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Chemotactic Behavior of *Azotobacter vinelandii*

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Chemotaxis was exhibited by *Azotobacter vinelandii* motile cells. Exposure of cells to sudden increases in attractant concentration suppressed the frequency of tumbling and resulted in smooth swimming. Cells responded chemotactically to a chemical gradient produced during metabolism. Motility occurred over a temperature range of 25 to 37°C with an optimum pH range of between pH 7.0 and 8.0. The average speed of motile cells was determined to be 74 μm/s or 37 body lengths per s. The speed of cells appeared to increase as a function of attractant concentration. Chemotactic systems for fructose, glucose, xylitol, and mannitol were inducible. *A. vinelandii* exhibited chemotaxis for a number of compounds, including hexoses, hexitols, pentitols, pentoses, disaccharides, and amino sugars. We conclude from these studies that *A. vinelandii* exhibits a temporal chemotactic sensing system.

The present study was undertaken to investigate the chemotactic behavior of *Azotobacter vinelandii*, an obligate aerobic, non-symbiotic nitrogen-fixing bacterium. *A. vinelandii* is a gram-negative bacterium about 4.5 μm long and 2.4 μm wide which possesses peritrichous flagella of normal wave shape.

The molecular biology of chemotactic behavior has been well studied in *Escherichia coli* and *Salmonella typhimurium* (22, 25, 31). Chemotaxis provides a means for bacteria to respond to chemical changes in the environment by using specific chemoreceptors (2, 3, 5, 6, 15, 16) and for the transduction of this information to the motor system of the bacterium (1, 21). The binding of a chemical to a specific chemoreceptor produces a chemical-chemoreceptor complex which in turn may bind to a specific class of membrane-bound methyl-accepting chemotaxis proteins, which are subsequently methylated (14, 19, 20, 32, 33, 36). Chemoreceptor methylation reactions appear to be a means by which receptor sensitivity is tuned to environmental changes (4, 35, 37). Bacteria sense a temporal change in either attractant (2) or repellent (39) concentrations and as a result move in a three-dimensional random walk composed of alternating tumbles and smooth projectors (7, 8, 26). Chemotactic migration therefore results from biasing the random walk in the preferred direction.

In the present article we describe for the first time chemotaxis in *A. vinelandii*. Using a quantitative tumble frequency assay and agar columns, we found *A. vinelandii* to exhibit chemotaxis for amino sugars, hexoses, hexitols, pentoses, penitols, trioses, and disaccharides. Our results indicate *A. vinelandii* uses a temporal sensing mechanism for chemotaxis.

MATERIALS AND METHODS

Media and strain maintenance. *A. vinelandii* OP was cultured with modified Burk's media (BM) as described previously (11, 27, 38). Cultures were routinely grown at 28°C with aeration. Solid BM plates contained 1.5% Difco agar and 2% of the appropriate carbon source. Burk's buffer (BB), pH 7.0, contained all the components of BM except for the carbon source. Chemicals used in this study were all reagent grade and commercially available.

Preparation of cultures for chemotactic studies. Cells assayed in chemotactic agar columns were pregrown for 12 to 14 h in BM-sucrose and subcultured (0.5% inoculum) in BM containing the appropriate carbon source. The cells were harvested by centrifugation (6,000 × *g*) at room temperature for 10 min and washed with 25 ml of BB. The washed cells were centrifuged, and the cell pellet was gently resuspended in 0.13% agar to an optical density of 0.8 at 590 nm (approximately 10^8 cells per ml). This bacterial agar suspension was used to inoculate chemotactic agar columns.

Cells used in temporal gradient studies were cultured as described above, filtered (Gelman GA-6 filter), and washed with 2 ml of BB. The culture turbidity was adjusted to 15 Klett units (filter no. 66). Test compounds were added at the appropriate concentration, an 80-μl aliquot of the suspension was transferred to a chamber as described by Spudich and Koshland (34), and photomicrographs were taken. Alternatively, cells were filtered through a Gelman GA-6 filter, washed with 2 ml of BB, resuspended in 25 ml of BB, and refiltered. These cells were resuspended to 80 Klett units (filter no. 66), and 0.9 ml of the washed cells was mixed with 0.1 ml of the test chemicals at the appropriate concentration and transferred to the chemotactic chamber.

