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Adsorption of *Rhodococcus* Strain GIN-1 (NCIMB 40340) on Titanium Dioxide and Coal Fly Ash Particles

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***Rhodococcus* strain GIN-1 (NCIMB 40340) can be used to enrich and isolate a titanium-rich fraction from coal fly ash. The gram-positive bacterium was isolated by its ability to adhere strongly and rapidly to suspended particles of pure titanium dioxide or coal fly ash. Adsorption depends on the salt concentration and occurs in seawater. Lowering of the salt concentration or washing of particles with pure water did not, however, cause desorption of the bacteria from TiO_2 particles; this was achieved by strong alkaline treatment or combined treatment with sodium dodecyl sulfate and urea but not with dilute acids, alcohols, or cationic or nonionic detergents. The bacterium exhibits higher affinity towards oxides of Ti and Zn than to other oxides with similar distribution of particle size. Moreover, it adheres much faster to TiO_2 than to magnetite (Fe_3O_4) or Al_2O_3. After about 1 min, more than 85% of the cells were adsorbed on TiO_2, compared with adsorption of only 10 and 8% to magnetite and Al_2O_3, respectively. Adsorption of the bacteria on TiO_2 occurs over a pH range of 1.0 to 9.0 and at temperatures from 4 to over 80°C. Scanning electron microscopy combined with X-ray analysis revealed preferential adherence of the bacterium to coal ash particles richer in Ti. Stronger adhesion to TiO_2 was also demonstrated in the translocation of bacteria, preadsorbed on magnetite, to TiO_2 particles. The temporary co-adhesion to magnetite and TiO_2 was exploited for the design of a prototype biomagnetic separation process in which bacterial cells serve as an adhesive mediator between magnetite and TiO_2 particles in a mixture of Al, Si, and Ti oxides that simulates their proportion in the ash.**

Coal fly ash (CFA) is produced in large quantities from coal-operated power plants. It consists of granular dry powder composed of multielemental complexes, generally including oxides and silicates (1, 5, 6, 11, 16, 18). The chemical composition of the ash depends on the source of the coal and the nature of coal blending before incineration (5, 11, 12, 16). Major elements in the CFA produced from Israeli power plants include silicon (25 to 32%), aluminum (15 to 18%), calcium and iron (5 to 7%), magnesium (2 to 3%), and titanium (1 to 2%). Toxic heavy metals such as cadmium and mercury are also present in the ash in small quantities, as in other coal ashes (5, 11, 16). Some particles within the ash are rich in certain elements, e.g., Ca, Fe, or Ti. The CFA powder is relatively inert to leaching and requires extremely acidic conditions (pH below 1) to release soluble metals into an aqueous environment. However, under mild conditions, e.g., in seawater, moderately agitated suspensions release metals such as Ca, Fe, Ni, Cd, Hg, and Ag, whose local concentrations may exceed the upper permissible levels. In addition, because of its high concentration of potentially useful metals such as aluminum and titanium, CFA is a source of valuable materials that can be extracted before its disposal.

Numerous studies have been devoted to analytical aspects of CFA content, but very few have dealt with exploitation of the ash as a source of useful materials (1, 6, 21, 28). Severe ecological constraints in Israel, together with the potential exploitation of CFA, prompted a search for novel treatment of the ash before its disposal or safe utilization. Our approach was to combine biotechnological and chemical procedures in the treatment of coal ash, with the aim of removing toxic metals and recovering valuable materials from the ash prior to its disposal. This work deals with one aspect of the biotechnological treatment, namely, the extraction of titanium-rich particles from the ash. We describe the isolation of a specific bacterium, its utilization for biosorption of titanium-rich solid particles, and the application of the procedure to a practical separation process.

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MATERIALS AND METHODS

Microbial strains. The bacterium *Rhodococcus* sp. strain GIN-1 (NCIMB 40340) was isolated and used in this study and is currently deposited as a patented strain in the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland, United Kingdom. The following microbial strains were used for comparison of adsorption to and desorption from TiO_2 and CFA: *Acinetobacter calcoaceticus* A2 and RAG-1, *Bacillus subtilis* 168, and *Escherichia coli* K-12 CHS 57 (kindly donated from the collection of the Department of Molecular Microbiology and Biotechnology, Tel-Aviv University, Tel-Aviv, Israel), *Pseudomonas aeruginosa* YS-7, *Saccharomyces cerevisiae* Y-567, and *Candida pseudotropicalis* IP-513 from our laboratory (Program for Biotechnology, Ben Gurion University of the Negev, Beer-Sheva, Israel).

Identification of bacterium. The bacterium was isolated and identified by the use of a standard identification kit (API 20 NE; Biomerieux, Montalie-Vercieu, France). Antibiotic sensitivity was determined by the use of standard kits (ATB G- 1401 and ATB Staph 1402-OF; Biomerieux). Additional chemotaxonomic analyses were carried out at the National Collection of Industrial and Marine Bacteria.

