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Mechanisms of Microbial Movement in Subsurface Materials

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The biological factors important in the penetration of *Escherichia coli* through anaerobic, nutrient-saturated, Ottawa sand-packed cores were studied under static conditions. In cores saturated with galactose-peptone medium, motile strains of *E. coli* penetrated four times faster than mutants defective only in flagellar synthesis. Motile, nonchemotactic mutants penetrated the cores faster than did the chemotactic parental strain. This, plus the fact that a chemotactic galactose mutant penetrated cores saturated with peptone medium at the same rate with or without a galactose gradient, indicates that chemotaxis may not be required for bacterial penetration through unconsolidated porous media. The effect of gas production on bacterial penetration was studied by using motile and nonmotile *E. coli* strains together with their respective isogenic non-gas-producing mutants. No differences were observed between the penetration rates of the two motile strains through cores saturated with peptone medium with or without galactose. However, penetration of both nonmotile strains was detected only with galactose. The nonmotile, gas-producing strain penetrated cores saturated with galactose-peptone medium five to six times faster than did the nonmotile, non-gas-producing mutant, which indicates that gas production is an important mechanism for the movement of nonmotile bacteria through unconsolidated porous media. For motile strains, the penetration rate decreased with increasing galactose concentrations in the core and with decreasing inoculum sizes. Also, motile strains with the faster growth rates had faster penetration rates. These results imply that, for motile bacteria, the penetration rate is regulated by the in situ bacterial growth rate. A sigmoidal relationship was found between the specific growth rates of all of the motile bacteria used in this study and the penetration rates through cores saturated with galactose-peptone medium.

Efficient and economical treatment of contaminated subterranean formations by in situ bioremediation processes is currently under investigation. These processes involve injection of specific nutrients into the subsurface to stimulate bacterial populations involved in transformation and/or mineralization of potentially toxic compounds (8, 17). The success of such processes depends on a fundamental understanding of the factors that influence the propogation, growth, and movement of bacteria within porous subsurface formations.

In flow systems, bacterial transport through porous media has been largely described as a function of the pore entrance size (20, 21) and, hence, adequately simulated by a filtration model (6, 9, 18). Under static conditions, however, a relationship between the bacterial penetration rate and the subsurface permeability has recently been demonstrated (11). Furthermore, a penetration mechanism based entirely on motility has been derived which successfully predicted penetration times for a motile *Bacillus* strain growing within Berea sandstone (11). Although motility was an important mechanism for bacterial penetration in this case, it was unknown whether other characteristics of motility, i.e., random or nonrandom movement, were also important. Directional or nonrandom movement is usually thought to occur only in chemotactic strains, since nonchemotactic mutants are known to move randomly (2). Nonmotile bacteria have also been shown to penetrate consolidated sandstone cores (11), although their penetration rates were considerably slower than that found for motile bacteria. The mechanisms by which nonmotile bacteria penetrate porous media are not known.

The aims of this study were to determine the biological factors which govern the movement of motile and nonmotile bacteria through unconsolidated porous media. To answer these questions, we compared the penetration times of *Escherichia coli* mutants defective in chemotaxis, flagellar synthesis, and gas production under anaerobic conditions.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The characteristics of the nine *E. coli* strains used in this study are listed in Table 1. The isogenic strains *E. coli* RP437 (a chemotactically wild-type strain), RP5232 (a Δ*cheY* mutant), RP1616 (a Δ*cheZ* mutant), and RP2912 (a nonmotile strain) were kindly provided by J. S. Parkinson. Strain RP487 (a chemotactic Δ*gal* mutant) was a gift from J. Adler. Motile strain RW262 and nonmotile strain MC4100, together with their respective isogenic mutants FM2629 and FM909, were kindly supplied by F. Zimain. The strains were grown in motility growth medium (MGM) which contained the following components (wt/vol): 0.45% galactose, 0.5% peptone, 0.025% methionine, 0.5% NaCl, 0.25% K_2HPO_4, 0.2% $(NH_4)_2SO_4$, 0.025% $MgSO_4$, and 0.00005% $Fe_2(SO_4)_3$. The pH of the medium was 7.0, and 0.1 mM EDTA was added to prevent inhibition of motility by heavy metal ions (1). The strains were maintained on slants containing MGM with 2% (wt/vol) agar. After growth, the slants were stored at 4°C until used as inocula. The motility of each strain was checked by inoculating MIO broth (Difco Laboratories). The chemotactic response of each strain toward galactose was measured by the method described by Adler et al. (1, 3).