Preparation and inoculation of chemotactic agar columns. Chemotactic agar columns consisted of 12.5-cm lengths of 8-mm (outside diameter) soft glass tubing fitted with serum vial stoppers. Sterile columns were filled with 3.2 ml of sterile 0.13% Difco agar in BB containing 30 mM NH_4Cl as a nitrogen source and the specific compound to be tested. The columns were refrigerated for 10 to 15 min and then overlaid with 100 μl of a bacterial agar suspension (prepared as described above) containing approximately 10^8 cells per ml. The agar columns were equilibrated at room temperature for 10 min, after which the interface between the bacterial overlay and the agar was marked to serve as a reference point for measuring chemotactic migration. Chemotactic migration was monitored at 28°C. The chemotactic band of cells was removed from chemotactic agar columns with a plunger.

Temporal gradient apparatus. The temporal gradient apparatus consisted of an American Optical series 20 Advanced Microstar microscope equipped for dark-field photomicroscopy. A mechanical strobe made from a black,

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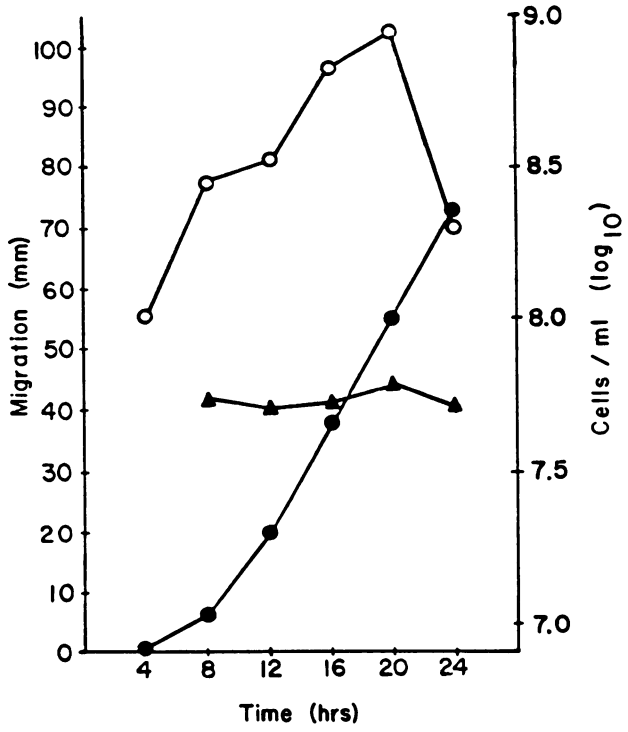

FIG. 1. Chemotactic migration of *A. vinelandii* in agar columns. Cultures pregrown on BM-glucose were overlaid onto agar columns containing 10 mM glucose, as described in Materials and Methods. The distance migrated by the band of cells was recorded at the indicated time (●). Viable counts of the bacteria in the band (▲) and overlay (○) were made by plating appropriate dilutions in BB and plating cells on BM-sucrose. Plates were incubated for 36 h at 28°C.

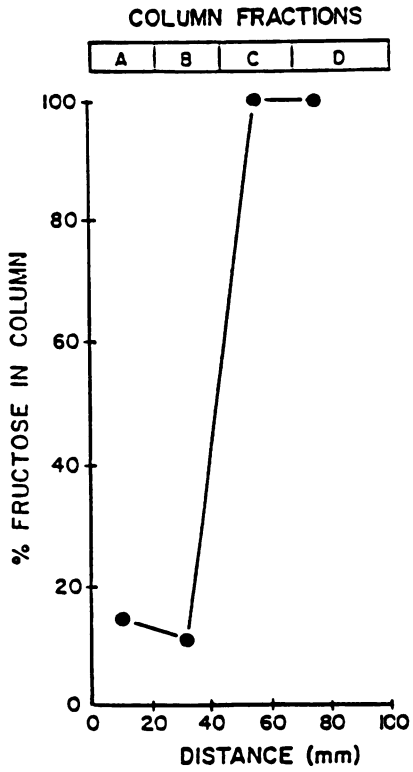

FIG. 2. Generation of a chemical gradient during chemotactic migration. Bacteria were prepared as described in Materials and Methods. Cells were loaded onto a column containing 1 mM fructose. A chemotactic band of cells was allowed to migrate 33 mm down the agar column, which was later fractioned into four sections: A, B, C, and D. Each fraction was reassayed for fructose by a modified resorcinol-thiourea-hydrochloric acid assay. The chemotactic band of cells was located between fractions B and C. The percent fructose remaining in each fraction was plotted as a function of the distance cells migrated in the agar column.