Media and growth conditions. The growth medium for *Rhodococcus* strain GIN-1 (NCIMB 40340) contained (per liter of deionized water) KCl (45 g), $K_2HPO_4 \cdot 3H_2O$ (8.9 g), KH_2PO_4 (2.9 g), $(NH_4)_2SO_4$ (4 g), $MgSO_4 \cdot 7H_2O$ (0.2 g),

sodium citrate (2.0 g), yeast extract (8.0 g), and glucose (10.0 g). The pH was adjusted to 6.8. Cultures were grown aerobically in shake flasks at 32°C in a gyrotory NBS G-24 shaker (New Brunswick Scientific Ltd.).

Seawater medium for enrichment culture. The medium used for the enrichment of adhesive microorganisms in continuous culture was prepared from seawater filtered through a Media-Kap-5 0.2-μm-pore-size hollow-fiber filter (Microgon Inc., Laguna Hills, Calif.). The filtered seawater was supplemented with the following ingredients (per liter): $K_2HPO_4 \cdot 3H_2O$, 2.2 g; KH_2PO_4, 0.72 g; $(NH_4)_2SO_4$, 1 g; yeast extract, 2.0 g; and glucose, 5.0 g.

Continuous culture for isolation of adhesive microorganisms. Enrichment of adhesive microorganisms was carried out in two consecutive continuous cultures. After enrichment, microbial cells that were retained on TiO_2 particles in the first culture served to inoculate the second continuous culture, which contained CFA. Both cultures were operated continuously for 3 weeks after inoculation in a 1-liter fermentor under the following conditions: dilution rate, 0.1 h^{-1}; agitation, 100 rpm; and aeration, 0.5 vol/vol/min. A 5% (wt/vol) suspension of TiO_2 or CFA was mixed into buffered seawater medium. Particle-free medium containing nonadsorbed cells was decanted from the widened upper section of an inverted U-shaped outlet of the fermentor, and attached cells were retained inside the fermentor. The primary source of microorganisms was a mixture of seawater samples collected from various sites near a power station (Maor David, Hadra, Israel), at which CFA is regularly conveyed, discharged, or dumped.

Measurement of growth and quantity of adsorbed cells. Cultures were monitored spectrophotometrically at 660 nm. The cellular dry weight was determined in cell samples after centrifugation (12,000 × *g*, 10 min). A_{660} of 1.0 corresponds to about 0.4 mg of cells (dry weight) ml^{-1}. The amount of cell protein was determined according to the method of Lowry et al. (19) after alkaline treatment of the cells (0.2 N NaOH, 100°C, 20 min). Viability counts were made by employing nutrient agar (Difco) and a spread-plate inoculation technique. Microscopic examinations were also used to verify growth and the number of microbial strains in the microbial population during both stages of continuous culture.

Measurement of cell hydrophobicity. A standard assay of bacterial adhesion to hexadecane droplets was used to evaluate cell hydrophobicity, as previously described (24).

CFA. CFA was obtained from Israel Electric Company, Ltd. Each 30-kg sample of CFA consisted of a blended mixture of four weekly samples collected at Maor David Power Station or Rutenberg Power Station, Ashkelon, Israel.

Oxide powders. The following analytically graded oxides were used for bacterial assays of adsorption: AgO, Al_2O_3, CdO, GeO_2, HgO, MgO, NiO, PbO_2, TiO_2, ZnO (all obtained from E. Merck AG, Darmstadt, Germany), and magnetite (Fe_3O_4; Sigma Chemical Co., St. Louis, Mo.). The particle size distribution of each oxide powder was restricted by collection of a sieved fraction of particles less than 20 μm in size.

Reagents for desorption of attached cells from TiO_2. Aqueous solutions of the following reagents were used for desorption of attached cells from TiO_2 particles: up to 4 M NaCl or KCl (Merck); up to 1 N hydrochloric acid (Fluka), up to 0.5 M sulfuric acid (Merck), up to 50% (wt/vol) ethanol or 2-propanol (Merck), 1% (wt/vol) sodium dodecyl sulfate (SDS; Sigma), 1% (wt/vol) cetyltrimethylammonium bromide (BDH), 8 M urea (Sigma), and 0.1 M NaOH (Merck).

Standard adsorption assay. The cell suspension (45 ml) in filtered seawater (or 3% NaCl) containing about 0.8 mg of cells (dry weight) ml^{-1} (~0.2 mg of protein ml^{-1}) was mixed with 800 mg of oxide or CFA to which 5 ml of filtered seawater had been added. The mixtures were incubated for 1 h at 30°C with gyratory shaking (100 rpm). Samples were taken periodically and centrifuged (200 × *g*, 4 min) to allow efficient separation of free and adsorbed cells (verified by microscopic examination), and the number of free or adsorbed cells was determined by measurement of cell protein in the separated fractions after their alkaline pretreatment as described above.