Anaerobic media and solutions were prepared and used as described previously (4). The gas phase of all anaerobically prepared media and solutions was that of the anaerobic chamber, about 1 to 5% H_2 with the balance being N_2. The specific growth rate of each strain under anaerobic conditions at 23°C was determined by transferring 0.1 ml of an aerobically grown culture into aluminum-seal culture tubes

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TABLE 1. Characteristics of the *E. coli* strains used

Strain	Genotype	Phenotype	Specific growth rate[a] (h^{-1})
Motile			
RP437	*thr*(Am) *leuB6 his-4 metF159*(Am)	Chemotactic wild type	0.27 ± 0.02
RP487	*gal* Δ*eda-50 metF159*(Am) Su^-	Chemotactic galactose mutant	0.2 ± 0.025
RP5232	Δ(*cheY*)m60-21	Nontumbling, smooth swimmer	0.5 ± 0.030
RP1616	Δ(*cheZ*)m67-25	Excessive tumbling, tumbly	0.35 ± 0.026
RW262	F^+ *mel-1 tanA supF58* λ^-	Gas producer	0.66 ± 0.040
FM2629	Same as RW262; Δ*fdhf*	Non-gas producer	0.57 ± 0.020
Nonmotile			
RP2912	Δ(*motA-tar*)*2211*	Flagellated	0.37 ± 0.036
MC4100	F^- *araD139* Δ(*lac*)*U169 ptsF25 deoC1 relA1 flb* B5301 *rpsL150* λ^-	Gas-producer	0.58 ± 0.026
FM909	Same as MC4100; Δ*fdhf*	Non-gas producer	0.46 ± 0.035

[a] Specific growth rates were determined anaerobically in MGM. Values represent means ± standard deviations of triplicate cultures.

(18 by 150 mm) containing 10 ml of anaerobically prepared MGM. Culture growth was measured as the change in A_{600}, and the specific growth rates were determined as described previously (14).

Inocula used for core experiments were prepared with a minimum number of transfers in liquid medium. Cells from a slant culture were inoculated into 100 ml of MGM and grown aerobically at 35°C until the mid-exponential phase of growth was reached (about 10^7 cells per ml). The culture was taken inside the anaerobic chamber, where the rest of the manipulations were performed. For each core inoculated, 1 ml of the culture was aseptically transferred to a sterile microcentrifuge tube and the cells were washed twice by centrifugation at 10,000 × *g* for 3 min and suspension of the cell pellet in anaerobically prepared motility growth buffer. Motility growth buffer contained 0.025% (wt/vol) $MgSO_4$, 0.1 mM EDTA, and 100 mM phosphate buffer (pH 7.0). The final cell pellet was suspended in 250 μl of motility growth buffer.

Viable cell counts were performed by serially diluting the sample in motility growth buffer and inoculating duplicate plates of MGM with 1.5% (wt/vol) agar. Colonies were counted after aerobic incubation of the plates for 24 to 48 h at 35°C.

Core experiments. Commercially available sand (100- to 200-mesh size) was suspended in concentrated HCl overnight to remove organic contaminants, rinsed with distilled water until the sand slurry had a pH of 7.0, and then dried at 125°C overnight. The core chambers were constructed by fusing together the top ends of two aluminum seal borosilicate culture tubes (18 by 150 mm) as shown in Fig. 1. Except for experiments in which the relationship between penetration and core length was studied, in which the core length ranged from 5 to 14 cm, 8-cm-long cores were used. The cores were prepared inside the anaerobic chamber (4). One end of the core chamber was fitted with a black rubber serum stopper. The core chamber was placed in a vertical position in a test tube rack and filled with anaerobically prepared MGM. For some experiments, modified MGM without galactose and/or peptone or with different galactose concentrations was used to saturate the cores. The test tube rack was placed in a sonicated water bath, and sand was added to fill each core. Excess medium in the core chamber was allowed to overflow into the water bath. After each chamber was filled with sand, sonication was continued for 10 min to release any gas bubbles trapped inside the cores. The open end of each core was then fitted with a grey butyl rubber serum stopper which had a filter plug insert to aid in uniform dispersion of the bacteria. To prevent liquid from escaping the core during sterilization, both ends of the core were crimp sealed and immersed inside a 2-liter glass bottle containing the same medium which saturated the inside of the core. To easily remove the cores from the bottle after autoclaving, each core was connected to the stopper of the bottle via rubber tubing. The bottle was stoppered, removed from the anaerobic chamber, and autoclaved at 121°C for 20 min. After cooling to room temperature, the cores were removed from the bottle inside the anaerobic chamber just before use.

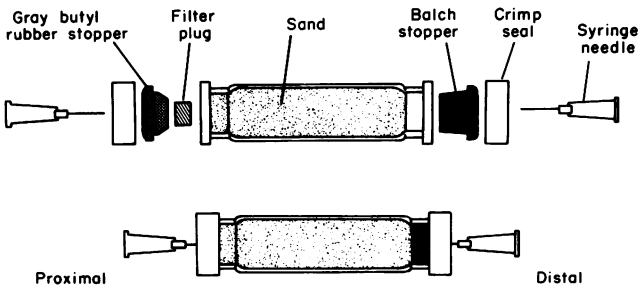

FIG. 1. Components of the core system used to study bacterial penetration through sand. Cores were inoculated at the proximal end, and samples were taken at the distal end. Core length, 8 cm; core diameter, 2.01 cm; filter plug, 0.5 by 0.3 cm.

