Effect of Substrate and Cell Surface Hydrophobicity on Phosphate Utilization in Bacteria

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We measured the rates of utilization of hydrophobic and hydrophilic phosphate compounds in gram-negative bacteria with different surface hydrophobicities, isolated from wetland habitats. Three hydrophobic and two hydrophilic bacterial species were selected for study by measuring cell adherence to hydrocarbons. The bacteria were grown under phosphorus-limited conditions with P_i , hydrophilic β -glycerophosphate, or hydrophobic phosphatidic acid as the phosphate source. Hydrophilic bacteria grew most rapidly on P_i , followed by β -glycerophosphate. Phosphatidic acid did not support growth or did so at a much later time (40 h) than did the other phosphate treatments. Although all hydrophobic species grew well on these substrates, the rate of growth of two *Acinetobacter baumannii* isolates on phosphatidic acid exceeded the rate of growth on phosphate or β -glycerophosphate. A membrane phospholipid and lipopolysaccharide were used as a source of phosphorus by hydrophobic species, whereas hydrophilic species could not use the membrane phospholipids and used lipopolysaccharide to a lesser extent. Besides hydrophobic interaction between cells and substrate, phosphatase activity, which was cell bound in hydrophilic species but 30 to 50% unbound in hydrophobic species, affected cell growth. Dialyzed culture supernatant containing phosphatase from hydrophobic species increased the phosphate availability to hydrophilic species. Additionally, cellular extracts from a hydrophilic species, when added to hydrophilic cells, permitted growth on hydrophobic phosphate sources. Naturally occurring amphiphilic humic acids affected the utilization of P_i and β -glycerophosphate in bacteria with hydrophilic surfaces but did not affect hydrophobic bacteria. Our results indicate that hydrophobic phosphate sources can be used by bacteria isolated from aquatic environments as the sole phosphorus source for growth. This utilization, in part, appears to be related to cell surface hydrophobicity and extracellular enzyme production.

Hydrophobic phosphates such as phospholipids are present in cells found in nature, yet most studies on nutrient mobilization focus on water-soluble compounds. For phosphorus, which is undoubtedly the most intensively studied nutrient in aquatic ecosystems (35), these studies in aquatic ecosystems include the cycling of P_i (see, e.g., references 19 and 26), availability based on molecular weight (12, 15), the release of phosphate from sediment-water (5, 8, 16, 27) and epiphytic boundary (6, 7, 28) layers, and the effect of organic phosphates on P_i uptake in bacteria and algae (4).

Phosphatase activity has been measured in an effort to link relative enzyme activity to nutrient availability from organic sources (see, e.g., references 14, 20, and 34). Enzyme-catalyzed reactions involving transfer of phosphoryl groups are central to metabolism. This class of enzymes can be divided into groups based on the nature of the phosphate-containing substrate or on the nature of the nucleophilic acceptor to which the phosphoryl moiety is transferred. Phosphatase and phosphodiesterase use water as the nucleophilic acceptor and therefore involve hydrolysis of phosphate monoesters and phosphate diesters, respectively, as substrates. These enzymes make P_i available from a variety of organophosphates.

The concentration of dissolved organic phosphorus in lake waters is 10 to 50 $\mu\text{g liter}^{-1}$ (32) and is at least equal to, and often much greater than, the concentration of dissolved P_i (35). Up to 20% of these compounds have a molecular mass greater than 50,000 Da and are hydrophilic compounds such as DNA, nucleotide fragments, or inositol phosphate associated

with high-molecular-weight organic matter (11, 13, 23). The availability of water-insoluble or hydrophobic organic phosphates, such as the cell membrane phospholipids from dying cells, as a source of phosphorus for living microorganisms is not well documented.

