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Cellobiose Chemotaxis by the Cellulolytic Bacterium *Cellulomonas gelida*

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In the course of a study on the bacterial degradation of plant cell wall polysaccharides, we observed that growing cells of motile cellulolytic bacteria accumulated, without attachment, near cellulose fibers present in the cultures. Because it seemed likely that the accumulation was due to chemotactic behavior, we investigated the chemotactic responses of one of the above-mentioned bacteria (*Cellulomonas gelida* ATCC 488). We studied primarily the responses toward cellobiose, which is the major product of cellulose hydrolysis by microorganisms, and toward hemicellulose hydrolysis products. We found that cellobiose, cellotriose, D-glucose, xylobiose, and D-xylose, as well as other sugars that are hemicellulose components, served as chemoattractants for *C. gelida*, as determined by a modification of Adler's capillary assay. Competition and inducibility experiments indicated that *C. gelida* possesses at least two types of separately regulated cellobiose chemoreceptors (Cb1 and Cb2). Cb1 binds cellobiose and xylobiose but does not bind D-glucose, and its synthesis is inducible. Cb2 binds cellobiose, cellotriose, xylobiose, and D-glucose, and it is constitutively synthesized. The presence in *C. gelida* of a constitutive response toward cellobiose and of at least two distinct cellobiose chemoreceptors has implications for the survival of this cellulolytic bacterium in nature. A possible mechanism for cellobiose-mediated bacterial chemotaxis toward cellulose is proposed. We suggest that, in natural environments, motile cellulolytic bacteria migrate toward plant materials that contain cellulose and hemicellulose by swimming up cellobiose concentration gradients and/or concentration gradients of other sugars (e.g., xylobiose, D-xylose, and D-glucose) formed by enzymatic hydrolysis of plant cell wall polysaccharides.

It has been estimated that photosynthesis yields annually up to 1.5×10^{11} tons of dry plant material worldwide (12 and references therein). This material consists primarily of plant cell wall polymers, such as cellulose, hemicelluloses, pectin, and lignin. The degradation of these enormous amounts of plant cell wall polymers, which is carried out mainly by microorganisms, is an important step in the cycling of carbon in the biosphere. Microbial degradation of cellulose, hemicelluloses, and pectin occurs aerobically or anaerobically, in environments such as swamps, marshes, ponds, forest soil, the rumen, the intestine of termites, and others. Significant biodegradation of lignin has been observed only aerobically.

We are investigating metabolic processes utilized by bacteria for the degradation of plant cell wall polymers (2–4, 10, 11, 19). In the course of studies on a mesophilic cellulolytic bacterium (*Clostridium* sp. strain C7), we observed that, when cellulose was the fermentable substrate, the supernatant fluid of broth cultures of this organism remained clear and the bacterial cells grew in association with the sedimented cellulose present on the bottom of the culture vessel (3). However, examination by light microscopy of wet-mount preparations showed that the bacterial cells did not adhere to the cellulose fibers. A similar behavior was observed for cultures of another cellulolytic bacterium, the facultative anaerobe *Cellulomonas gelida* ATCC 488. A literature search revealed that Hungate had described this same behavior for cellulolytic bacteria that he had isolated (7). It seemed likely that the accumulation of cellulolytic bacteria near the cellulose fibers was due to some type of chemotactic response. Because cellulose is insoluble in water and therefore cannot elicit a direct chemotactic response, it appeared probable that the response was due to a soluble cellulose hydrolysis product formed by the activity of an extracellular cellulase bound to the cellulose fibers. This possibility was of great interest because chemotactic responses toward products of cellulose hydrolysis may play an important role in the overall process of cellulose degradation in natural environments. Furthermore, it seemed possible that, in nature, hydrolysis products of polymers present together with cellulose in plant cell walls could serve to attract motile cellulolytic bacteria toward cellulose-containing plant material. For example, various cellulolytic bacteria and noncellulolytic sugar-fermenting bacteria synthesize extracellular enzymes (xylanases) that hydrolyze xylan, a major component of hemicelluloses. Presumably, products resulting from the hydrolysis of xylan could serve as chemoattractants for cellulolytic bacteria.

In view of these considerations, we initiated an investigation of the chemotactic responses of the cellulolytic bacterium *C. gelida* ATCC 488, with emphasis on the responses toward cellobiose, which is the major product of cellulose hydrolysis by microorganisms, and toward hemicellulose hydrolysis products. The results of this investigation are described in this paper.

(A preliminary report of part of this work was presented previously [6].)