The cores were placed in a horizontal position inside the anaerobic chamber and inoculated as follows. The anaerobically prepared cell suspension (about 10^7 cells in 250 μl) was drawn into a 1-ml syringe fitted with a 22-gauge needle and then inserted through the grey butyl rubber stopper without disturbing the contents of the core. The tip of the needle had a small drop of liquid to ensure that an air bubble which would prevent movement of bacteria into the core did not form in the needle during inoculation. A syringe fitted with a 22-gauge needle filled with sterile MGM was inserted through the other (distal) side of the core in an identical manner. The cores were incubated inside the anaerobic chamber at room temperature. At hourly intervals, the presence of viable cells in the distal syringe was determined by removing the syringe and aseptically inoculating 0.1 ml of the syringe contents into a sterile tube containing 3 ml of MGM. The syringe was then carefully reinserted into the distal side of the core by avoiding the introduction of gas bubbles or mixing of the core contents. New sterile syringes

TABLE 2. Penetration rates of motile and nonmotile *E. coli* strains through sand-packed cores containing MGM with or without galactose

Strain	Penetration rate (cm/h)[a]	
	With galactose	Without galactose
Motile		
RP437	0.073 ± 0.03	ND[b]
RP5232	0.28 ± 0.02	0.31 ± 0.042
RP1616	0.16 ± 0.007	0.24 ± 0.027
Nonmotile (RP2912)	0.086 ± 0.003	ND

[a] Values represent means ± standard deviations of triplicate cultures through 8-cm-long cores. The core volume was 25.4 cm^3, and the liquid volume was 7 ml.
[b] ND, Not detected after 672 h; penetration rate, less than 0.01 cm/h.

filled with MGM were used as needed. Tubes inoculated with the contents of the distal syringe were incubated aerobically at 35°C and examined for the presence or absence of growth after 48 h. Tubes with growth were checked for purity microscopically and by plating onto MGM with 1.5% agar.

The penetration or breakthrough time (t_p) is the time (in hours) from inoculation of the core until a sample of the distal syringe contents had viable cells. The penetration rate (r_p) (in centimeters per hour) is the length of the core divided by the penetration time and represents average rates, not instantaneous rates. Unless specified otherwise, triplicate cores were used for each treatment variable. After each experiment, the permeability (k) and liquid volume (pore volume) of each core were determined as described previously (11).

RESULTS

Effect of motility on bacterial penetration. The average penetration rates of four isogenic *E. coli* strains through unconsolidated sand cores under static conditions are shown in Table 2. Both with and without galactose, the penetration rates of chemotactic wild-type strain RP437 resembled those of the nonmotile mutant RP2912. Significant and independently faster rates of penetration (as determined by the Ryan-Eindt-Gabriel-Welsch general linear model at $P < 0.05$ [16]) were found for the excessively tumbling mutant RP1616 and the smoothly swimming bacterium RP5232. Without galactose, only the two nonchemotactic motile strains were able to penetrate the cores with rates exceeding 0.01 cm/h. Furthermore, the rates of penetation by these strains were slightly higher when galactose was removed from both the core and the distal syringe (Table 2).

Importance of chemotaxis. The fact that both nonchemotactic strains, RP1616 and RP5232, had faster penetration rates than did wild-type strain RP437 (Table 2) indicated that chemotaxis was not required for penetration through the core under conditions of nutrient saturation. In the subsurface, however, nutrients may be localized, and under quiescent conditions, effective biotransformation may occur only if bacteria are able to move toward nutrients by a mechanism such as chemotaxis. To test this possibility, the substrate location was varied by altering the composition of the medium inside the core and the distal syringe. To eliminate the possible effect of substrate utilization, as shown by strain RP437, and therefore to determine whether a chemotactic strain is able to reach a localized substrate, we used strain RP487, which is capable of chemotaxis toward but not metabolism of galactose (Table 3). When both the core and the distal syringe contained MGM, the penetration rate was three times slower than when galactose was deleted from the medium in both the core and the distal syringe. However, when galactose was removed from the medium in the core but remained in the distal syringe, the penetration rate was similar to that of the condition without galactose. Penetration of RP487 was not observed within 336 h (r_p, <0.024 cm/h) when both galactose and peptone or galactose, peptone, and methionine were deleted from the core but remained within the distal syringe. It is interesting that a chemotactic bacterium which was only 8 cm from the source of a fermentable substrate was not able to reach the substrate, even in the presence of methionine, which is required by this strain for chemotaxis. Furthermore, when galactose was present in the proximal end of the core, penetration of RP487 was not observed, even though the core contained peptone and methionine. These results suggest that translational movement of bacteria decreases as a function of substrate localization.

TABLE 3. Penetration rates of *E. coli* RP487 through sand-packed cores with or without nutrient gradients

Medium component deleted[a]		Penetration rate[b] (cm/h)
Core	Distal syringe	
None	None	0.032 ± 0.005
Galactose	Galactose	0.10 ± 0.006
Galactose	None	0.09 ± 0.005
Galactose and peptone	None	<0.024
Galactose, peptone, methionine	None	<0.024
Galactose[c]	Galactose	<0.024

[a] The composition of MGM in the core or distal syringe was altered as indicated to establish substrate localization. The distal syringe contained 0.1 ml of the indicated medium.
[b] Penetration rates are average results (± standard deviations) from two core experiments.
[c] Galactose was located only at the proximal end of the core by addition of an extra syringe containing 0.1 ml of MGM.