Preparation of magnetite biosorbent. Washed cells of *Rhodococcus* strain GIN-1 were mixed with magnetite at a cell-to-magnetite ratio of 1 to 10 (wt/wt) in filtered seawater. The mixture was incubated for 1 h to ensure complete adsorption. The magnetite biosorbent was then collected under a magnetic field of 1 kG with a model EDT magnetic separator (Electromagnetic Davis Tube tester; Eriez Manufacturing Company, Erie, Pa.) equipped with a rotating glass tube through which washing liquids can be flushed or circulated.

Novel biomagnetic separation of TiO_2 from a mixture of oxides. Magnetite biosorbent (20 mg in 2 ml of filtered seawater) was mixed with 200 mg of a mixture of Al_2O_3, SiO_2, and TiO_2 (42, 55, and 3% (wt/wt), respectively) suspended in 48 ml of filtered seawater. A similar quantity of the magnetite biosorbent was mixed with each of the oxides separately. Following incubation for 5 min at 32°C with gyratory shaking, each of the mixtures was subjected to a magnetic field of 2 kG, while the mixed suspension was circulated through the rotating tube by means of a Masterplex peristaltic pump (Cole Parmer Instrument Company, Niles, Ill.) and a pellet was magnetically immobilized near the poles of the magnetic fields. The pellet was then released by interruption of the field and resuspended in fresh filtered seawater, and the suspension was vigorously mixed to release the magnetite. A second magnetic separation was carried out under identical conditions to immobilize the magnetite and wash out a conjugate of the bacterium and oxide. Cell protein was measured to assess the number of bacteria in each fraction. The elemental content of each fraction was determined after the oxide pellets were washed with distilled water.

Determination of elemental content in oxide mixtures and CFA. Elemental analysis was carried out as described by Davison et al. (11). Following a strong acidic digestion, the amounts of the elements in each sample were determined with the aid of an inductively coupled plasma atomic emission spectrometer (model Spectroflame; Spectro, Klaeva, Germany).

Scanning electron microscopy coupled with elemental analysis. For specific elemental analysis of particles with and without bacteria, electron micrographs were taken following standard critical point fixation in a Jeol Electron Microscope (model 840A) equipped with a Link X-Ray Scanning System (High Wycomb, Buckinghamshire, England).

RESULTS

Isolation of bacteria that adhere to titanium dioxide and CFA in continuous culture. A single bacterial species was enriched in the two-stage continuous culture. It predominantly colonized the surfaces of the solid particles and adhered strongly to TiO_2 and CFA granules. Viability counts and cell protein determination indicated that more than 60% of the microbial cells were associated with the particles. Most of the free cells also consisted of this bacterium (with other types of bacteria accounting for less than 0.0001%). After 2 weeks in the presence of TiO_2, other forms of microorganisms (e.g., yeasts or fungi) were not detected in the continuous cultures despite their presence in the inocula. This suggests either that such forms could not grow under the existing conditions or that

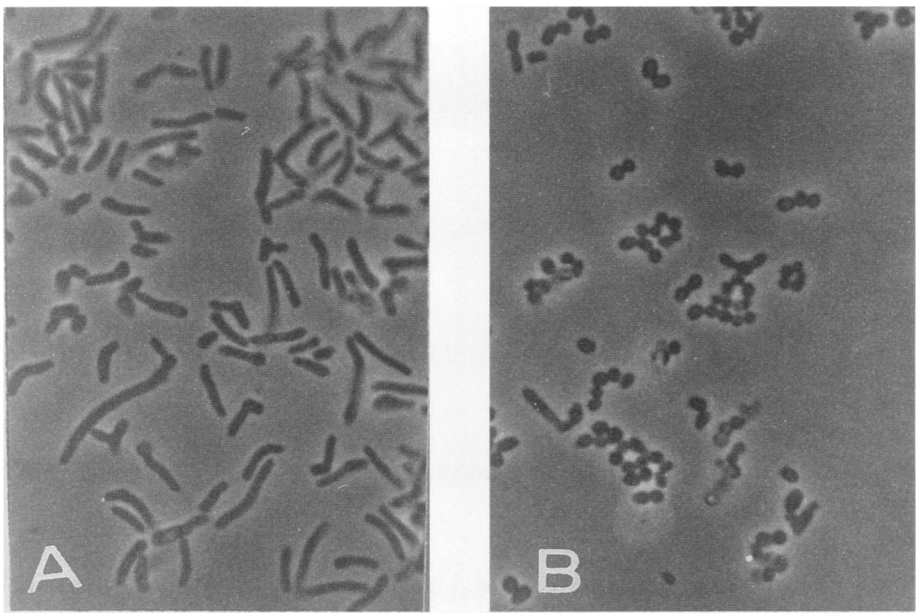

FIG. 1. Phase-contrast micrographs of *Rhodococcus* strain GIN-1 cells from exponential phase (A) and stationary phase (B). Magnification, ×1,200.

they failed to adhere and therefore were washed out of the fermentor. During the second stage, i.e., enrichment in the presence of CFA, the continuously operating culture took on the characteristics of a pure culture of the *Rhodococcus* strain GIN-1 bacterium (verified by viability counts and microscopic examination), presumably possessing advantageous properties under the selective conditions imposed by the CFA.