Cell surface hydrophobicity has been recognized as a physical, measurable characteristic of bacteria (25). This property has been commonly investigated in the adherence of bacteria to surfaces (e.g., biofilms, tooth plaque) (29). Although hydrophobic cell surface interaction with hydrophobic compounds may account for enhanced biodegradation of carbon substrates, such as alkanes (37), no investigations have considered compound hydrophobicity with respect to nutrient utilization. In this study, the utilization of hydrophobic phosphate sources by bacteria isolated from a freshwater ecosystem was examined. The bacteria were separated into two groups, one with hydrophilic surfaces and the other with hydrophobic surfaces. We were interested in determining if hydrophobic phosphate sources would be utilized more rapidly by hydrophobic bacteria.

Bacterial outer membranes show unusual functional properties in that there is very low permeability toward lipophilic solutes and high permeability toward hydrophilic solutes (24). Humic acids are large, condensed polyphenolics which are amphiphilic. Recent reports have indicated that at high concentrations (7.4 g liter⁻¹), they form micellar structures (17). Molecules with these surface-active properties could potentially interact with membrane surfaces and alter membrane permeability.

Humic substances are formed largely from the decomposition of vascular plants. Most (50 to 90%) of the dissolved organic carbon is composed of humic substances (36), and the concentration in the colored waters of wetlands is typically 10

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FIG. 1. Growth of bacterial isolates (mean $\pm$ standard deviation [SD]; $n = 3$) with P_i (●), β GP (■), and PA (▼) substrates under phosphate-limiting conditions. Bacteria with hydrophilic surfaces were *E. cloacae* (A) and *P. putida* (B); those with hydrophobic surfaces were *A. baumannii* 1 (C), *A. baumannii* 2 (D), *P. aureofaciens* (E).

to 50 mg of C liter⁻¹. Of the humic substances in dissolved organic carbon-rich waters, 15 to 35% can be humic acids (HA) (32). At acidic pH values, the carboxylic acid functional groups of HA are protonated, thus making these molecules

hydrophobic. However, at ambient pH values, the carboxylate groups are deprotonated. Under these conditions, HA are amphiphilic and, like surfactants, are capable of lowering the surface tension of water. Surface-active molecules, such as

TABLE 1. Bacteria, isolation characteristics, and cell surface hydrophobicity

Species	Similarity index	Sample location	Culture enrichment	% Adsorption to:	
				Toluene	Hexadecane
<i>Acinetobacter baumannii</i> 1	0.789	Sediment	Hexadecane	88.2	88.0
<i>Acinetobacter baumannii</i> 2	0.541	Sediment	Hexadecane	84.2	75.0
<i>Enterobacter cloacae</i>	0.789	Sediment	Glucose	0.0	0.0
<i>Pseudomonas aureofaciens</i>	0.732	Biofilm	Oleic acid	72.3	71.6
<i>Pseudomonas putida</i>	0.321	Biofilm	Oleic acid	0.0	0.0

FIG. 2. Bacterial growth (mean $\pm$ SD; $n = 3$) on the membrane phosphates LPS (▼) and PE (■) and on P_i as a control (●). Bacteria with hydrophilic surfaces were *E. cloacae* (A) and *P. putida* (B); those with hydrophobic surfaces were *A. baumannii* 1 (C) and *P. aureofaciens* (D).

detergents, can affect biomembrane permeability. It has previously been suggested that the surface activity of humic substances on cell membranes results from the presence of both hydrophilic and hydrophobic sites that interact with phospholipids to aid in the transport of nutrients into cells (9, 10). If HA are capable of such action, it may be possible to observe a

difference in the utilization of different phosphorus sources in the presence of these surface-active molecules.

The objectives of this study were to determine if water-insoluble phosphate compounds would be used differently by bacteria with dissimilar surface hydrophobicities and how rates of utilization of P_i and hydrophobic and hydrophilic organic phosphates compared in the presence and absence of humic acids.