Effect of gas production on penetration. Large amounts of gas were observed inside cores saturated with MGM. To determine whether gas production was important for bacterial penetration through the sand-packed cores, the abilities of motile (RW262) and nonmotile (MC4100) *E. coli* strains and their isogenic, non-gas-producing derivative strains (FM2629 and FM909), which lack formate dehydrogenase, to penetrate through sand-packed cores were compared (Table 4). Cores saturated with MGM with or without galactose were used. The penetration rates of the two motile strains, RW262 and FM2629, through cores containing medium with or without galactose were similar. However, the importance of gas production for penetration of the nonmotile strains was clearly demonstrated. In cores saturated with MGM, the penetration rate of strain MC4100 was about five times faster than that of non-gas-producing strain FM909. Furthermore, when galactose was deleted from the medium used to saturate the cores, i.e., when gas production was minimal, penetration of strain FM909 was not observed after 672 h (r_p, <0.01 cm/h).

Importance of substrate concentration. The effect of the substrate concentration on the bacterial penetration rate through the cores was investigated by using the nonchemotactic strain RP1616 (Fig. 2). Cores were prepared as described above, except that the concentration of galactose inside the core and in the distal syringe ranged from 0 to 10 g/liter. When the galactose concentration was increased, the

TABLE 4. Penetration rates for motile and nonmotile wild-type *E. coli* and isogenic formate dehydrogenase mutants under different nutrient conditions

Strain	Phenotype	Galactose	Penetration rate[a] (cm/h)
RW262	Motile gas producer	−	0.50 ± 0.052
		+	0.42 ± 0.03
RM2629	Motile non-gas producer	−	0.50 ± 0.052
		+	0.38 ± 0.06
MC4100	Nonmotile gas producer	−	0.031 ± 0.001
		+	0.085 ± 0.004
FM909	Nonmotile non-gas producer	−	ND[b]
		+	0.015 ± 0.001

[a] Values represent means ± standard deviations of triplicate cultures through 8-cm-long cores. The core volume was 25.4 cm^3, and the liquid volume was 7 ml.

[b] ND, Not detected after 672 h; penetration rate, less than 0.01 cm/h.

penetration rate decreased linearly from 0.22 cm/h in cores without galactose added to 0.13 cm/h in cores with 10 g of galactose per liter. The change in the penetration rate cannot be attributed to differing growth rates, since the growth rates of RP1616 at all of the substrate concentrations used in the experiment were identical (data not shown). A decrease in the penetration rate of RP5232 was also observed when the galactose concentration inside the core was increased (data not shown).

If bacterial movement within unconsolidated porous media is restricted by the concentration of the available substrate, then altering the size of the initial bacterial population should influence the rate of movement by affecting the rate of substrate utilization. This hypothesis was tested by using nonchemotactic strain RP5232 to preclude any ambiguity which may result from chemotaxis. Penetration rates increased with an exponential increase in cell concentration (Fig. 3). These data imply that bacterial penetration was a function of cell concentration. It may also indicate that there was some finite retention, i.e., absorption, of cells within the core that may be overcome only by increasing cell numbers before effective penetration can occur.

Relationship to pore volume and length. A previous study (11) using consolidated sandstone showed that growth inside

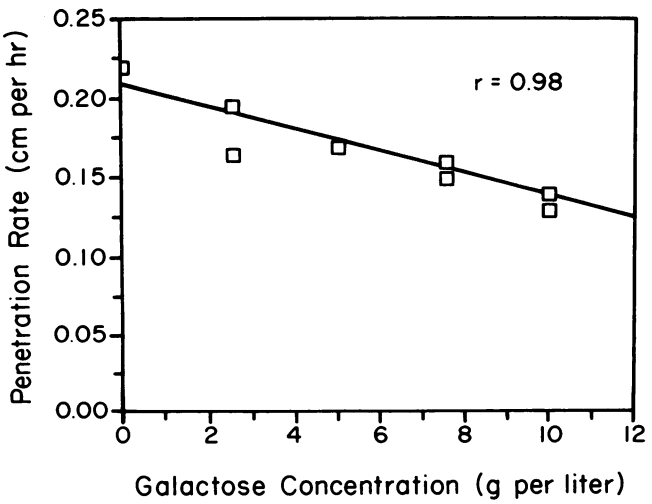

FIG. 2. Effect of galactose concentration on the penetration rate of *E. coli* RP1616. The solid line is the relationship predicted by regression as follows: penetration rate = 0.21 + (0.0007 × galactose concentration).

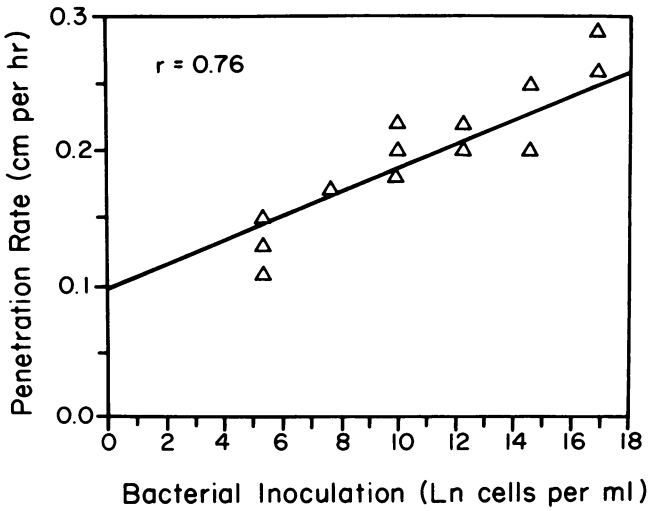

FIG. 3. Effect of initial cell concentration on the penetration rate of *E. coli* RP5232. The solid line is the realtionship predicted by regression as follows: penetration rate = 0.098 + (0.009 × bacterial inoculation).