Identification of isolated bacterium. The isolated bacterium was gram positive and strictly aerobic, with irregularly shaped short or long rod cells (Fig. 1). Its identity as a *Rhodococcus* species was indicated by the use of standard identification kits. The bacterium formed orange-colored colonies on nutrient agar and accordingly was termed *Rhodococcus* strain GIN-1. Chemotaxonomic analysis of the cell wall composition and fatty acid profile verified the above genus identity. The diamino acid in the cell wall was *meso*-diamino pimelic acid (*meso*-DAP), and mycolic acids were present. The fatty acid profile showed that the major components are straight-chain saturated and unsaturated acids together with branched-chain acids with the CH_3 group on C-10, in particular, tuberculosic acid (10-methyloctadecanoic acid). The bacterium exhibited a low protein-to-cell (dry weight) ratio of 0.26 (wt/wt), indicating that the cells accumulate other polymers during growth. The bacterium is capable of growth at temperatures up to 45°C. Some of its physiological properties, the decomposition of organic compounds as carbon and nitrogen sources, and some of its functional biochemical properties suggested that the most closely fitting profile is that of *Rhodococcus rhodochrous* (13), despite the atypical finding of maltose, sodium adipate, testosterone, glycerol, and trehalose as the sole sources of carbon utilization. The *Rhodococcus* strain GIN-1 bacterium was cultivated readily in a defined medium with glucose as the carbon source. Small amounts of yeast extracts (~0.4 g/liter) were essential for growth, and larger amounts (>4 g/liter) promoted the growth significantly. The specific requirement supplied by the yeast extract is not yet known.

Growth cycle and adhesive properties of *Rhodococcus* strain GIN-1. During a growth cycle, the bacterial cells underwent morphological changes that affected their adhesiveness and other properties of binding to TiO_2 or CFA particles and among themselves. Fractions of adhesive cells from the different growth phases are presented in Fig. 2. During the logarithmic phase, the cells changed from relatively nonadhesive short rods into highly adhesive, long branched cells. More than 90% of the cells were able to adhere to TiO_2. At the late-log and stationary phases, the cells became shorter again, and their adhesive capacity dropped correspondingly. Less than 40% of the stationary phase cells adhered to the oxide particles.

Salt requirement for adsorption. Adsorption of *Rhodococcus* strain GIN-1 to TiO_2 or CFA particles occurred only when the concentration of salt, e.g., NaCl or KCl, was raised above about 10 mM (Fig. 3). This was observed in the standard assay, in which 31 mg of cells (7.7 mg of cell protein) was blended with 800 mg of the oxide. About 95% of the cells were adsorbed on the particles (~9 mg of protein g of oxide^{-1}). No significant

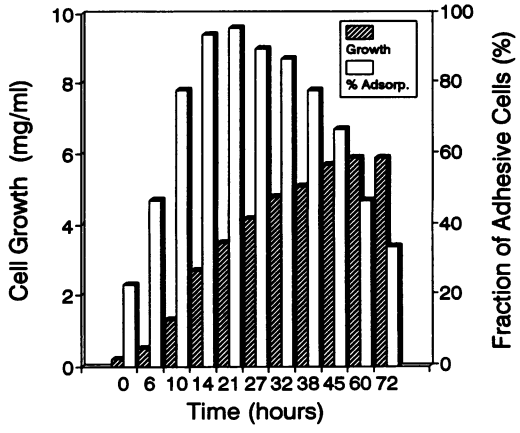

FIG. 2. Adhesion of *Rhodococcus* strain GIN-1 cells to TiO_2 at different stages of the growth cycle. Adsorp., adsorption.

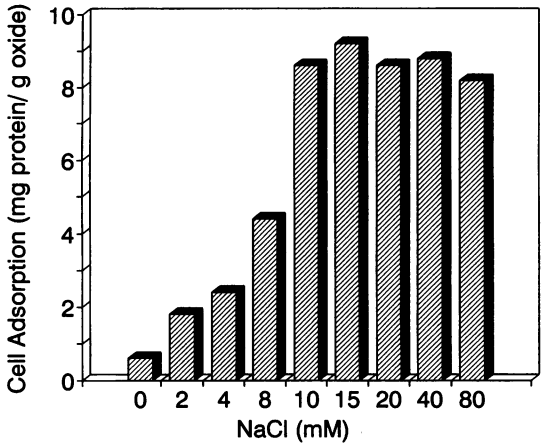

FIG. 3. Dependence of adherence of *Rhodococcus* strain GIN-1 to TiO_2 on salt concentration.