MATERIALS AND METHODS

Bacterial strain. The cellulolytic bacterium used in these studies was obtained from the American Type Culture Collection, in which it was listed under the name "*Cellulomonas subalbus*" (*Cellulomonas subalba*) ATCC 489. However, investigations of phenotypic characteristics and DNA-DNA homology by Stackebrandt and Kandler (18) have shown that "*C. subalbus*" ATCC 489 is identical to *C. gelida* ATCC 488. Inasmuch as the specific epithet *gelida*

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antedates *subalbus* on the basis of page priority (9, 18), the epithet *gelida* should be used for this species (18). Thus, we will refer to the cellulolytic bacterium used in this investigation as *C. gelida* ATCC 488. This strain stained gram positive. Most cells measured approximately 0.5 by 2 μm, but both longer and shorter cells were present in cultures. The colonies had a yellow pigmentation.

Media and growth conditions. Cells for capillary assays were grown in minimal salts medium (medium MS) to which an energy and carbon source was added. Medium MS had the following composition: salts solution, 98 ml; vitamin solution, 1 ml; and amino acid solution, 1 ml. The salts solution contained (in milligrams per 100 ml of distilled water) the following: KH_2PO_4, 30; Na_2HPO_4, 15; $(NH_4)_2SO_4$, 100; $MgSO_4 \cdot 7H_2O$, 25; $CaCl_2 \cdot 2H_2O$, 1.5; $FeSO_4 \cdot 7H_2O$, 0.3; and $Na_2MoO_4 \cdot 2H_2O$, 1. The vitamin solution contained (in milligrams per 100 ml of distilled water) the following: thiamine · HCl, nicotinic acid, and hemi-Ca salt of D-pantothenic acid, 5 each; riboflavin, B_{12}, and pyridoxine · HCl, 1 each; folic acid, 0.1; and biotin, 0.05. The amino acid solution contained 100 mg of Casamino Acids (Difco Laboratories, Detroit, Mich.) per 100 ml of distilled water. Amino acids were added because it was observed that cell motility was enhanced in their presence. The medium was prepared by adding the filter-sterilized vitamin and amino acid solutions to the salts solution, previously sterilized by autoclaving. Carbon and energy sources were added as separately sterilized solutions. The pH of the medium was approximately 7.

Medium MS-CB consisted of medium MS to which cellobiose was added to a final concentration of 5 mM as a carbon and energy source. Medium MS-glycerol was medium MS containing 5 mM (final concentration) glycerol. Cellulose was added to MS medium in the form of either ball-milled filter paper (10) (0.12 g, dry weight, per 100 ml of medium) or Avicel (a crystalline cellulose preparation) (0.2 g per 100 ml of medium; type PH 105; 20-μm particles; FMC Corp., Marcus Hook, Pa.). Medium MS-X consisted of medium MS to which larchwood xylan (Sigma Chemical Co., St. Louis, Mo.) was added (0.1 g of xylan per 100 ml of medium). Xylan was routinely washed in 70% (wt/vol) ethanol before use.

TY-CB agar medium, used for viable count plates in the capillary assays, contained (in grams per 100 ml of distilled water) the following: tryptone (Difco) and yeast extract (Difco), 0.5 each; cellobiose, 0.01; and agar (Bacto; Difco), 1.5. Cells used for the detection of cellulose hydrolysis products were grown in TY-C medium, containing (in grams per 100 ml of distilled water) the following: cellulose (ball-milled filter paper) (10), 0.12 (dry weight); and tryptone and yeast extract, 0.01 each.

Cells for capillary assays were grown in test tubes (18 by 150 mm; 10 ml of medium per tube). For other experiments, cells were grown in 250-ml Erlenmeyer flasks each containing 100 ml of medium. Cells were grown aerobically, unless otherwise indicated. Aerobic cultures in liquid media were incubated in air on a New Brunswick Scientific Co. rotary shaker (model VS-100) operating at 200 rpm. Anaerobic cultures were incubated in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, Mich.; 10% CO_2, 7% H_2, 83% N_2 [vol/vol/vol]). All cultures were incubated at 30°C.

Products of the enzymatic hydrolysis of cellulose and xylan. Cells were grown in TY-C medium until all the cellulose was utilized (1 to 2 days). The cells were sedimented by centrifugation, and the protein-containing supernatant fluid was concentrated 30 times by use of an Omega Cell 150 (Filtron; nominal molecular weight limit, 10,000) at 0°C. Enzyme activities were assayed by incubating 0.35 ml of the concentrated supernatant fluid with 0.1 ml of substrate suspension or solution (7 mg of substrate per ml of distilled water), 0.025 ml of 0.8 M potassium phosphate buffer (pH 7), 0.025 ml of 10% (wt/vol) NaCN, and 0.35 ml of distilled water for 2.5 h at 42°C. Substrates were larchwood xylan (washed) or cellulose in the form of either Avicel or ball-milled filter paper. After incubation, the assay mixture was centrifuged to remove the sediment, and the hydrolysis products in the supernatant fluid were detected by high-performance liquid chromatography (HPLC) with an SP8430 refractive-index detector (Spectra-Physics, San Jose, Calif.) and an Aminex HPX-87P column (Bio-Rad Laboratories, Richmond, Calif.).

