_4Cl		

[a] All compounds were tested at concentrations ranging from 10^{-6} to 10^{-1} M, with the following exceptions. Bile, bovine serum albumin, and mucin were tested at concentrations ranging from 0.001% to 1.0% and saliva and serum were tested at concentrations ranging from a 10^{-5} dilution to undiluted sample. Crevicular fluid was tested at dilutions from 10^{-5} to 10^{-2}.

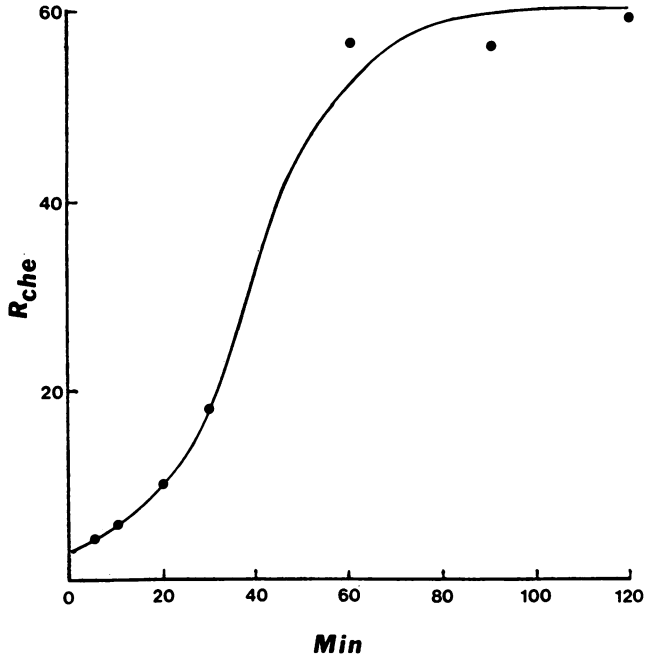

FIG. 3. Effect of incubation time on chemotaxis by *C. concisus* 288 to 0.1 M formate. After only 5 min, a significant number of cells (R_{che} of about 4) migrated into the capillary tubes, and the number reached a maximum by 60 min.

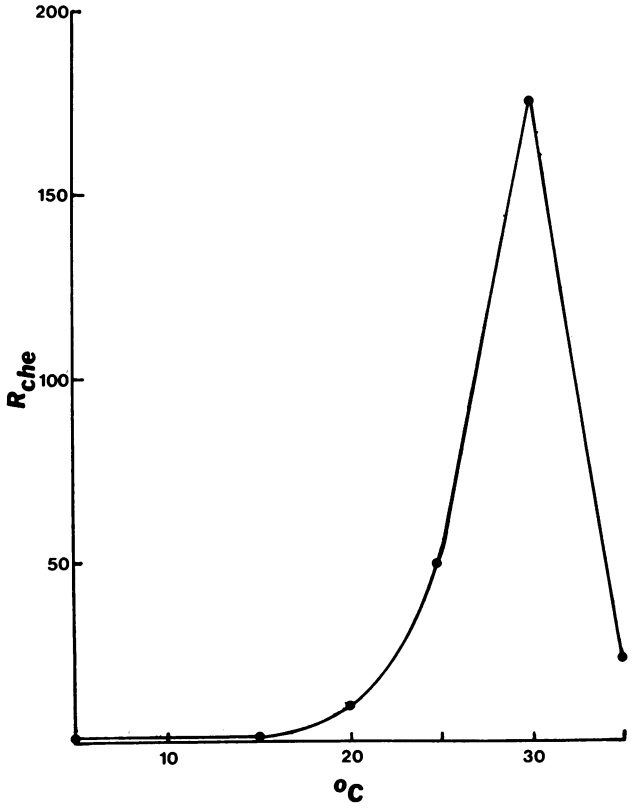

FIG. 4. Effect of temperature on chemotaxis by *C. concisus* 288 to 0.1 M formate.

fatty acids (Table 1) at concentrations up to 0.1 M did not inhibit formate chemotaxis of *C. concisus* 288.