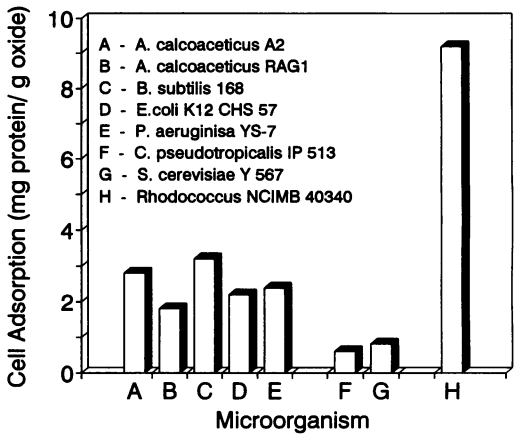

FIG. 5. Comparison of adsorption of various microorganisms on TiO_2.

adsorption was observed in distilled water. Attempts to remove the adsorbed cells by dilution of the salt content or even by extensive washing of the particles with distilled water were unsuccessful. The maximum weight of cells that can be immobilized by TiO_2 may reach as high as 0.6 to 0.7 g of cells g of oxide^{-1}.

Effects of pH and temperature. The effect of pH on adsorption of *Rhodococcus* strain GIN-1 to TiO_2 was examined in the standard assay in a 3% NaCl solution. The pH levels of the solutions were adjusted with HCl or NaOH. Adhesion of the cells (~30 mg of cells [dry weight]) was almost unaffected by pH. More than 90% of the cells adhered to the oxide over a wide pH range of 1 to 9. Adsorption was slightly hindered (lower by ~10%) at low pHs of 1 to 3. The weight of cells adsorbed per gram of TiO_2 was independent of temperature over a range of 4 to 80°C.

Specificity of adsorption. The specificity of adsorption of *Rhodococcus* strain GIN-1 was examined in two sets of adsorption assays (see Materials and Methods). In the first set of experiments, we compared the surface adhesion of *Rhodococcus* strain GIN-1 cells among different oxides. Equal weights of *Rhodococcus* strain GIN-1 cells (30 mg) were exposed to identical amounts (800 mg) of each oxide in seawater. The relative weights of cells that were adsorbed on each of the oxides after 1 h are presented in Fig. 4, which illustrates moderate differences in the adhesive capacity of *Rhodococcus* strain GIN-1 to the various oxides. The highest degree of adsorption was obtained with titanium and zinc oxides.

In a second set of experiments, the adhesion of *Rhodococcus* strain GIN-1 cells to TiO_2 was compared with that of other microorganisms. The results (Fig. 5) show a high efficiency of adsorption of the bacterium on this oxide (more than 97% of added cells). The efficiency of adsorption obtained with other bacteria was much lower (up to 15% of added cells). Most of these bacteria died upon adsorption and were easily desorbed during treatment with mild detergents or acid solutions. Yeast strains were adsorbed on TiO_2 particles to a smaller extent than the bacteria and could be easily desorbed from the particles by any of the treatments used.

Kinetically selective adsorption to TiO_2. The differences in the kinetics of adsorption of *Rhodococcus* strain GIN-1 to TiO_2, Al_2O_3, and magnetite are of great interest from a practical point of view. The results (Fig. 6) pointed to extremely rapid adsorption on TiO_2, a moderate rate of adsorption on magnetite, and rather slow adsorption on Al_2O_3. When a suspension of cells (23 mg of cells [dry weight]/g of oxide) was

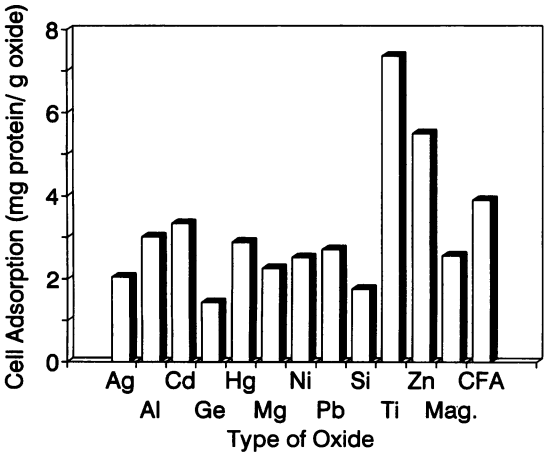

FIG. 4. Adsorption of *Rhodococcus* strain GIN-1 on various oxides. Mag., magnetite.