Chemotaxis assays and competition experiments. For chemotaxis measurements, we used a modification of Adler's capillary assay described by Weis et al. (21). Cells were grown to the mid-logarithmic phase in medium MS-CB or in medium MS containing an energy and carbon source other than cellobiose, harvested by centrifugation, and washed twice with chemotaxis buffer (0.01 M potassium phosphate buffer [pH 7], 0.15 M NaCl, 0.1 mM potassium EDTA, 1 mg of Casamino Acids per 100 ml of chemotaxis buffer). The cells were then suspended in chemotaxis buffer to a final concentration of approximately 10^8 cells per ml, and 250 μl of this cell suspension was dispensed into each microcentrifuge tube (microcentrifuge tubes served as reservoirs for the bacteria in the assay). Capillary tubes (1-μl micropipettes; Drummond Scientific Co., Broomall, Pa.) were filled with solutions of chemicals at various concentrations in chemotaxis buffer. Then, the open end of the capillary tube was inserted into the bacterial suspension within the microcentrifuge tube. After 25 min of incubation at room temperature (23 ± 1°C), suitable dilutions of the capillary tube contents were made with a sterile tryptone solution (0.5 g/100 ml of distilled water) and samples of the dilutions were spread on TY-CB agar plates. Colonies on the plates were counted after 2 days of incubation. Chemotaxis measurements are reported as averages for duplicate plate counts in three separate capillary assays.

The following definitions apply to the capillary assay-related terminology used in this article. "Background" is the number of bacteria accumulating in the capillary tube containing only chemotaxis buffer (no attractant present). "Response" refers to the accumulation of bacteria (in excess of the background) in an attractant-containing capillary tube. The response to an attractant is reported in this article as the "relative response" (20), which is the number of cells that accumulate in an attractant-containing capillary tube divided by the background. "Threshold concentration" is the lowest attractant concentration (in the capillary tube) that elicits a response. "Peak concentration" is the concentration of an attractant (in the capillary tube) that elicits the highest response. "Peak relative response" is the relative response at the peak concentration. The standard deviation of response measurements at the peak concentration for replicate assays was approximately 10%.

In competition experiments (1), in addition to the attractant present in the capillary tube, a second attractant was present (at the peak concentration) in both the capillary tube and the bacterial suspension. Under these conditions, if the two attractants bind to the same chemoreceptor, the response to the attractant added only to the capillary tube is inhibited, although inhibition may occur through causes other than competition for binding sites (14, 15). However, lack of inhibition is strong evidence that the two attractants bind to different chemoreceptors (1, 14).

Inducibility experiments. Inducibility experiments are based on evidence indicating that the induction of a chemoreceptor results in enhancement of the response toward attractants that bind to the same chemoreceptor, as compared with the response without induction (1). In contrast, the response toward attractants that bind to a different, uninduced chemoreceptor is not enhanced (1).

Cells were grown in media that differed only in their carbon and energy source and then were tested for their chemotactic responses toward cellobiose and D-glucose by means of the capillary assay.

Motility. The swimming behavior of *C. gelida* cells was observed by video microscopy as described by Weis and Koshland (22). Cell velocity was determined as previously described (5). For visualization of flagella, cells negatively stained for 30 s with 1% (wt/vol) uranyl acetate or 1% (wt/vol) uranyl oxalate were examined with a JEOL 100S transmission electron microscope operating at 80 kV.

Sugars. Sugars were obtained from various commercial sources. Cellobiose, D-galactose, and D-mannose used in capillary chemotaxis assays were reference grade. All other sugars used in capillary assays and all sugars used in growth media were research grade.

Reference-grade cellobiose contained 0.013 mol% glucose or less, as determined by HPLC with an LC-NH_2 column from Supelco Inc. (Supelco Park, Bellefonte, Pa.). Reference-grade D-galactose and D-mannose contained 0.029 mol% glucose or less, as determined by HPLC with an Aminex HPX-87P column from Bio-Rad. L-Arabinose, arbutin (4-hydroxyphenyl β-D-glucopyranoside), and salicin [2-(hydroxymethyl)phenyl β-D-glucopyranoside] contained less than 0.001 mol% glucose, and D-xylose contained approximately 0.07 mol% glucose, as determined by the glucose oxidase assay (Sigma Diagnostics; catalog no. 315-100).

