Effect of bacterial heterogeneity on adhesion to uniform collectors by monoclonal populations

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Abstract: Transport of bacteria over significant distances through aquifer sediments occurs primarily among bacteria with low affinity for sediment materials. Bacterial affinity for a uniform collector surface has been represented quantitatively by a collision efficiency (α), defined as the fraction of colliding cells that adhere to the collector surface. Using a new method for estimating α during advective transport of monoclonal bacterial populations through a uniform bed of 40-μm borosilicate glass spheres, we found that α decreased 10-fold over a bed depth of only 1 cm. Depth-dependent differences in α were not related to variation in bacterial size or intra-strain genetic variation. Intra-population heterogeneity in biocolloid-collector affinity may be an important determinant of subsurface bacterial transport characteristics, with critical implications for pathogen transport and dispersal of bacteria for the remediation of hazardous waste.

Key words: Bacterial heterogeneity; Bacterial adhesion; Bacterial transport

Introduction

The recent discovery of a rich subsurface microfauna at depths measured in hundreds of meters [1–3] led to questions relative to the origin of deep subsurface communities [4]. Principal theories are (i) that bacteria were transported to depth with local groundwaters, and are therefore removed from their near-surface relatives for periods on the order of 1–5 × 10^4 years; or (ii) that deep-subsurface bacteria were laid down with strata that now comprise local sediments and managed to survive to the present, absent genetic contact for 7–8 × 10^7 years with the near-surface bacterial world that was known prior to their discovery [5]. To accept the former theory we must also accept the idea that bacteria can be transported through porous media that comprise aquifers for tens to hundreds of kilometers—that is, that bacteria can avoid long-term retention on sediments, making possible their transport over great distances with flowing groundwater. Experiments described here suggest that even the major determinants of bacterial sorption/transport through porous media are not completely known. Our findings have implications for other practical questions such as subsurface dissemination of genetically engineered bacteria, bioaugmentation for remediation of hazardous waste, and oilfield repressurization.

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Materials and Methods

Bacterial species A1264 from the United States Department of Energy (DOE) Subsurface Microbial Culture Collection [6] was grown aerobically in batch culture, 5% peptone, tryptone, yeast extract, glucose (PTYG) medium, to a final, stationary-phase cell density of about \(10^9\) ml\(^{-1}\). Growth was monodisperse with a log-phase doubling time of about 2 h. Suspensions were diluted to \(10^6\) cell/ml\(^{-1}\) with 3-(N-morpholino)-propanesulfonic acid, sodium salt (MOPS) buffer (4.624 g l\(^{-1}\) with 50 mg l\(^{-1}\) MgSO\(_4\) \(\cdot\) 7H\(_2\)O, 1000 mg l\(^{-1}\) NH\(_4\)Cl, 17.4 mg l\(^{-1}\)K\(_2\)HPO\(_4\), 88.5 mg l\(^{-1}\) CaCl\(_2\) \(\cdot\) H\(_2\)O, 66.65 mg l\(^{-1}\) FeCl\(_3\) \(\cdot\) 6H\(_2\)O; pH = 7.7). \(^{3}H\)Leucine was added to the buffered suspension (0.040 mCi per 100 ml), and the dilute culture was agitated at 150–200 rpm for 18 h to permit label uptake. During the labeling periods, bacteria doubled 2–4 times, re-entering stationary phase prior to use in transport experiments.

Transport experiments were conducted using the microbe and radiolabel kinesis (MARK) method [7]. Column media were comprised of 40-\(\mu\)m borosilicate glass beads. The beads were prepared by soaking in a 10% solution of H\(_2\)SO\(_4\) on a shaker table at 150 rpm for 3 h. After settling, the supernatant was poured off and deionized water (Milli-Q\(^{\text{\textregistered}}\) Water Treatment System) added. This solution was placed in a centrifuge (Beckman Model J2-21) for 15 min at 6000 rpm to separate the beads. The excess liquid was poured off and more deionized water added. The centrifugation process was repeated three times before the beads were dried at 105°C to remove all the remaining liquid and stored dry until used.