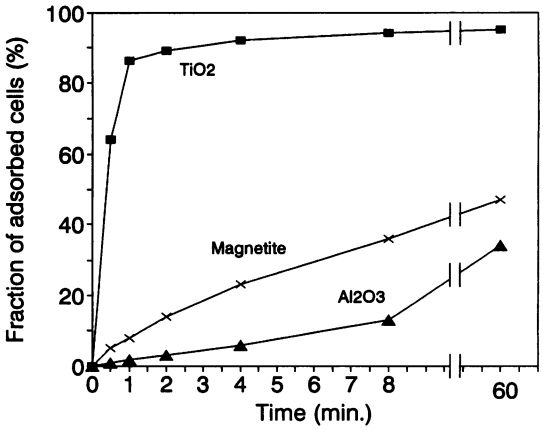

FIG. 6. Kinetics of adsorption of *Rhodococcus* strain GIN-1 on TiO_2, Al_2O_3, and magnetite.

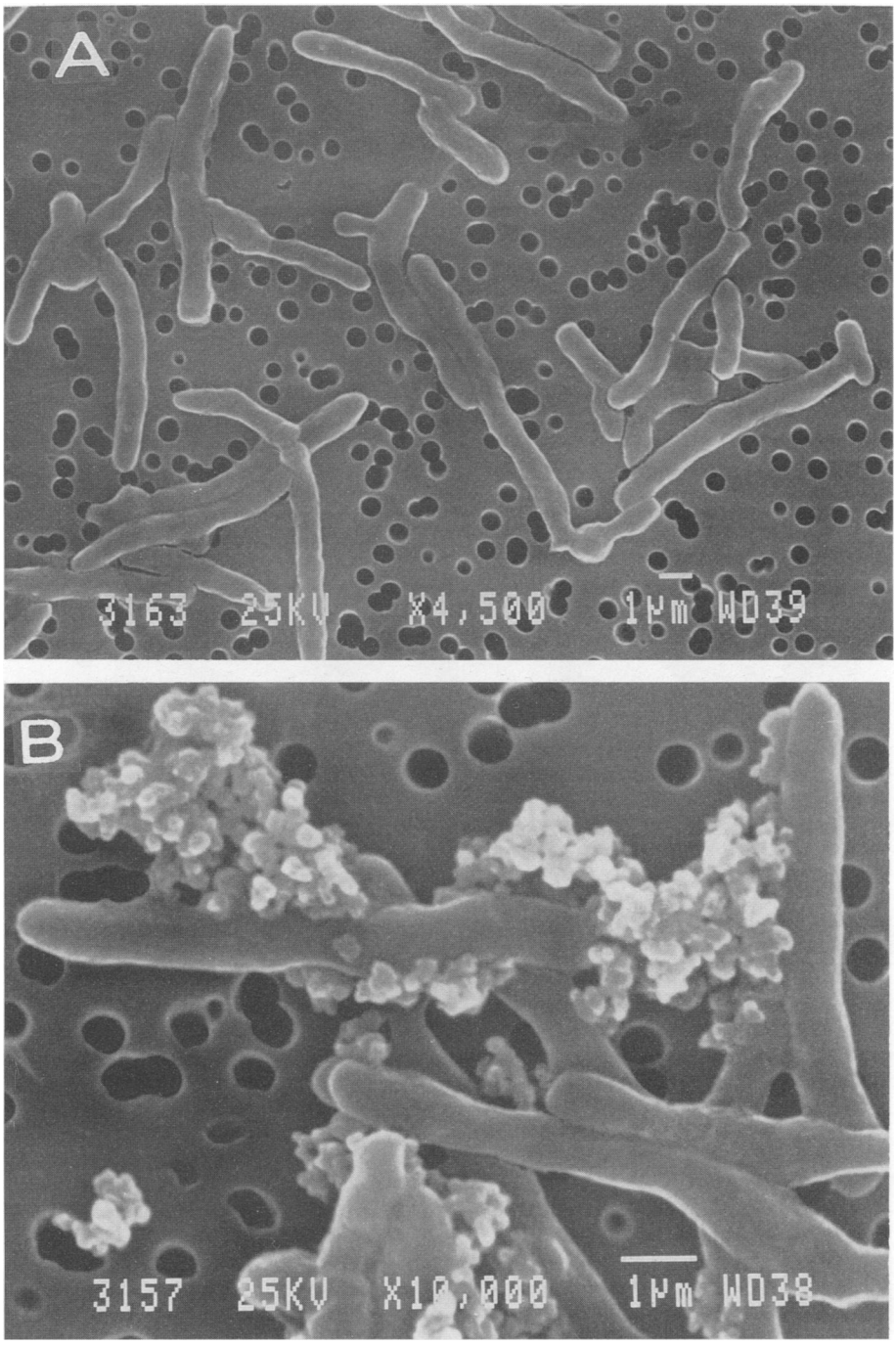

FIG. 7. Scanning electron micrographs of *Rhodococcus* strain GIN-1. (A) Free cells; (B) cells with adsorbed TiO_2 particles.

mixed with each of the oxides in seawater (at an initial ratio of 24 of mg of cells [dry weight]/g of oxide, corresponding to 6 mg of protein/g of oxide), more than 85% of the cells (~20 mg of cells [dry weight]/g of oxide, or ~5 mg of protein/g of oxide) were adsorbed on TiO_2 after 1 min. In contrast, about 10% of the cells adhered to magnetite and less than 8% of the cells were adsorbed on Al_2O_3 during the same period. Scanning electron micrographs of the free bacteria and bacteria with adsorbed TiO_2 particles are shown in Fig. 7.