Chemotactic responses toward sugars treated with glucose oxidase. The chemotactic responses of *C. gelida* toward commercial sugar preparations were compared with the responses toward the same sugar preparations that had been treated with glucose oxidase (Sigma type VII-S; product no. G-7016). The capillary assays were performed as described above. Reference-grade cellobiose or D-galactose and research grade D-glucose were used. Sugar concentrations in the capillary tubes were 1×10^{-2} M cellobiose, 1×10^{-2} M D-galactose, and 1.5×10^{-6} M D-glucose. Cells were grown in medium MS-glycerol, harvested by centrifugation, and washed twice with chemotaxis buffer. Cells to be used in assays that included the enzyme were resuspended in chemotaxis buffer containing 3 U of glucose oxidase per ml (final concentration). Sugars were treated by being dissolved in chemotaxis buffer containing 3 U of glucose oxidase per ml and were incubated at 35°C for 30 min prior to the assay. According to product specifications, 1 U of the enzyme oxidizes 1 μmol of D-glucose per min at pH 5.1 and 35°C.

RESULTS

Motility. Electron microscopy of negatively stained preparations indicated that *C. gelida* cells were peritrichous, with most cells having five or six flagella each (Fig. 1). Individual flagella in the preparations measured up to 7.6 μm in length. Observation of videotaped freely swimming cells showed that *C. gelida* had a motility pattern similar to that of *Escherichia coli*, i.e., smooth swimming interrupted by tumbles that were accompanied by changes in direction (13). The average velocity of growing log-phase cells was approximately 28 μm/s. More than 80% of the cells were motile in cultures used for the chemotaxis assays.

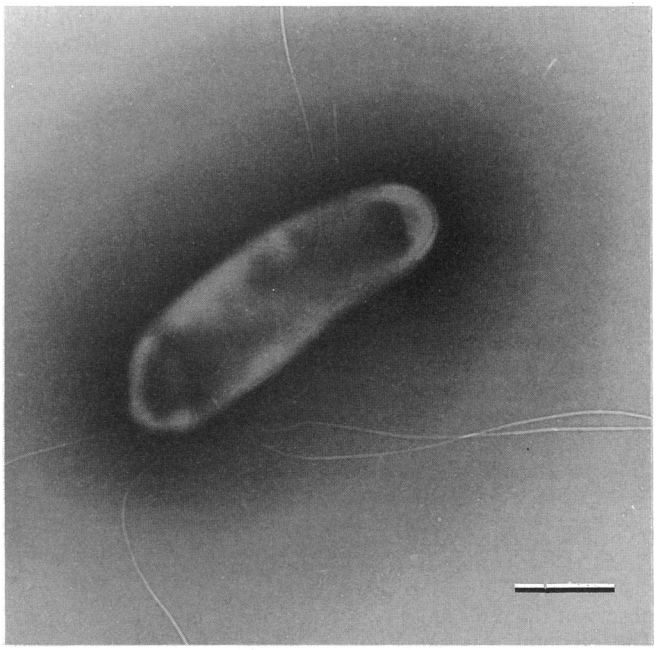

FIG. 1. Electron micrograph of a negatively stained peritrichous cell of *C. gelida*. Six flagella are visible. Bar, 0.5 μm.

Utilization of plant cell wall polysaccharides for growth. *C. gelida* utilized cellulose, xylan, or sugars that are hydrolysis products of these plant cell wall polysaccharides (e.g., cellobiose, D-glucose, and D-xylose) as carbon and energy sources for aerobic and anaerobic growth (Table 1). Our strain of *C. gelida* utilized, as a growth substrate, cellulose provided in the form of either ball-milled filter paper or

TABLE 1. Chemotactic responses of *C. gelida*[a]

Chemical	Growth[b] (doubling time, in h)	Threshold concn (M)	Peak concn (M)	Peak relative response
D-Glucose	2.5	10^{-7}	10^{-5}	9.4
Cellobiose	2.1	10^{-5}	10^{-2}	17.7
Cellotriose	ND	10^{-5}	10^{-2}	8.0
D-Xylose	2.5	10^{-7}	10^{-5}	9.9
Xylobiose	ND	10^{-5}	10^{-2}	14.9
L-Arabinose	2.9	10^{-5}	10^{-2}	9.6
D-Galactose	3.5	10^{-4}	10^{-2}	11.3
D-Mannose	2.8	10^{-5}	10^{-3}	13.8
Arbutin	SG	10^{-4}	10^{-2}	7.6
Salicin	SG	10^{-4}	10^{-2}	10.6
2-Deoxy-D-glucose	NG	10^{-5}	10^{-3}	13.4
Sodium acetate[c]	SG			1.0
Glycerol[d]	2.6			1.0

[a] Cells for capillary assays were grown aerobically in medium MS-CB. The background was 15,000 cells per capillary tube. Cells for growth studies were grown in medium MS containing an 8 mM final concentration of the chemical indicated. The following compounds did not support growth and did not elicit a response from cells grown in medium MS-CB: raffinose, L-rhamnose, D-ribose, and sodium propionate.