slotted cardboard disk was mounted on a Cole-Parmer Micro V magnetic stirrer. The disk was 10 in. (1 in. = 2.54 cm) in diameter and contained a 1-in.-wide slot cut for 180° on its perimeter. This disk was placed between the light source and the condenser of the microscope. Photomicrographs were made by using Kodak recording film 2475 with a 1-s exposure strobed at 8 flashes per s. On multiple photomicrographic exposure bacteria appeared as tracks, each track representing the path generated by a single motile bacterium. The track index is calculated as the ratio of the number of tracks observed for a test sample of motile cells to the number of tracks of the control. The velocity index is expressed as the ratio of the average velocity of motile bacteria of a test sample to the average velocity of motile bacteria of the control. These studies were performed at room temperature and pH 7.

Assay for fructose. Fructose utilization by *A. vinelandii* during chemotactic migration in a fructose (1 mM) agar column was determined by using the modified resorcinol-thiourea-hydrochloric acid assay of Nakamura (30).

RESULTS

Chemotactic response of *A. vinelandii*. The agar column technique was developed as a rapid means to assess *A. vinelandii* chemotactic behavior. Over the course of an experiment cells migrated in the column as a single band (Fig. 1). The number of cells in the band remained at around 5×10^7 per ml. The number of cells in the agar overlay gradually increased during the first 20 h of incubation and then decreased. *A. vinelandii* cells migrating in a fructose agar column created a chemical gradient (Fig. 2). Similar results were obtained when cells were overlaid onto glucose-chemotactic agar columns. *A. vinelandii* did not migrate, however, in agar columns containing the glucose analog α-methyl-D-glucoside of 2-deoxy-D-glucose. Metabolic inhibitors such as sodium azide or cyanide also inhibited chemotactic migration.

In order to assess the chemotactic response of *A. vinelandii* independent of metabolism, we used a quantitative tumble frequency assay. The response of bacteria subjected to sudden changes in concentrations of test compounds was recorded photographically. (See inserts of Fig. 4A and B for illustrations of the typical swimming behavior observed for motile *A. vinelandii*.) A culture of motile cells contained some bacteria which swam smoothly, producing runs or tracks, whereas other bacteria in the culture failed to demonstrate motility and appeared as splotches of light. These splotches resulted from bacteria which were tumbling and as such produced successive images near the same position. Photomicrographs resulting from a 10-s exposure showed that motility tracks of some cells varied with respect to path lengths (photomicrographs not shown). Some cells in the same focal plane produced longer tracks than others. This suggested that individual cells of a culture were capable of

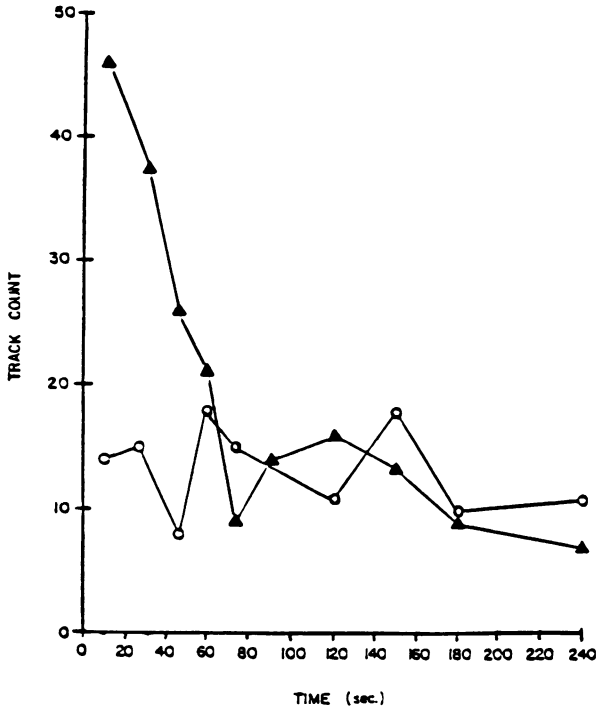

FIG. 3. Temporal response of *A. vinelandii* to glucose. Cells were grown on BM-sucrose with ammonium acetate. Samples were washed via filtration and resuspended into BS–10 mM glucose (▲) or BS without carbon source (○). Each sample was monitored for 4 min, and the results were analyzed as track count per second.