Adsorption to CFA. Light and electron microscopic observations of CFA particles following short-term adsorption of *Rhodococcus* strain GIN-1 cells revealed their nonuniform attachment to the particles (Fig. 8). Combined scanning electron microscopy and X-ray diffraction elemental analysis of more than 200 CFA particles with adsorbed bacteria, as shown in Fig. 8, indicated that the surfaces of cell-carrying particles were at least twice as rich in titanium (2 to 4%) as those of naked CFA particles (0.5 to 1.0%). Other elements (e.g., Al, Ca, Fe, and Si) did not appear to vary significantly from their average concentrations in CFA.

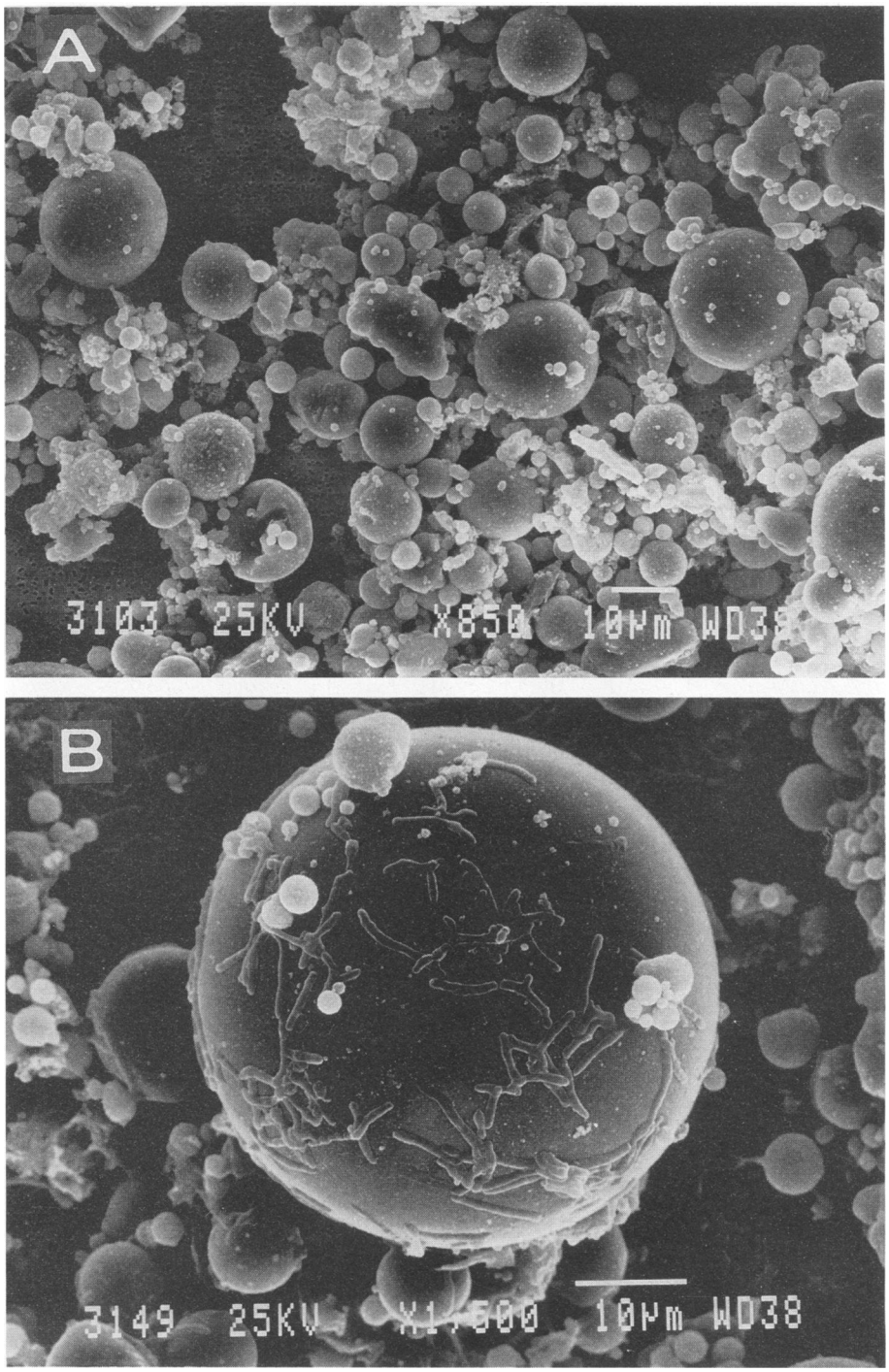

FIG. 8. Scanning electron micrographs of *Rhodococcus* strain GIN-1 cells adsorbed on CFA particles. (A) General view of CFA particles; (B) *Rhodococcus* strain GIN-1 cells adsorbed selectively on a fraction of titanium-rich particles in CFA; (C) closer view of adsorbed cells.