[b] ND, not determined; SG, slow growth; NG, no growth.

[c] Cells grown in medium MS-acetate were not motile.

[d] Cells grown in medium MS-glycerol did not exhibit a chemotactic response toward glycerol.

Avicel. Larchwood xylan (e.g., in medium MS-X) served as a carbon and energy source. D-Mannose, D-galactose, and L-arabinose, which are components of plant cell wall polysaccharides (hemicelluloses and pectin), also served as carbon and energy sources for *C. gelida* (Table 1).

Products of cellulose or xylan hydrolysis. *C. gelida* produced extracellular enzymes that hydrolyzed cellulose (Avicel or ball-milled filter paper) and xylan. Incubation of culture supernatant fluid with crystalline cellulose (Avicel) yielded cellobiose and small amounts of glucose. The major hydrolysis product from xylan was xylobiose, with minor amounts of xylose also being detected. The cellulolytic and xylanolytic extracellular enzymes were produced not only when cellulose was the carbon and energy source but also when xylan, cellobiose, or glycerol was the growth substrate.

Survey of chemotactic responses of *C. gelida*. Components of plant cell wall polysaccharides and other chemicals were tested for their ability to elicit chemotactic responses from *C. gelida* cells. D-Glucose, cellobiose, and cellotriose, which are products of cellulose hydrolysis (4), served as chemoattractants (Table 1). D-Xylose and xylobiose, which are hydrolysis products of xylan (a major component of hemicelluloses), also were attractants for *C. gelida* (Table 1). Other attractants were sugars present in hemicelluloses and pectin (L-arabinose, D-galactose, and D-mannose), as well as arbutin and salicin (Table 1), which are β-glucosides naturally occurring in plants. 2-Deoxy-D-glucose, an analog of D-glucose, served as a chemoattractant but did not support growth, whereas glycerol supported growth but was not an attractant (Table 1). Other compounds that did not serve as chemoattractants are given in Table 1, footnote *a*.

At their peak concentration (10^{-2} M), the cellobiose and D-galactose solutions may have contained as much as 1.3×10^{-6} M and 2.9×10^{-6} M contaminating glucose, respectively (see Materials and Methods). Inasmuch as the threshold concentration for D-glucose was 10^{-7} M, it was possible that part of the response toward the cellobiose or D-galactose preparations was elicited by contaminating glucose. However, as described below, experiments in which the cellobiose and D-galactose solutions were treated with glucose oxidase, as well as induction experiments, indicated that the chemotactic responses toward the cellobiose and D-galactose preparations were not due to glucose contamination.

Cells grown in a medium containing 5 mM (final concentration) glycerol and 0.5 g each of tryptone (Difco) and yeast extract (Difco) per 100 ml of distilled water exhibited a chemotactic response toward L-aspartate (peak relative response = 6.8 at 10^{-4} M potassium aspartate). Cells grown in a similar medium in which cellobiose (5 mM final concentration) was present instead of glycerol exhibited a weak chemotactic response toward L-serine.

Competition experiments. To obtain information on the cellobiose chemoreceptor(s), we carried out competition experiments (see Materials and Methods). Unless otherwise indicated, the cells used in these experiments were grown in a medium containing cellobiose as the carbon and energy source (medium MS-CB).

Xylobiose abolished the chemotactic response toward cellobiose (Fig. 2A), and the response toward xylobiose was likewise abolished in the presence of cellobiose (Fig. 2B), as would be expected if both disaccharides were to bind to the same chemoreceptor(s).

In the presence of D-glucose, the chemotactic response toward cellobiose was partially inhibited (Fig. 3B), and in the presence of cellobiose, partial inhibition of the response toward D-glucose was observed (data not shown). The chemotactic response toward cellotriose was eliminated by cellobiose or D-glucose, the response toward cellobiose was inhibited only partially by cellotriose, and D-glucose partially inhibited the response toward xylobiose (data not shown).