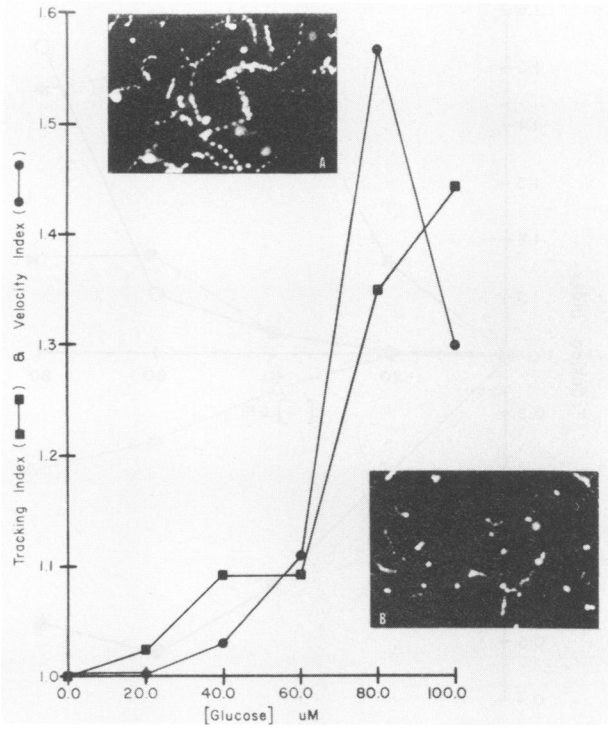

FIG. 4. *A. vinelandii* response curve to glucose. Cells were grown on BM-sucrose with ammonium acetate. Samples were prepared by the Swinnex wash procedure and resuspended into BM at various glucose concentrations. Photomicrographs were made within the first minute. Results were analyzed by both tracking index and velocity index methods. Inserts A and B are photomicrographs taken at 80 and 20 μM glucose, respectively.

traveling at different speeds. Several chemical and physical parameters which affected motility and chemotaxis of *A. vinelandii* were identified. The pH optimum for motility was between pH 7.0 and 8.0. Oxygen was absolutely required for motility, and chemotactic migration for all compounds tested was optimum between 25 and 37°C.

Response and recovery times of chemotactic cells. The temporal response of *A. vinelandii* is shown in Fig. 3. *A. vinelandii* cells initially tumbled constantly, and very few smooth tracks were observed. However, after exposure (t = 0.5 min) to a sudden increase in attractant concentration, e.g., glucose, 0.0 to 10 mM, essentially all the bacteria swim smoothly. In the presence of 10 mM glucose the tumbling frequency was suppressed within 15 s and the number of motile cells increased (Fig. 3). Cells returned to their original tumbling frequency 75 s after exposure to a sudden increase in the stimulus. Cells not exposed to a sudden increase in glucose concentrations did not exhibit the initial suppression of tumbling and therefore failed to exhibit an increase in the number of tracks.

***A. vinelandii* exhibits a temporal chemotactic sensing system.** A temporal gradient technique was used to study the chemotactic response of *A. vinelandii* to various compounds. Figure 4 shows the chemotactic response curve of *A. vinelandii* to glucose. The threshold concentration of glucose was 20 μM. Increasing glucose concentrations caused a corresponding increase in the tracking index of motile cells. This resulted from the suppression of tumbling and subsequent smooth swimming. Individual cells tumbled more frequently at glucose concentrations below the threshold (Fig. 4B). An increase in the number of motility tracks resulted when cells were exposed to higher glucose concentrations, e.g., 80 μM (Fig. 4A).