Chemotaxis of motile bacteria harvested from subgingival plaque. Several selected compounds were surveyed as potential chemoattractants for motile bacteria harvested from subgingival plaque. Formate and, to a lesser extent, lactate were effective attractants for some cells, as determined qualitatively and quantitatively (Table 4). Some compounds, such as delta-aminovaleric acid, an intermediate amino acid commonly found in saliva (4), and glutamine, appeared to be weak chemoattractants (Table 4). Microscopic examination showed that cells which responded chemotactically to formate or lactate were morphologically similar to *Selenomonas* species (e.g., they had tufts of flagella from the inner curve of the cell) and *Campylobacter* or *Wolinella* species (i.e., small, highly motile cells). However, further characterization is necessary to establish positive identification.

DISCUSSION

The optimal conditions for motility and chemotaxis of species of *Campylobacter* and *Wolinella* were similar to those described for other motile bacteria (2, 11, 14). These conditions included an optimum cell concentration, pH range, time of incubation, and temperature. In our assays, KCl provided the potassium requirement. Potassium phos-

TABLE 2. Electron acceptors in formate chemotaxis by *C. concisus* 288[a]

Electron acceptor	Optimal concn[b]
Sodium thiosulfate ($Na_2S_2O_3$)	0.1%
Sodium bisulfite ($Na_2S_2O_5$)	0.1%
Sodium sulfite (Na_2SO_3)	0.1%
Sodium sulfate (Na_2SO_4)	0.1%
Potassium nitrate	0.1%
Postassium thiocyanate (KSCN)	0.01 M
Sodium thiocyanate	0.01 M
Sodium fumarate	0.01 M

[a] $MgSO_4$, Ca_2SO_4, and trimethylamine-*N*-oxide dihydrate were not efective as electron acceptors for chemotaxis.

[b] Compounds with optimal concentrations of 0.1% were tested over a range form 0.001 to 1%, and those with optima of 0.01 M were tested over a range from 0.001 to 0.1 M.

phate buffers were suitable for chemotaxis of *C. concisus* 288; however, better responses were observed with C buffer. DTT or mercaptoethanol, probably acting as reducing agents, was necessary in C buffer and in saliva to maintain motility for the duration of the chemotaxis assay.

Growing cells of *Campylobacter* and *Wolinella* utilize compounds such as fumarate and KNO_3 as electron acceptors (26). Potential electron acceptors (Table 2) were also required for formate chemotaxis in C buffer. It is interesting that KSCN, which is commonly found in saliva (3), worked in our system (Table 2).

It was demonstrated that the chemotactic behavior of *C. concisus* 288 is remarkably specific. Cells were attracted to formate but not to any of a wide range of other compounds tested. Other oral strains of *Campylobacter* and *Wolinella* were also chemotactic to formate. Since motile bacteria harvested from subgingival plaque also responded chemotactically to formate (and lactate) (Table 4), this phenomenon would appear to play an important role in the oral cavity. It is probably not a coincidence that the formate concentration which gave the optimal chemotactic response of *C. concisus* 288 (5×10^{-2} M) is the mean formate concentration reported present in dental plaque (10). Chemotaxis to formate, which is primarily a microbial metabolite and not a host-derived component, may enable *Campylobacter* and other motile species to locate and subsequently colonize plaque-laden periodontal pockets.

Since species of *Campylobacter* and *Wolinella* utilize formate as an energy source (26), it is not surprising that these bacteria were chemotactic to this compound. Hydrogen gas may be a possible chemoattractant inasmuch as these bacteria can also utilize H_2 as an electron donor (26). However, H_2 was not tested in our chemotaxis assays due to the difficulties of manipulating the gas as a chemoattractant. Campylobacters and wolinellas are asaccharolytic, but it is not known whether they can derive energy for growth by utilizing amino acids (26).