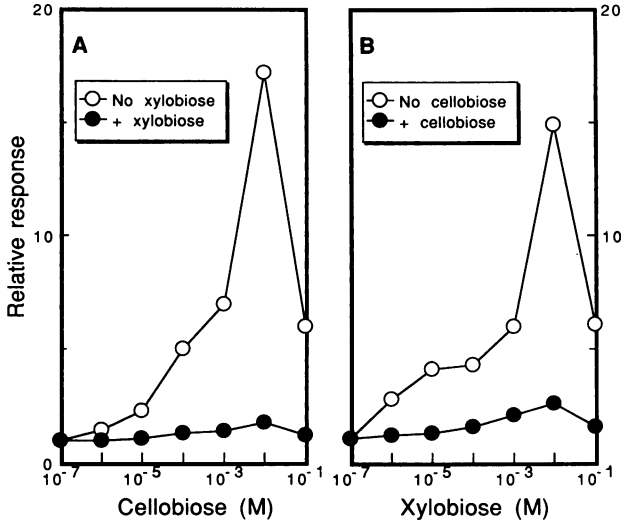

FIG. 2. (A) Inhibition of taxis toward cellobiose by xylobiose (10^{-2} M). (B) Inhibition of taxis toward xylobiose by cellobiose (10^{-2} M). *C. gelida* cells used in panels A and B were grown in medium MS-CB.

These observations suggested that cells of *C. gelida* possess at least two types of cellobiose chemoreceptors. One type (Cb1) binds cellobiose and xylobiose but does not bind D-glucose and cellotriose. The other type (Cb2) binds cellobiose, cellotriose, xylobiose, and D-glucose. Furthermore, a

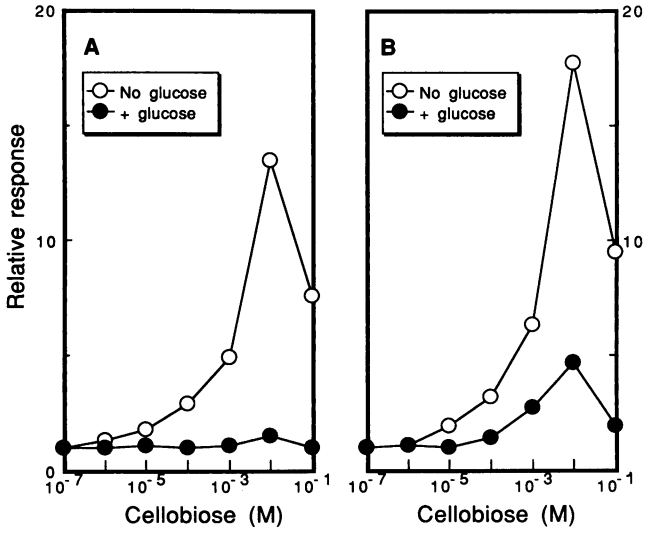

FIG. 3. (A) Inhibition of the tactic response toward cellobiose by D-glucose (10^{-5} M) in *C. gelida* cells grown in medium MS-glycerol. (B) Partial inhibition of the tactic response toward cellobiose by D-glucose (10^{-5} M) in *C. gelida* cells grown in medium MS-CB.

TABLE 2. Cellobiose chemotaxis in cells grown on different substrates[a]

Growth substrate	Threshold concn (M)	Peak concn (M)	Peak relative response
Cellobiose	10^{-5}	10^{-2}	17.7
Cellobiose[b]	10^{-5}	10^{-2}	16.2
D-Glucose	10^{-4}	10^{-2}	6.5
D-Xylose	10^{-4}	10^{-2}	12.7
D-Mannose	10^{-4}	10^{-2}	10.8
D-Galactose	10^{-4}	10^{-2}	9.5
Glycerol	10^{-4}	10^{-2}	13.2
Tryptone[c]	10^{-4}	10^{-2}	6.3

[a] Cells were grown aerobically in medium MS containing the substrate indicated (5 mM final concentration), except as indicated otherwise. The background was 15,000 cells per capillary tube.

[b] Cells were grown anaerobically in prereduced medium MS-CB containing 5 mM cellobiose (final concentration) in an N_2 atmosphere. Chloramphenicol (C. P. Pfizer & Co. Inc., New York, N.Y.; 20-μg/ml final concentration) was added to the chemotaxis buffer used in the capillary assay.

[c] The concentration of tryptone (Difco) was 0.5 g/100 ml of medium.

type of D-glucose chemoreceptor that does not bind cellobiose may be present.

Inducibility of the chemotactic response toward cellobiose. The chemotactic response toward cellobiose of *C. gelida* cells grown in medium MS containing one of various carbon and energy sources was determined (Table 2). Cells grown with cellobiose as the carbon and energy source had the highest chemotactic response and the lowest threshold concentration (Table 2). The response toward cellobiose was present whether the cells were cultured aerobically or anaerobically (Table 2), indicating that cellobiose chemoreceptors were synthesized both in the presence and in the absence of O_2. A response toward cellobiose was detected with all carbon and energy sources tested (Table 2). This observation is consistent with the conclusion that a significant level of cellobiose chemoreceptor(s) is constitutively synthesized in *C. gelida*. Furthermore, in vivo methylation experiments indicated that *C. gelida* synthesizes a constitutive methyl-accepting protein for cellobiose chemotaxis (6a).