MATERIALS AND METHODS

Cell culturing. Laboratory bacterial cultures were established by collecting samples with sterile apparatus from two locations in the Talladega Wetland Ecosystem, a National Science Foundation study site located in the Talladega National Forest, Ala. The samples were flocculent aerobic sediment, vortexed at high speed for dispersal, and submerged biofilm from the base of the dominant emergent aquatic macrophyte *Juncus effusus*, which was scraped into a sterile glass tissue grinder and homogenized. Coarse particles were allowed to settle from the solutions by sedimentation. Subsamples of the respective supernatants were placed in an essential nutrient solution enriched with 0.02 mM sodium phosphate and with hexadecane, glucose, or oleic acid as the carbon source. Essential nutrient solution (ENS) consisted of (per liter) 1 ml of stock salt solution (3), 1.0 g of NH_4Cl , 0.1 g of $MgSO_4$, 0.005 g of $FeNH_4(SO_4)_2$, 0.3 g of KNO_3 , and 0.05 g of $CaCl_2$ and was buffered with 50 mM MES (2-[N-morpholino]ethanesulfonic acid; pH 6.1) (Sigma Chemical Co.). Cultures were plated on LB top agar, and individual colonies were isolated in LB medium (also Luria broth or Lenox broth) (2).

Culture characterization. Individual isolates were identified by fatty acid methyl ester analysis on a Microbial Identification System at MIDI, Newark, Del., and the results are reported as a similarity index to identified bacterial strains. Uncontaminated parent cultures used in experimentation were maintained temporarily on LB top agar at 4°C or permanently in 85% glycerol at -80°C (31).

Cell surface hydrophobicity was determined by the bacterial adherence to hydrocarbons assay (30, 37). In this study, the hydrocarbons were toluene and the more hydrophobic *n*-hexadecane, which were added to a subsample of the culture and mixed, and the mixture was allowed to return to a biphasic system. The percentage adhesion was calculated as follows: $100 \times (OD_{600} \text{ before mixing} - OD_{600} \text{ after mixing}) / OD_{600} \text{ before mixing}$, where OD_{600} is the optical density at 600 nm.

Experimental procedure. Experimental starter cultures, consisting of a parent culture inoculum and essential nutrient solution with a carbon source and P_i , were grown overnight to produce bacterial cultures in exponential growth phase. Treatment flasks (125-ml Erlenmeyer flasks) contained 15 ml of essential nutrient solution and final concentrations of 0.2% glucose or 5 mM succinate, 0.02

FIG. 3. Optical density of *E. cloacae* (mean $\pm$ SD; $n = 3$) cultured for 72 h in essential nutrient solution and one of the phosphate substrates P_i , PA, PE, or LPS. Control cultures were inoculated media with no external phosphorus source. Solution amendments to each of the five treatments were none (□), *E. coli* alkaline phosphatase (▨), *E. cloacae* cellular extract (▤), and *A. baumannii* 2 culture supernatant (■).

TABLE 2. Phosphatase activities under limiting and nonlimiting phosphate conditions and percentage of phosphatase not bound to cells

Species	Cell surface	Sp act (nmol of product/min/mg of protein) ^a		% Free activity ^a
		Limiting (0.04 mM PO ₄ ³⁻)	Nonlimiting (2.0 mM PO ₄ ³⁻)	
<i>Acinetobacter baumannii</i> 1	Hydrophobic	63.72 ± 4.09	ND ^b	42.1 ± 3.7
<i>Acinetobacter baumannii</i> 2	Hydrophobic	79.10 ± 2.37	ND	30.8 ± 2.1
<i>Enterobacter cloacae</i>	Hydrophilic	1.98 ± 9.21	2.09 ± 0.18	ND
<i>Pseudomonas aureofaciens</i>	Hydrophobic	0.13 ± 0.01	ND	54.3 ± 6.6
<i>Pseudomonas putida</i>	Hydrophilic	0.58 ± 0.03	ND	ND

^a Values are means ± SD for triplicate samples^b ND, not detected.

mM phosphate substrates, and 50 µl of diluted (1:60) starter culture. Bacteria isolated on long-chain alkanes required succinate as a soluble carbon source in aqueous cultures. Cells were grown at 25°C with constant rotary shaking at 90 rpm. Growth was monitored by periodic measurement of OD₆₀₀ on a Beckman DU-650 spectrophotometer. All experiments were repeated at least twice.