the core occurred in a restricted manner, as demonstrated by the dependence of penetration time on the pore volume of the sandstone. If growth were perceived to occur in an unrestricted manner, then the penetration time would be independent of the actual liquid volume within a core, i.e., resembling growth in a flask of medium. With sandstone cores with permeabilities ranging from 55 to 520 millidarcys (1 darcy = 1 μm^2), it was found that the penetration time increased linearly with pore volume for cores with permeabilities greater than 100 millidarcys, whereas for cores with permeabilities lower than 100 millidarcys, the penetration time increased exponentially (11). To determine the relationship between penetration and pore volume in our unconsolidated sand-packed systems, the penetration times of nonchemotactic strain RP5252 through cores of different lengths ranging from 5 to 14 cm were determined. In unconsolidated sand-packed cores which had permeabilities ranging from 4.1 to 11.2 darcys, the penetration time increased linearly with pore volume, with the y intercept close to 0 (Fig. 4A). These data showed that penetration time was not independent of pore volume, and thus growth inside the core did not occur in an unrestricted manner, as it would in a flask. This supposition was confirmed by measuring the penetration times of strains RP487, RP5232, RP1616, and RP2912 through 8-cm-long cores filled with MGM but without sand. Penetration occurred very rapidly, with detection of viable cells in the distal syringe within 1 to 3 h after inoculation, compared with penetration times of 28 to 114 h for these strains through cores containing sand.

Adler and Dahl (2) demonstrated that movement of nonchemotactic bacteria through capillary tubes could be described by an equation for diffusion of a thin layer of cells in a column of liquid, for which the plot of time versus the square of the distance that the fastest cells have moved from the origin (L^2) gives a straight line with a y intercept of 0. Our assay is similar to the frontier assay used by Adler and Dahl (2), in which the penetration time is a function of the time for the fastest cells to penetrate a sand-packed core of known length (11). When the penetration times of RP5232 were plotted as a function of the square of the core length, a curvilinear relationship was obtained (Fig. 4B). When these data were plotted as a function of the length of the core, a

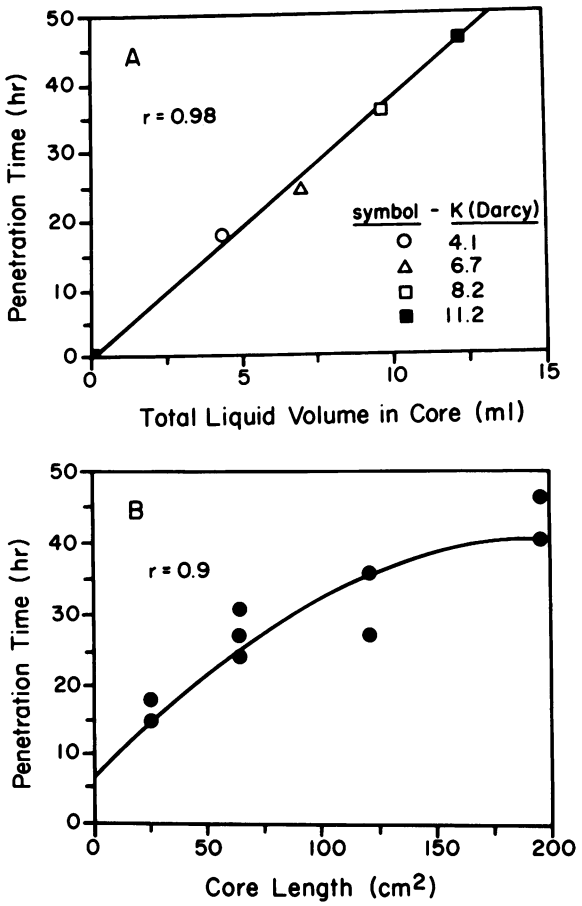

FIG. 4. (A) Relationship between pore volume and penetration time of *E. coli* RP5232. The solid line is the relationship predicted by regression as follows: penetration time = 3.8 · total liquid volume. (B) Relationship between core length squared and penetration time of *E. coli* RP5232. The data were fitted to a second order polynomial curve with the general form $y = a + bx - ce^{4x}$ ($R = 0.95$ when $a = 6.640$, $b = 0.345$, and $c = 8.753$).

straight line with a y intercept of 0 was obtained (data not shown). These analyses showed that the movement of RP5232 through sand-packed cores did not resemble a random process such as diffusion. The relationship between penetration time and the square of the length was indicative of a nonrandom process resembling chemotaxis (2). However, a description of penetration based on chemotaxis can be excluded, since this experiment was conducted with nonchemotactic mutant RP5232.