A lower peak response toward cellobiose was detected when *C. gelida* cells were grown in glucose-containing medium than in cellobiose-containing medium (Table 2). The lower response possibly occurred because cells growing with D-glucose as a carbon and energy source synthesized only the type of chemoreceptor that binds both cellobiose and D-glucose (Cb2). Thus, the observation of a lower response toward cellobiose in glucose-grown cells was consistent with the results of competition experiments suggesting the presence of a cellobiose chemoreceptor that does not bind D-glucose.

The inducibility of chemotaxis toward D-glucose was also studied with cells grown in medium MS containing D-glucose, cellobiose, D-xylose, glycerol, or tryptone as the carbon and energy source. In all cases, a response toward D-glucose was detected, with the peak relative responses ranging between 9.5 and 10.5. Thus, the response toward D-glucose apparently was constitutively expressed, and there was no growth substrate-dependent enhancement of the response toward D-glucose.

Additional evidence indicating the presence of two distinct types of cellobiose chemoreceptors was obtained by comparing the tactic responses of glycerol-grown cells with those of cellobiose-grown cells. As mentioned above, glycerol serves as a carbon and energy source for *C. gelida*, but it is not an attractant (Table 1). Competition experiments showed that, in glycerol-grown cells, the tactic response toward cellobiose was totally inhibited by D-glucose (Fig. 3A). In contrast, in cellobiose-grown cells, the tactic response toward cellobiose was only partially inhibited by D-glucose (Fig. 3B). Furthermore, the peak relative response of glycerol-grown cells was approximately 13.2 (Fig. 3A), whereas the peak relative response of cellobiose-grown cells was 17.7 (Fig. 3B). Our interpretation of these results is as follows. In cellobiose-grown cells, both types (Cb1 and Cb2) of cellobiose chemoreceptors are synthesized, and D-glucose blocks only the response attributable to Cb2. Thus, the response of cellobiose-grown cells observed in the presence of D-glucose (Fig. 3B) is due to Cb1, the type of cellobiose chemoreceptor that does not bind D-glucose. In contrast, in glycerol-grown cells, only Cb2 is synthesized; therefore, D-glucose totally inhibits the response toward cellobiose (Fig. 3A). These results indicate that *C. gelida* possesses two types of cellobiose chemoreceptors (Cb1 and Cb2) and that their syntheses are separately regulated.

Chemotactic responses toward glucose oxidase-treated cellobiose, D-galactose, and D-glucose solutions. As mentioned above, contaminating glucose possibly present in the commercial cellobiose and D-galactose used in chemotactic assays might have been responsible for part of the chemotactic response of *C. gelida* cells toward preparations of the latter two sugars. The maximum concentration of contaminating glucose that might have been present in a 10^{-2} M (peak concentration) solution of commercial cellobiose or D-galactose was 1.3×10^{-6} M or 2.9×10^{-6} M, respectively. Inasmuch as the D-glucose threshold concentration was 10^{-7} M, the observed peak response toward cellobiose and D-galactose might have been due, in part, to the presence of contaminating glucose.

Cells grown in medium MS-CB (containing cellobiose) exhibited a relative response toward 1.3×10^{-6} M D-glucose that was 40% their relative response toward 1×10^{-2} M cellobiose. Cells grown in medium MS-glycerol had a relative response toward 1.3×10^{-6} M D-glucose that was 64% their relative response toward 1×10^{-2} M cellobiose. Thus, even if contaminating glucose was present at the maximum possible concentration, *C. gelida* cells exhibited a chemotactic response toward cellobiose under the conditions used.

Cellobiose and D-galactose solutions were treated with glucose oxidase (see Materials and Methods) to oxidize possible contaminating glucose. As a control, a D-glucose solution (1.5×10^{-6} M) was subjected to the same treatment. Capillary assays were used to compare the chemotactic responses of *C. gelida* cells toward treated and untreated solutions of each of the three sugars. The peak relative response toward cellobiose (11.4) or to D-galactose (15.8) was unaffected by the treatment, whereas very little or no response toward glucose oxidase-treated D-glucose (1.5×10^{-6} M) was observed. The relative response toward the untreated D-glucose solution was 9.8. The results indicated that the observed chemotactic responses of *C. gelida* cells toward cellobiose or D-galactose solutions were, in fact, responses toward these sugars and not toward any contaminating glucose.