Different sets of experiments evaluated rates of growth as a function of the phosphate substrate used under phosphate-limiting conditions. The experiments were arranged into two sets to differentiate substrate utilization on the basis of size. In the first set, substrates were P_i (Sigma Chemical Co.), the hydrophilic organic phosphate β-glycerophosphate (βGP) (Fluka Chemical Co., Buchs, Switzerland), and the hydrophobic organic phosphate phosphatidic acid (PA) (1,2 dicaproyl-*sn*-glycero-3-phosphate; Avanti Polar Lipids, Inc.). P_i and βGP are monomeric in solution, and hence their size should not exclude their transport. PA can be monomeric or micellar, and in micellar form it may be too large to be transported. At the concentration used in this study (0.02 mM), the short-carbon-chain PA was monomeric. Millipore Q-H₂O was used as a control. All treatment solutions were sterile filtered (pore size, 0.22 µm) before use.

Complex membrane phosphate compounds from gram-negative bacteria were used as the phosphorus source in the second set of experiments. A 1.0 mM phospholipid (1-α-diocetyl phosphatidylethanolamine [PE] [Avanti Polar Lipids, Inc.]) stock solution was dispersed as liposomes by sonication (maximum output at 60 Hz for 1 min with a Fisher model 300 Sonicator) and diluted in cultures to 0.02 mM (final concentration). The other treatment was lipopolysaccharide (LPS) from *Escherichia coli* serotype O55:B5 (Sigma Chemical Co.). Persulfate digestion of the LPS stock solution, followed by quantitative analysis for phosphorus by the ascorbic acid method (1), allowed us to adjust the final phosphorus concentration to 0.02 mM in culture. It was thought that because *E. coli* LPS had an approximate molecular mass of 4,000 Da its size might prevent cellular internalization. Likewise, the long-chain PE (18:1)₂ was micellar at 0.02 mM, which may preclude its transport into the cell.

The rate of growth of bacteria given P_i, βGP, and PA alone was compared with that for similar treatments with the addition of 40 mg of Suwannee River HA (HA standard; International Humic Substances Society, Golden, Colo.) liter⁻¹. This concentration was estimated from reported wetland amounts (50 mg of humic substance C · liter⁻¹)(2 mg of humic substance/1 mg of C)(1 mg of dissolved organic carbon/0.9 mg of humic substance) (0.35 mg of HA/mg of dissolved organic carbon) = 38.9 mg of HA · liter⁻¹. OD was corrected by subtracting the OD₆₀₀ of the HA control from treatment OD measurements.

Enzyme assay. Phosphatase activity was determined by adding a 0.2 mM 4-methylumbelliferyl phosphate (Sigma Chemical Co.) solution, diluted from a 5 mM stock in ethylene glycol monomethyl ether, to the sample in 20 mM Tris buffer (pH 8). Fluorescence was measured on a model LS50B Perkin-Elmer Fluorometer with 5-nm slit widths and excitation and emission wavelengths of 365 and 455 nm, respectively.

Total phosphatase specific activity was measured in bacterial cultures under phosphate-limiting (0.04 mM P_i) and nonlimiting (2.0 mM P_i) conditions. In addition, free-enzyme activity was measured in the supernatant after centrifugation (10,000 × g, for 10 min in a Beckman JA-17 rotor). Protein was assayed by the bicinchoninic acid method (Sigma Chemical Co.) with a bovine serum albumin standard.