Not only was there an increase in the number of tracks projected by motile cells, but the path length of the individual tracks also increased (Fig. 4A). The increase in the lengths of tracks suggests that the speed of individual cells had increased as a function of attractant concentration. There was a decrease in the speed of cells at glucose concentrations above 80 μM. However, the overall chemotactic response of the cells to glucose at these concentrations was not impaired. The speed of attractant-stimulated cells was 37 body lengths per s or 74 μm/s.

The response of *A. vinelandii* to gluconate, mannose, galactose, xylose, and glucose is shown in Fig. 5. An increase in the number of motility tracks in response to mannose, gluconate, and glucose was observed. The threshold for these compounds was 40, 20, and 60 μM, respectively.

Attractants are known to bind to bacterial chemoreceptors and initiate a signal that promotes smooth swimming, whereas chemicals that act as repellents tend to increase tumbling. The response of *A. vinelandii* to gluconate, mannose, and glucose (Fig. 5) suggests that compounds act as attractants, since they suppressed tumbling and increased smooth swimming. Galactose and xylose acted as repellents and as such tended to increase tumbling and to decrease the tracking index (Fig. 5). Xylose increased tumbling more effectively at lower concentrations than galactose.

Survey of the chemotactic response of *A. vinelandii* to various carbohydrates. The chemotactic response of *A. vine-*

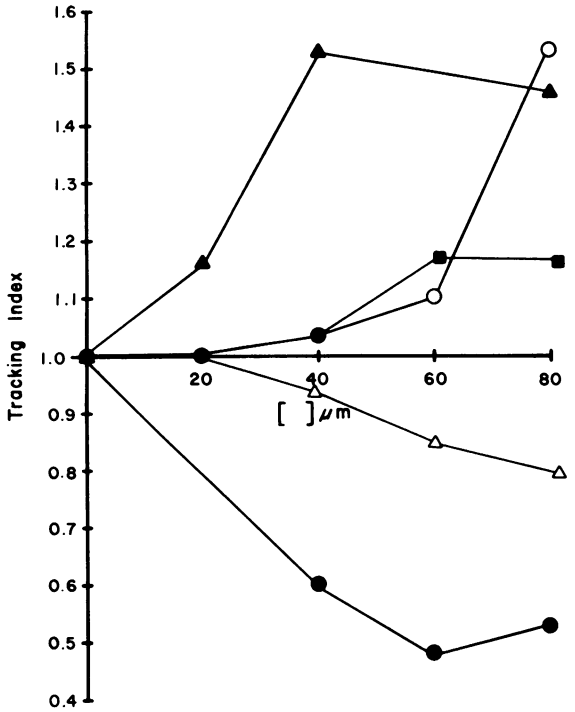

FIG. 5. *A. vinelandii* response curve to glucose, gluconate, galactose, mannose, and xylose. Cells used for glucose, gluconate, and galactose taxis were grown on the respective carbon sources. Cells for xylose and mannose taxis were grown on sucrose media. Samples were prepared by the batch wash procedure and resuspended into BS-glucose (○), BS-gluconate (▲), BS-galactose (△), BS-mannose (■), and BS-xylose (●) at various concentrations. Photomicrographs made within the first minute were analyzed by the tracking index method.

landii to various carbohydrates is shown in Table 1. These compounds were placed into one of three classes. Class I compounds elicited a positive chemotactic response as detected by the agar column or tracking chemotactic assay. All of these compounds can serve as a sole carbon source for the growth of *A. vinelandii*. Class II compounds include trehalose and glyceraldehyde. These compounds did not elicit a chemotactic response in agar columns or in the tracking assay. Class III compounds consist of those chemicals which elicited only a chemotactic response as determined by using the tracking assay.

TABLE 1. Chemotactic response[a] of *A. vinelandii* to various compounds

Compound class[b]	Response		
	Growth	Agar disk	Tracking
I	+	+	+
II	−	−	−
III	−	−	+

[a] Chemotactic response was determined by the agar disk and temporal gradient methods. Cultures used in the agar disk method were grown on BM-sucrose. Tracking results were obtained from cultures prepared by the batch wash procedure which were tested for a response to the various compounds at 10 mM concentrations within the first minute of exposure.