DISCUSSION

We found that soluble sugars that are components of cellulose or other plant cell wall polysaccharides serve as chemoattractants for *C. gelida*. Among these sugars, cellobiose is of special interest, because this disaccharide is the

major product of cellulose hydrolysis by *C. gelida* and other cellulolytic microorganisms.

Our data show that at least two types of cellobiose chemoreceptors (Cb1 and Cb2) are present in *C. gelida*. Cb1, which binds cellobiose but not D-glucose, is inducible, being synthesized by cellobiose-grown cells but not by glycerol-grown cells. In contrast, Cb2, which binds both cellobiose and D-glucose, is constitutively synthesized. Both Cb1 and Cb2 bind xylobiose, which is a major product of the hydrolysis of xylan (a hemicellulose component). The chemotactic response toward cellobiose was observed whether the cells were grown aerobically or anaerobically (Table 2), indicating that cellobiose chemoreceptors are synthesized both in the presence and in the absence of molecular oxygen. This observation implies that the chemotactic response toward cellobiose occurs in both aerobic and anaerobic natural environments. Furthermore, the observations that *C. gelida* cells exhibit a constitutive chemotactic response toward cellobiose and possess at least two distinct cellobiose chemoreceptors indicate that chemotaxis toward cellobiose is important to this bacterium with respect to its survival in nature.

On the basis of our results, we propose that the following mechanism for bacterial chemotaxis toward cellulose may occur in natural environments. Extracellular cellulases produced by bacteria that inhabit these environments bind to cellulose present in plant material and hydrolyze this polysaccharide. The hydrolysis of cellulose generates cellobiose concentration gradients. In response to the cellobiose concentration gradients, motile cellulolytic bacteria migrate toward cellulose, utilizing their constitutively synthesized cellobiose chemoreceptors (e.g., Cb2 in *C. gelida*). At the same time, in the presence of cellobiose, inducible cellobiose chemoreceptors (e.g., Cb1) are synthesized by the cellulolytic bacteria. As a result, the chemotactic response is enhanced. Finally, cellulolytic bacteria either attach to cellulose fibers or remain in their vicinity, where the highest cellobiose concentrations are present. It appears that the cellobiose gradients persist as the cellulolytic bacteria multiply, as indicated by the observation that, in laboratory cultures, growing cells of cellulolytic bacteria remain in the vicinity of cellulose fibers.

Enzyme systems that hydrolyze crystalline cellulose are synthesized during growth of microorganisms in media containing cellulose as the carbon and energy source and are synthesized, either at low or at relatively high levels, by various cellulolytic bacteria growing in media in which cellobiose is the carbon and energy source (e.g., 3, 8, 17). It is believed (16) that cellulolytic bacteria form an as-yet-unidentified low-molecular-weight product of cellulose degradation (or a derivative thereof) that serves as an inducer of cellulases. This inducing compound is generated from cellulose by cellulolytic enzymes synthesized constitutively at low basal levels (16). Presumably, the activity of these constitutively produced enzymes initiates the process of cellulose degradation, with the concurrent production of an inducer and the subsequent synthesis of inducible cellulases. In the chemotaxis mechanism proposed above, the low basal level of constitutive extracellular cellulases would bind to cellulose present in plant material and hydrolyze it, with the resultant production of an inducer by the cellulolytic bacteria, enhanced cellulase synthesis, and the generation of cellobiose gradients. We found that, in *Clostridium* sp. strain C7 (3), the synthesis of a relatively high level of the extracellular cellulase system was induced in the presence of cellobiose concentrations as low as 29 μM (15a).

The functioning of the chemotaxis mechanism proposed above requires that the cellulose-hydrolyzing proteins become dispersed in the aqueous portion of the environments in which the cellulolytic bacteria are present. In these environments, the dispersion of cellulases may occur by diffusion, by sedimentation, and/or by convective mixing resulting from thermal fluctuations that generate temperature gradients. Furthermore, the motion of organisms present in the environments may contribute to the dispersion of cellulases.

Our survey of the chemotactic responses of *C. gelida* showed that D-glucose, xylobiose, D-xylose, and other products of cellulose and hemicellulose hydrolysis serve as chemoattractants for this bacterium. These observations suggest that, in nature, *C. gelida* cells and those of other motile cellulolytic bacteria migrate toward cellulose- and hemicellulose-containing plant material not only in response to cellobiose gradients but also by swimming up concentration gradients of other sugars formed by the hydrolytic activities of the cellulases and hemicellulases (e.g., xylanases) that they produce. It should be noted that *C. gelida*, as well as other cellulolytic bacteria (3), utilizes xylan as a carbon and energy source for growth.

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