DISCUSSION

To understand how to manipulate the growth of bacteria in the subsurface, it is important to know the mechanisms which control bacterial penetration. Previously, we showed that the average penetration rate of a motile *Enterobacter aerogenes* strain through Berea sandstone was eight times faster than that of a taxonomically similar, nonmotile enteric bacterium, *Klebsiella pneumoniae* (11). However, because of the metabolic and physiological differences between these two strains, it was not clear whether motility alone could account for the differences in penetration rates. If the penetration rates of all of the strains used in this study are compared, then motile *E. coli* strains penetrated the cores under nutrient-rich conditions (with galactose), on the average, four times faster than did nonmotile strains. However, although this does indicate the importance of motility for bacterial penetration, not all of these strains are isogenic, so other factors are also involved. One important factor seems to be the growth rate of the strain. In general, the strains with the fastest growth rates in MGM under anaerobic conditions were also the strains with the fastest penetration rates through cores saturated with this medium. Interestingly, the two nonchemotactic mutants, RP5232 and RP1616, had faster rates of penetration than the parental strain, RP437 (Table 2). The penetration rate of RP437 was similar to that of its isogenic nonmotile mutant, RP2912. The reason why the chemotactic strains had slower penetration rates may be because they sense a nutrient-rich environment in all three directions and thus grow throughout the entire pore volume of the core. Nonchemotactic strains are unable to sense nutrient gradients, and thus the route of the fastest moving cells may be less tortuous than that of chemotactic cells. Further work is needed to determine the mechanisms by which chemotactic and nonchemotactic strains penetrate. This discussion shows that although the factors which affect bacterial penetration may be manifold, motility and bacterial growth rate seem to be important.

Our results suggest that the mechanisms that control movement of nonmotile bacteria are growth, as indicated by the increase in the penetration rate of MC4100 in the presence of galactose, and, to a larger extent, gas production (Table 4). It is probable that during fermentation, enough gas is generated inside the pores that localized pressure gradients form which act to push nonmotile cells through larger pores. The penetration rate of FM909 under nutrient-rich conditions (with galactose) was much slower than that of MC4100, which differs from FM909 only in its ability to produce gas. Thus, the enzymatic ability to produce gas and the appropriate culture conditions to allow gas production to occur are important factors that determine the rates of penetration of nonmotile strains.

One possible mode of penetration of nonmotile cells through porous medium would be if growth were perceived to occur in a filamentous fashion. An equation which describes this type of process has been described previously (11), in which the penetration time would be as follows:

$$t_p = t_d[\ln (l_x/l_c)/\ln 2] \quad (1)$$

where l_x and l_c are the lengths of the core and the cell, respectively, and t_d is the doubling time (in hours) of the bacterium. This represents the time required for the bacterium to traverse the length of the core. Assuming an average cell length of 3 μm and a doubling time of 2.7 h (Table 1) for strain FM909, the expected penetration time for an 8-cm-long core on the basis of equation 1 would be 31.9 h. The actual average penetration time for FM909 was 513.5 h. Thus, penetration times are much slower than that which would be predicted by a model for filamentous growth, which probably indicates that the actual path taken by the bacterium is much more tortuous than that predicted by equation 1. If the penetration time is a function not only of the core length but also of the pore volume and the cell volume, then an equation that describes the penetration of nonmotile cells without gas production would be follows:

$$t_p = t_d\, l_x\, [\ln (V_x/V_c)/\ln 2] \quad (2)$$

where V_x and V_c are the liquid volume of the core per centimeter and the average cellular volume, respectively.

For an *E. coli* cell with an average dimension of 3 by 0.75 μm (12), the cell volume is given by the following equation (19):

$$V_c = \pi R^2(L - 2/3R) \quad (3)$$

where L is the length and R is the radius of the cell. Therefore, the predicted time required for a single cell with a volume of 1.2 μm^3 to traverse an 8 cm-long sand-packed core containing 7 ml of liquid volume would be 511 h. This is very close to the actual penetration time of 513.5 h for FM909. This indicates that nonmotile cells penetrate the core by growing throughout all of the available pore volume and gas production acts to increase the rate of penetration.

The involvement of chemotaxis in bacterial penetration through subsurface formations has been reported by Yen (22). Our data suggest that for motile cells, chemotaxis is not required for bacterial penetration through unconsolidated systems (Tables 2 and 3). The penetration rates of the motile cells used in our study increased in a curvilinear fashion with the square of the core length (Fig. 4B), suggesting ordered or bandlike movement of cells, as would be found in chemotactic strains (2). However, since a nonchemotactic strain was used in this experiment, the involvement of chemotaxis can be excluded. The ordered or bandlike movement of these nonchemotactic cells may be due to the available substrate concentration and, hence, the specific growth rate of the organism. The penetration rate decreased when the substrate concentration in the core was increased, although the specific growth rate of the strain was unaltered (Fig. 2). Therefore, the rates of substrate utilization and, consequently, the growth rates of individual bacterial species may ultimately determine penetration rates under nutrient-saturated conditions. Movement of bacteria through subsurface formations has been related to the movement of nutrients in seeping wastewater (7). At high nutrient concentrations, nonchemotactic *E. coli* mutants are known to pause for greater periods in the presence of attractants than in the presence of repellents (13). Under conditions in which growth is restricted, as in sand-packed cores, motile bacterial cells would consume practically all of the locally available substrates, thereby creating a substrate gradient. Concomitantly accumulated fermentation end products would act as repellents and decrease the duration of the pauses. Both of these conditions would lead to an increase in cellular movement through the core. This mode of substrate utilization resembles that found in plug flow reactors (15). Thus, bandlike movement through the core would depend not on chemotaxis but on bacterial growth and substrate utilization rates. The fact that motile strains which had markedly different growth rates also had significantly different penetration rates (Tables 2 and 4) supports this conclusion.