Immediately prior to the experiment, the beads were packed into columns that were fashioned from 3-cc syringe barrels and pre-rinsed with 2 ml of the MOPS buffer. 2 ml of the dilute, labeled suspension were then passed through the bead-filled columns. Unassimilated label was rinsed from the column with 4 ml of the buffer solution. Corrections were obtained for sorption of unassimilated label on the glass beads by running parallel columns that received a filtered solution (0.2 \(\mu\)m polycarbonate Millipore filter) of labeled suspension. Following the filtration/rinse procedure, beads and attached bacteria were extruded and cut into 1-mm sections for depthwise measurements of retained label. All measurements of retained radiolabel were made with a scintillation counter (Beckman LS 3801 Liquid Scintillation System).

Total assimilated radiolabel was estimated by passing 2-ml samples of the dilute, labeled suspension through 0.2-\(\mu\)m polycarbonate filters. Unassimilated label was rinsed from the filters prior to measurement. Again, a correction was obtained for retained liquid-phase label. The corrected total filter count provided a baseline measure for estimates of fractional retention in individual columns or column sections [7]. Collision efficiency was calculated from the fraction of bacteria retained, based on the clean bed filtration model of Yao [8], using the semi-empirical methods of Rajagopalan and Tien [9,10].

Size distribution measurements of bacteria were made with a particle counter (Coulter Multisizer II). Measurements were obtained for unlabeled cells, before and after filtration through a borosilicate glass bead column. All other procedures were identical to those used to estimate fractions of retained bacteria.

To test whether \(a\) distributed values were due to intra-strain genetic variation, unlabeled bacteria were passed sequentially through four columns of the type described. Effluent cells from the fourth column were grown on solid media consisting of 5% PTYG agar. Resultant colonies were used to initiate standard radiolabel/transport experiments.

Results and Discussion

Our work shows that cell affinity for a uniform collector surface was broadly distributed in stationary-phase, monoclonal batch cultures of bacteria. \(^{3}H\)leucine-labeled bacteria were sequentially passed through two columns containing 40-\(\mu\)m borosilicate glass beads. Collision efficiency for the deep-subsurface bacterium A1264 decreased from 0.068 (top cm of reactor 1) to 0.010 (top cm of reactor 2). By slicing a 1-cm column
Fig. 1. Measurement of stickiness using a stationary-phase bacterial population. $^3$H(leucine)-labeled bacteria (A1264, a Savannah River deep subsurface bacterium from the DOE Subsurface Microbial Culture Collection [SMCC] [6]) were passed through columns containing 40-$\mu$m borosilicate glass spheres. The column of beads was dissected into 1-mm layers, and retained label was directly measured in each layer using a scintillation counter. From the fraction of bacteria retained in each slice, collision efficiency ($\alpha$) was calculated using the model of Rajagopalan and Tien [9,10]. Cells applied to each column section were estimated by subtracting the cells retained in upstream slices from the original cell number. (A) Collision efficiency as a function of column depth or slice number. Results of two identical experiments, each involving three replicate columns. Column length was 1 cm. (B) Collision efficiency versus depth in a 2-cm column (no replicates).
into ten 1-mm layers, we found that the effective population $\alpha$ decreased by a factor of 9.4 for strain A1264 over a 1-cm column length (Fig. 1A), from 0.234 in the top 1-mm slice to 0.025 in the bottom slice. The effective $\alpha$ for the entire 1-cm reactor was 0.063. In a parallel, identical experiment, the average $\alpha$ decreased by a factor of 9.0 across the top cm of the reactor—from 0.216 in the top 1-mm slice to 0.024 in the tenth layer.