Preparation of enzyme extracts. The effect of adding enzyme sources on the growth of *Enterobacter cloacae* with P_i, PA, PE, or LPS was examined. The sources used were purified *E. coli* alkaline phosphatase (Sigma Chemical Co.), as well as the cell-free supernatant which resulted from growth of *Acinetobacter baumannii* 2 in P_i-limited media. This supernatant was dialyzed against three changes of 100 volumes each of growth media lacking carbon and phosphorus. A phosphorus determination of the dialyzed supernatant revealed that 8 µM P_i was present. The supernatant was diluted 40-fold when added to cultures, to give a final P_i concentration of 0.2 µM. A cellular extract was prepared from *E. cloacae* by pelleting cells and then performing lysozyme treatment and sonication. The resulting solution was centrifuged at 30,000 × g for 1 h. Ammonium sulfate was added to the supernatant to reach 70% saturation. The resulting solution was centrifuged at 10,000 × g for 30 min, and the protein pellet was resuspended in

ENS with no carbon or phosphorus. The solution was dialyzed three times against 500 volumes of the same medium for 2 h each time.

E. cloacae cultures received one of three growth amendments. These amendments were supernatant from *A. baumannii* 2, cell extract from *E. cloacae*, and purified *E. coli* alkaline phosphatase. All enzyme amendments were added to give 0.1 U of phosphatase activity. One unit of enzyme hydrolyzed 1 µmol of 4-methylumbelliferyl phosphate per min. The concentration of organophosphates used in the growth experiments was 0.02 mM, or 0.2 µmol in a 10-ml reaction volume. Phosphate should be released rapidly, because these phosphatases hydrolyze the organophosphates at a similar rate to their hydrolysis of 4-methylumbelliferyl.

RESULTS

Five gram-negative bacteria, three with hydrophobic and two with hydrophilic cell surfaces, were selected from cultures isolated from wetland habitats. Of these species (Table 1), both *A. baumannii* isolates and *Pseudomonas aureofaciens* had hydrophobic surfaces. Solvents more polar than hexadecane have been used to differentiate between cells which do not adsorb to hexadecane. Neither *E. cloacae* nor *Pseudomonas putida* had any adsorption to the alkane. In these cases, however, neither species was shown to adsorb to toluene either.

Phosphate utilization by bacteria. Three phosphate substrates with different chemical characteristics were tested with bacteria with different cell surface hydrophobicities. *E. cloacae* grew most rapidly on P_i and βGP (Fig. 1A). Exponential growth on βGP started about 32 h after growth had started on P_i. No growth of *E. cloacae* was observed when PA was provided as the sole phosphate source. The observed growth of *P. putida* on βGP started 18 h after it had been observed on P_i (Fig. 1B). *P. putida* was capable of utilizing PA as a phosphate source; however, growth started 40 h after growth was initially observed on P_i.

Both *A. baumannii* isolates grew well on P_i, βGP, and PA (Fig. 1C and D). There was no delay in the onset of growth with PA, as was observed with hydrophilic cells, and both isolates began exponential growth at the same time as or slightly sooner than they did on P and βGP. The growth rates of *P. aureofaciens* on P_i and βGP were virtually identical (Fig. 1E). Detection of growth on PA was delayed relative to that on P_i and βGP, but this effect was much smaller than was observed for hydrophilic cells.

Growth on cytoplasmic and outer membrane phosphates was evaluated with two hydrophilic and two hydrophobic bacteria. P_i was used as a positive control to compare treatment results. Neither strain of hydrophilic bacteria was able to use PE for growth, but these species were able to grow on LPS (Fig. 2A and B). In contrast, bacteria with hydrophobic surfaces were able to utilize both substrates for growth (Fig. 2C and D). The major difference between the two hydrophobic strains tested occurred when PE was used as a phosphorus source. The exponential growth of *A. baumannii* 1 on PE was

FIG. 4. Effect with (■) and without (●) HA on the growth of phosphate-limited bacteria (mean $\pm$ SD; $n = 3$) with hydrophilic surfaces, *E. cloacae* (A to C) and *P. putida* (D to F), grown on three different phosphate substrates: P_i , β GP, and PA.

delayed by approximately 34 h when compared with its growth on P_i . This delay was not observed to the same extent in the exponential growth of *P. aureofaciens* on PE compared with P_i . The two phosphate sources LPS and PE were not expected to be readily permeable to bacteria. LPS has a molecular mass of about 4,000 Da, and PE, at the concentration used in this study, was not monomeric but, rather, was micellar. Therefore, the utilization of these phosphorus sources probably required extracellular enzymes.