The relationship between the penetration rates and the growth rates under nutrient-rich conditions (galactose present) for all of the motile *E. coli* strains used in this study (Fig. 5) can be described by the following sigmoidal relationship:

$$r_p = e^{\alpha\mu}/(1 + e^{\alpha\mu} + \beta\mu^{\delta}) \quad (4)$$

where μ is the specific growth rate (per hour), α is the upper limit of the sigmoidal relationship (estimated from Fig. 5 as 0.5), β represents the midpoint of the sigmoidal curve, and δ is an empirical constant (estimated from Fig. 5 as −2.95). A sigmoidal expression was chosen to represent the relationship between penetration rate and growth rate for several reasons. That an upper limit to this expression exists seems reasonable, since one would expect that physiochemical or other factors would limit the penetration rate of even very fast-growing organisms. Equation 4 predicts that the penetration rate markedly decreases when the specific growth rate of the bacterium decreases below 0.15 to 0.2/h. This seems reasonable, since one would expect some penetration to occur, albeit very slowly, even at very slow growth rates. Also, as fewer cells are formed with time, it is probable that less cells are available to penetrate further into the formation, thus giving progressively slower penetration rates. Anaerobes which have slow rates of growth, such as methanogenic and sulfate-reducing bacteria, would be expected to penetrate porous material very slowly. The penetration rate of *Desulfovibrio desulfuricans* and a sulfate-reducing consortium which had growth rates of about 0.03/h through Berea sandstone was about 0.01 cm/h (A. D. Montgomery, M.S. thesis, University of Oklahoma, Norman, 1987). When a medium with higher lactate concentrations which supported a faster growth rate was used, a faster rate of penetration of *D. desulfuricans* was observed (M. Rozmin and M. J. McInerney, unpublished data). These data are consistent with the predictions of equation 4.

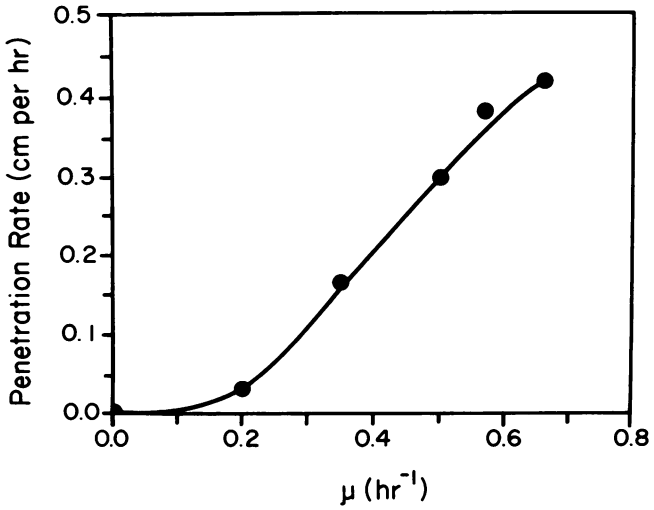

FIG. 5. The relationship between the growth rate and penetration rate of all motile strains of *E. coli* used in this study through sand-packed cores containing galactose medium. The sigmoidal curve was generated from equation 4.

Previous studies by Jenneman et al. (11) and Jang et al. (10) reported penetration rates of 0.4 cm/h for motile *Bacillus* species growing through Berea sandstone with permeabilities above 100 millidarcys. In both of these studies, the penetration time included the time for the bacterial population to reach visible turbidity in a receiving flask. From the cell concentration at the time when faint turbidity was observed and the specific growth rate, it was possible to estimate the breakthrough time by assuming that one cell entered the receiving flask (11). However, if more than one cell reaches the receiving flask at any time, i.e., if the cells arrive as a front or band, then the actual penetration rate may be underestimated. From equation 4, we can calculate a penetration rate of 0.54 cm/h for a motile *Bacillus* strain (μ, 0.94/h) (11) growing through an unconsolidated sand-packed core with a porosity of 27.6% (7-ml liquid volume). If the penetration rate is linearly related to pore volume (Fig. 4A), then the penetration rate for this same strain growing through Berea sandstone with a porosity of 3% (0.7-ml liquid volume) would be 10 times faster, 5.4 cm/h. This is much faster than the penetration rates reported in these studies (5).

Although Fig. 4A predicts a linear relationship between penetration rate and pore volume, it is possible that this relationship does not hold for all porous media, especially those with low porosity. In general, as the porosity decreases, so does the average pore entrance size, thereby restricting penetration (11). Nevertheless, the penetration rates obtained in earlier studies (9, 11) need to be remeasured in a system analogous to the one used here, in which the number of cells exiting the core would not affect the calculation of the penetration rate.

From a practical standpoint, the method developed in this and our earlier studies (11) can be used to obtain accurate measurements of bacterial movement within the subsurface under a variety of biological and physiochemical conditions. Such studies may provide valuable data needed to evaluate and/or construct models designed to (i) predict the fate of genetically engineered organisms released into the environment, (ii) study the fate of bacterial contamination of groundwater due to septic tank seepage or land application of municipal wastewater, and (iii) provide an understanding of the factors which influence the movement of bacteria during in situ bioremediation of contaminated groundwaters or microbially enhanced oil recovery processes.