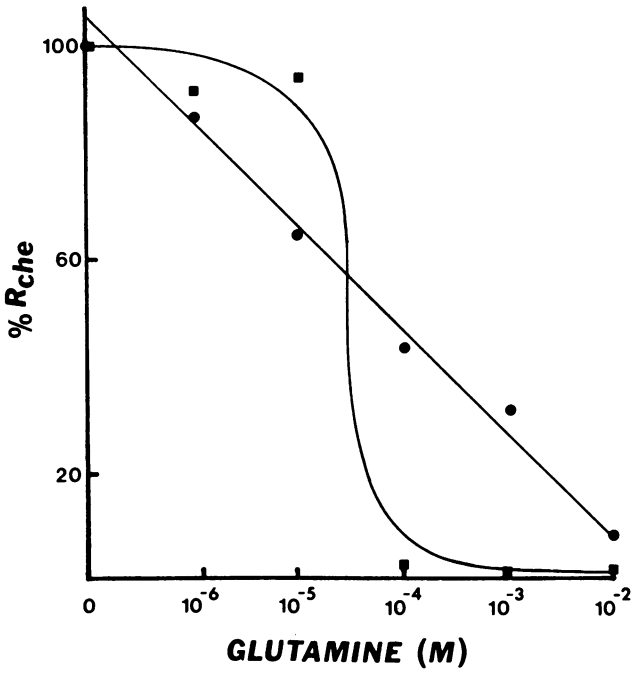

FIG. 5. Effect of glutamine on chemotaxis by *C. concisus* 288 to 0.1 M formate. Cells were suspended in C buffer (■) or whole saliva (●). R_{che} was approximately 100 in controls with no glutamine.

The biochemical basis for the inhibitory effect of amino acids on formate chemotaxis by *C. concisus* 288 is not understood. It is possible that they serve as chemorepellents, but we have not been able to detect this in preliminary studies with techniques previously described (28). However, amino acid inhibition may enable cells to regulate their chemotactic response to formate when they enter the gingival crevice area, which contains serumlike crevicular fluid, likely rich in amino acids.

Further studies are being pursued to determine whether chemotaxis by *C. concisus* 288 plays an essential role in colonization or facilitates its attachment to oral surfaces.

TABLE 3. Chemotaxis of *Campylobacter* and *Wolinella* strains to formate

Strain	No. of cells per capillary[a] at formate concn (M):					
	0	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
C. concisus 288	−	−	−	1+	3+	4+
C. concisus 484	−	−	−	+/−	2+	3+
W. recta	−	−	+/−	1+	3+	5+
W. curva	−	−	−	1+	3+	4+
W. succinogenes	−	−	+/−	1+	3+	6+
C. fetus subsp. *jejuni*	−	−	−	−	−	−
C. fetus subsp. *intestinalis*	−	−	−	−	−	−

[a] −, Cells randomly distributed (see Fig. 1C); +/−, cells beginning to cluster; 1+ to 6+, visible clusters of cells, ranging from about 2×10^4 (1+) to about 1×10^6 (6+) cells per capillary tube.

TABLE 4. Chemotaxis of in vivo-motile plaque bacteria

Attractant	Optimal concn[a] (M)	Response	
		Qualitative[b]	R_{che}
None		−	1.0
Formate	0.1	1+–2+	8.4–28.0
Lactate	0.1	1+	4.0–6.2
Glucose	0.1	−	1.1
Mix of 20 amino acids	0.05	−	1.0
δ-NH_4 *n*-valeric acid	0.001	+/−	2.0–2.5
Aspartic acid	0.001	−	1.0
Glutamine	0.001	+/−	2.2

[a] Concentrations from 10^{-5} to 10^{-1} M were tested.

[b] Ranged from randomly distributed cells (−) to visible clusters of up to 2×10^4 cells per capillary (2+).

ACKNOWLEDGMENTS

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