Phosphatase activity and the effect of externally added enzymes on growth rates. All bacteria showed phosphatase specific activity under limiting phosphate conditions (Table 2). *E. cloacae* had the same amount of activity under nonlimiting conditions, which suggested that phosphatase enzyme activity was constitutive. Phosphatase was induced in all other species under phosphate-limiting conditions. One-third to one-half of

the phosphatase activity measured was not associated with the cell in bacteria with hydrophobic surfaces.

To further evaluate the ability of hydrophobic strains to successfully utilize phosphate sources which hydrophilic bacteria could not use, we examined the growth of *E. cloacae* when externally added enzyme sources were presented to cultures with hydrophobic phosphates. Results of these experiments are shown in Fig. 3. Dialyzed (molecular weight cutoff, 12,000 to 14,000) supernatant from *A. baumannii* 2, when added to a culture of *E. cloacae*, allowed growth on both PA and PE, substrates which were not utilized without enzyme addition. The addition of purified *E. coli* alkaline phosphatase allowed growth on PA, suggesting that only extracellular hydrolysis of the phosphate source was required for growth. These results suggested to us either that the hydrophobic phosphate source was not being transported into *E. cloacae* or that this organism

lacked the enzymatic ability to hydrolyze PA or PE. Although the latter possibility seemed remote, we prepared an enzyme extract from *E. cloacae* and presented it to a culture of the same cells in the presence of PE or PA phosphate sources. This design was similar to that using *A. baumannii* 2 supernatant and purified *E. coli* alkaline phosphatase for growth experiments with PA or PE as the sole phosphate source. The results clearly indicated that when cellular barriers are removed, *E. cloacae* contains the enzymes necessary to hydrolyze both PA and PE.

Effects of HA on phosphate utilization. The addition of 40 mg of HA liter⁻¹ to *E. cloacae* decreased the time needed to reach exponential growth on P and β GP by 24 h (Fig. 4A and B). Although HA did not affect the growth of *P. putida* on P_i, addition of HA decreased the time required by *P. putida* to reach exponential growth on β GP by 25 h (Fig. 4D and E). HA produced a marginal growth increase in *E. cloacae* when PA was used as a phosphate source, but it slightly increased the time required by *P. putida* to reach exponential growth on PA (Fig. 4C and F). HA had little or no effect on the growth of bacteria with hydrophobic surfaces (data not shown).

DISCUSSION

The outer membrane of gram-negative bacteria allows the transport of molecules up to about 600 Da (33). Although the sizes of all three phosphate substrates in the first set of experiments fell below this nominal threshold (P_i, 40 Da; β GP, 216 Da; PA, 389 Da), growth responses differed significantly. These results indicated that factors other than molecular size, such as the enzymatic hydrolysis of organic phosphate and the interaction of the substrate with the cell surface, were important for cell utilization of phosphate.

The delay in growth of bacteria with hydrophilic surfaces (e.g., *P. putida*) on β GP relative to growth on the P_i treatment may be the result of enzyme induction or the rate of hydrolysis reaction for *P. putida*. For *E. cloacae*, enzyme induction can be ruled out since the enzyme is constitutively expressed. Properties of the outer bacterial layers may explain the differences in the respective times needed to reach exponential growth on PA and β GP.