When the column of beads was increased to 2 cm in length so that collision efficiencies could be calculated for each of 20 1-mm layers, the effective population $\alpha$ decreased by a factor of 11.3 across the first cm of the column and by 18.5 over the entire 2-cm length (Fig. 1B). The average bacterial collision efficiency decreased monotonically with distance traversed through the packed bed in each of these experiments. The rate of decrease of $\alpha$ was greatest in the top few millimeters of the reactor. Observations are consistent with hypotheses that: (i) there was a distributed affinity (collision efficiency) that governs cell-collector attachment, even in the extreme

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**Fig. 2.** Size distribution measurements in a stationary-phase population of A1264. Coulter Multisizer II measurements showed essentially no difference in size distributions representing diluted cell suspensions before and after filtration through a 2.5-cm column of 40-μm borosilicate glass beads. Measurements were obtained for unlabeled cells; all other procedures were identical to those used to estimate fractions of retained bacteria. (A) Particle number (cells/ml) as a function of diameter in pre- and post-filtration cell suspensions. (B) Pre- and post-filtration distribution of particle sizes in cell suspensions.
case of a uniform collector surface and monoclonal bacterial population; (ii) large \( \alpha \) values in the top layers of the reactor were due to a relatively few microorganisms with exceptional affinity for the collector; and (iii) the effective population \( \alpha \) decreased as higher affinity, stickier microorganisms were preferentially removed in the upper bed.

These results have potentially critical implications for predictions of biocolloid transport through porous media including deep-bed filters and aquifer sediments. It is evident, for example, that estimates of bacterial \( \alpha \) values depend on the length of the column used to measure fractional retention of the biocolloid. In our experiment in the 2-cm reactor, we calculated an overall, effective \( \alpha \) of 0.031 using cells retained within the entire column length. Had we considered only the top cm, that value would have been 0.050, and, in the extreme case, the effective \( \alpha \) in the top mm was 0.203. Predictions of travel distances for specific levels of bacterial attenuation are sensitive to measurement of \( \alpha \). To illustrate this point, the estimated distance for a 3-log removal using \( \alpha = 0.031 \) is 6.6 times the distance required for comparable attenuation given \( \alpha = 0.203 \). The exercise suggests that reasonable prediction of bacterial transport characteristics in porous media will depend on accurate representation of the distribution of affinities between the biocolloid and collector surface under the prevailing aqueous chemical conditions.

Particle size distribution measurements indicated that there was significant variation in size among stationary-phase cells that were representative of those used in transport experiments. However, size distributions obtained before and after passage through the glass bead column were essentially identical (Fig. 2). Therefore, variations in \( \alpha \) within the monoclonal population cannot be explained on the basis of preferential retention of larger cells.

To determine whether distributed \( \alpha \) values were genetic in origin, unlabeled bacteria were passed sequentially through four reactors of the type described, resulting in a \( 10^4 \) (overall) attenuation in suspended cell numbers. Effluent cells from the fourth reactor were grown on solid media consisting of 5% PTYG agar. Two of the resultant colonies were selected for use in standard radionuclide/transport experiments that produced calculated \( \alpha \) values of 0.061 and 0.075 (1-cm reactor length). A parallel (control) experiment that was carried out using the original A1264 culture produced a collision efficiency in the same range, \( \alpha = 0.063 \). Based on these results, it is unlikely that observed heterogeneities in bacterial affinity for the borosilicate glass collector were the result of intra-population genetic variation.

Establishment of microorganisms in subsurface environments does not require the transport of large numbers of bacteria. A few relatively non-adhesive cells could colonize deep subsurface formations following transport over long distances. The broad distribution of \( \alpha \) values in monoclonal populations, heretofore unsuspected, offers clues to the mechanism of bacterial transport in the subsurface, including (perhaps) transport to deep sediments. Similarly, the distribution of collision efficiencies, particularly in the very low range of \( \alpha \), may dominate the dispersal and the eventual extent of pathogens and genetically engineered bacteria in sediments.

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References