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LITERATURE CITED

1. **Adler, J.** 1966. Chemotaxis in bacteria. Science **153**:708–715.
2. **Adler, J., and M. M. Dahl.** 1967. A method for measuring the motility of bacteria and for comparing random and non-random motility. J. Gen. Microbiol. **46**:161–173.
3. **Adler, J., G. L. Hazelbauer, and M. M. Dahl.** 1973. Chemotaxis toward sugars by *Escherichia coli*. J. Bacteriol. **115**:824–847.
4. **Balch, W. E., and R. S. Wolfe.** 1976. New approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. Appl. Environ. Microbiol. **32**:781–791.
5. **Chang, P. L., and T. F. Yen.** 1984. Interaction of *Escherichia coli* B and B/4 and bacteriophage T4D with Berea sandstone rock in relation to enhanced oil recovery. Appl. Environ. Microbiol. **47**:544–550.
6. **Civan, F., R. M. Knapp, and M. J. McInerney.** 1988. Modeling growth and transport of microorganisms in porous formations, p. 676–679. *In* R. Vichnevetsky, P. Borne, and J. Vignes (ed.), IMACS 1988 Proceedings of the 12th World Congress on Scientific Computation, vol. 3. International Association for Mathematics and Computers in Simulation, Paris.
7. **Corapcioglu, M. Y., and A. Haridas.** 1984. Transport and fate of microorganisms in porous media: a theoretical investigation. J. Hydrol. (Wellington North) **72**:149–169.
8. **Edgehill, R. U., and R. K. Finn.** 1983. Microbial treatment of soil to remove pentachlorophenol. Appl. Environ. Microbiol. **45**:1122–1125.
9. **Jang, L.-K., P. W. Chang, J. E. Findley, and T. F. Yen.** 1983. Selection of bacteria with favorable transport properties through porous rock for the application of microbial-enhanced oil recovery. Appl. Environ. Microbiol. **46**:1066–1072.
10. **Jang, L.-K., M. M. Sharma, J. E. Findley, P. W. Chang, and T. F. Yen.** 1982. An investigation of the transport of bacteria through porous media, p. 60–70. *In* E. C. Donaldson and J. B. Clark (ed.), Proceedings of the 1982 International Conference on the Microbial Enhancement of Oil Recovery. Bartlesville Energy Technology Center, Bartlesville, Okla.
11. **Jenneman, G. E., M. J. McInerney, and R. M. Knapp.** 1985. Microbial penetration through nutrient-saturated Berea sandstone. Appl. Environ. Microbiol. **50**:383–391.
12. **Kreig, N. R., and J. G. Holt.** 1984. Bergey's manual of systematic bacteriology. The Williams & Wilkins Co., Baltimore.
13. **Lapidus, I. R., M. Welch, and M. Eisenbach.** 1988. Pausing of flagellar rotation is a component of bacterial motility and chemotaxis. J. Bacteriol. **170**:3627–3632.
14. **McInerney, M. J., M. P. Bryant, and N. Pfennig.** 1979. Anaerobic bacterium that degrades fatty acids in syntrophic association with methanogens. Arch. Microbiol. **122**:129–135.
15. **Pfeffer, J. T.** 1980. Anaerobic digestion processes, p. 187–203. *In* D. A. Stafford, B. I. Wheatley, and D. E. Hughes (ed.), Anaerobic digestion. Applied Science Publishers Ltd., London.
16. **Ray, A. A. (ed.).** 1982. SAS user's guide, p. 39–84. SAS Institute Inc., Cary, N.C.
17. **Schwerdinger, R. B.** 1968. Reclamation of soil contaminated with oil. J. Inst. Petroleum **54**:182–197.
18. **Torbati, H. M., E. C. Donaldson, G. E. Jenneman, R. M. Knapp, M. J. McInerney, and D. E. Menzie.** 1985. Depth of microbial plugging and its effect on pore size distribution in Berea sandstone cores, p. 213–225. *In* J. E. Zajic and E. C. Donaldson (ed.), Microbes and oil recovery, vol. 1. Bioresources Publishing, El Paso, Tex.
19. **Trueba, F. J., and C. L. Woldringh.** 1980. Changes in cell diameter during the division cycle of *Escherichia coli*. J. Bacteriol. **142**:869–878.
20. **Updegraff, D. M.** 1982. Plugging and penetration of reservoir microorganisms, p. 80–85. *In* E. C. Donaldson and J. B. Clark (ed.), Proceedings of the 1982 International Congress on the Microbial Enhancement of Oil Recovery. Bartlesville Energy Technology Center, Bartlesville, Okla.
21. **Updegraff, D. M., and G. B. Wren.** 1954. The release of oil from petroleum-bearing materials by sulfate-reducing bacteria. Appl. Microbiol. **2**:309–322.
22. **Yen, T. F.** 1984. Bacterial transport through porous media. Annual report for the period ending December 31, 1984. Bartlesville Project Office, U.S. Department of Energy, Bartlesville, Okla.