[b] Class I compounds include maltose, arabitol, gluconate, melibiose, xylitol, mannitol, fructose, sorbitol, sucrose, and glycerol. Class II compounds are trehalose and glyceraldehyde. Class III compounds are glucoronate, 2-deoxy-D-ribose, ribose, 2-deoxy-galactose, mannose, ribitol, glucose-6-phosphate, *N*-acetyl-D-glucosamine, arabinose, 2-deoxy-D-glucose, α-methyl-D-glucoside, and *N*-acetyl-D-mannosamine.

Neither class II nor class III compounds served as growth substrates for *A. vinelandii*.

A. vinelandii demonstrated a positive chemotactic response to hexoses (e.g., glucose, fructose, and mannose), hexitols (e.g., sorbitol and mannitol), pentoses (e.g., arabinose and ribose), and pentitols (e.g., ribitol, xylitol, and arabitol). *A. vinelandii* also exhibited a chemotactic response to glycerol and the amino sugars (e.g., *N*-acetyl-D-mannosamine and *N*-acetyl-D-glucosamine) as well as to disaccharides (e.g., maltose, melibiose, and sucrose). Analogs of glucose (α-methyl-D-glucoside and 2-deoxy-D-glucose), ribose (i.e., 2-deoxy-D-ribose), and galactose (i.e., 2-deoxy-galactose) elicited a positive chemotactic response. None of these compounds served as growth substrates for *A. vinelandii*.

Chemotaxis of *A. vinelandii* for some compounds was inducible. Cells pregrown on glucose or fructose were induced for glucose and fructose chemotaxis, respectively. However, these cells failed to exhibit a chemotactic response to either mannitol or xylitol. Mannitol-pregrown cells, however, were induced for both mannitol and xylitol chemotaxis. These results suggest cellular components essential for chemotaxis of these compounds are inducible in *A. vinelandii*.

DISCUSSION

Much of the genetics, biochemistry, and bioenergetics of bacterial chemotaxis has been elucidated from studies of *E. coli*, *Bacillus subtilis*, *Streptococcus lactis*, *S. typhimurium* (21, 28), *Vibrio alginolyticus* (9, 10, 12), and *Spirochaeta aurantia* (13). We report here the first studies of chemotaxis in *A. vinelandii*.

Our results indicate that *A. vinelandii* uses a temporal sensing mechanism for chemotaxis. Motile cells suppressed tumbling in the presence of attractants. The tumbling frequency of these cells was suppressed within 15 s in the presence of glucose. The time required for the tumbling frequency of the population of bacteria to return to prestimulus levels was 75 s, which is very close to the recovery time for *E. coli* (21).

The ability of *A. vinelandii* to respond to chemoattractants and repellents was demonstrated by the modulation of its tumbling frequency in response to various compounds tested in this study. When cells encountered an attractant there was a decrease in the tumbling frequency of cells, whereas repellents increased the tumbling frequency (8, 20, 24, 26). Attractants elicit a counterclockwise rotation of flagella which produces smooth swimming, and repellents elicit a clockwise rotation, causing the cell to tumble (24). We found that *A. vinelandii* exhibits a positive chemotactic response for glucoronate, ribose, glucose, *N*-acetyl-D-glucosamine, arabinose, arabitol, glycerol, sucrose, fructose, sorbitol, xylitol, melibiose, and maltose. Xylose and galactose, at low concentrations, were found to increase the frequency of tumbling in *A. vinelandii*. The genetics and biochemistry of many of these chemotactic systems have been delineated in *E. coli* and *S. typhimurium*. The speeds of many bacteria have been calculated by using tracking photomicroscopy (31, 40). The mean velocities of *Bacillus licheniformis*, *E. coli*, and *Sporosarcina ureae* were reported as 21.4, 16.5, and 28.1 μm/s, respectively. *Pseudomonas aeruginosa* and *Thiospirillum jenense* have mean velocities of 55.8 and 86.5

μm/s which, respectively, correspond to movement of 37 and 2 body lengths per s. Our results show *A. vinelandii* moves at speeds of 74 μm/s and 37 body lengths per s. This is about four to five times faster than *E. coli*. How this is accomplished is presently not known. In prokaryotes motility has been shown to be driven by a proton motive force (12, 13, 23, 24, 29) or a sodium motive force (9, 10, 17, 18).

The present study provides a basis for future investigations of the genetic and biochemical features of chemotaxis in *A. vinelandii*.

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