The most extensive differences we observed occurred when the growth rates of hydrophilic cells presented with hydrophobic and hydrophilic phosphate sources were compared. An obvious difference, besides cell surface hydrophobicity between our cells, was the occurrence of extracellular phosphatase activity in hydrophobic cells. All phosphatase activity of both hydrophilic species was cell associated. *P. putida* was capable of using PA; however, the onset of growth was delayed significantly when compared with growth on P_i or β GP. This may have resulted from (i) reduced substrate-surface interaction because of hydrophobic forces and/or (ii) reduced PA concentrations entering the cell because of slow diffusion of the larger substrate through the outer layers of the cell. This explanation is reasonable if the substrate had to move through the amorphous capsule to reach the cell-bound enzymes typically concentrated in the periplasmic space.

If the constitutively produced phosphatase in *E. cloacae* was able to contact the organic phosphate (PA), phosphate should have become available for growth. It was expected that this process would be relatively fast, because the enzyme does not have to be induced, as was the case in the other species studied. Since no phosphatase was released from the cell and no growth occurred with PA, this suggested that the highly viscous, anionic extracellular polysaccharide capsule of *E. cloacae* (22) created a barrier between the substrate and the enzyme.

To further examine the inability of *E. cloacae* to use hydrophobic phosphate sources, experiments were conducted in which cultures of *E. cloacae* containing hydrophobic organophosphates were given various enzymatic amendments. We first added extracellular enzymes from the dialyzed supernatant of hydrophobic *A. baumannii* 2. This amendment allowed growth of *E. cloacae* on both PA and PE. The supernatant also made phosphate from LPS more available than in the untreated, enzyme-bound control culture. The addition of purified *E. coli* alkaline phosphatase resulted in the ability of *E. cloacae* to grow on PA but not on PE, which is consistent with the catalytic activity of the externally added enzyme. These results indicated that the presence of extracellular organophosphate-hydrolyzing enzymes allows for growth of *E. cloacae* and suggest that in their absence, hydrophobic phosphate sources cannot cross cell barriers to reach cell-associated enzymes. However, the possibility existed that the organophosphate-hydrolyzing enzymes of *E. cloacae* have low or nonexistent affinity for hydrophobic phosphate substrates. To directly address this possibility, we prepared an enzyme extract from lysed *E. cloacae* cells and used this preparation in similar growth studies. The results indicated that *E. cloacae* has the enzymatic potential to liberate P_i from PA, PE, and LPS. We therefore concluded that substrate availability to the appropriate enzymes, rather than enzymatic potential, was limiting for growth of *E. cloacae* on hydrophobic phosphates.

Further investigation of potential effectors to bacterial surfaces was done by examining the effects of HA on the growth of our isolated bacteria with different P_i sources. HA possess both hydrophobic and hydrophilic regions, and these amphiphilic molecules have been demonstrated to possess surface-active properties. Additionally, HA and other polyphenolic compounds have been shown to interact with proteins by both ionic and hydrophobic interactions (18). Some bacteria have specialized porin proteins for substrate uptake. The porin PhoE is induced by phosphate limitation and acts as a very effective molecular pore that facilitates transport of phosphorus through the outer membrane of *E. coli* K-12 (21). Therefore, both its surface-active properties and its ability to interact with protein indicate that HA can alter phosphate uptake in bacteria with different surface hydrophobicities.

HA enhanced growth of both hydrophilic species on hydrophilic organic phosphate and also decreased the time required for *E. cloacae* to reach exponential growth on P_i. Bacteria with hydrophobic surfaces showed very little response to HA addition (data not shown), in contrast to hydrophilic bacteria. From these preliminary studies, we speculated that HA may increase the transport of P and β GP into hydrophilic cells by (i) altering the capsule composition and decreasing the barrier to transport (especially in *E. cloacae*), (ii) changing the outer membrane permeability, or (iii) acting as a transport-enhancing molecule by affecting membrane proteins (i.e., porins). Further studies on the complex interaction of bacterial cell surfaces with HA are required before a detailed understanding of the effects of HA is fully realized.

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