Desorption of *Rhodococcus* strain GIN-1 cells from TiO_2. The attachment of *Rhodococcus* strain GIN-1 cells to TiO_2 appears to be extremely strong. Attempts to achieve desorption of the bound cells from the particles by the use of high or low salt concentrations or alcohol solutions (Fig. 9), or with dilute hydrochloric or sulfuric acids (results not shown), were unsuccessful. Treatment with pure SDS or cetyltrimethylammonium bromide (CTAB) detergents (1% wt/vol) resulted in release of less than 10% of the attached cells, and the desorbed cells lost their regular shape. Strong alkaline treatment removed the bound cells but led to their severe disruption. High concentrations of urea (8 M), especially in the presence of 1% SDS, enabled removal of most of the attached cells (>90%). Microscopic examination after such treatment revealed that most TiO_2 particles were free of cells (data not shown); this was further verified by the fact that the amount of cell protein

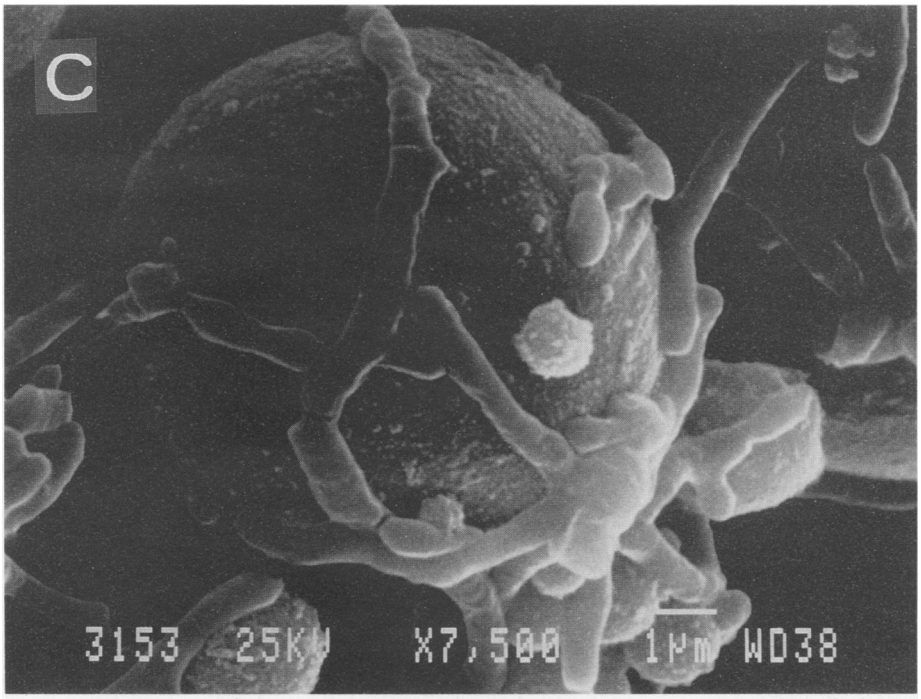

FIG. 8—*Continued.*

released from the particles corresponded to the amount initially loaded during adsorption.

Translocation of cells from magnetite to TiO_2. *Rhodococcus* strain GIN-1 cells were easily translocated from magnetite to TiO_2 particles, but the reverse migration did not occur (Table 1). This unidirectional translocation was observed during exposure of TiO_2 particles to cells that were previously attached to magnetite. The results presented in Table 1 suggest that binding to magnetite is weaker than that to TiO_2.

Biomagnetic separation. A fraction rich in titanium oxide (Table 2) was isolated from a mixture of three oxides, SiO_2, Al_2O_3, and TiO_2 (oxide mixture, Table 2), whose elemental proportions simulated that in CFA (see Materials and Methods). Short-term interaction of the magnetite-bacterium conjugate (magnetite-biosorbent, Table 2) with the above mixture was immediately followed by magnetic separation, which left Al_2O_3 and SiO_2 unbound (nonadsorbed oxides, Table 2) and enabled us to collect mainly TiO_2 associated with the magnetite-biosorbent (adsorbed oxides, Table 2). The temporary triple conjugate was resuspended in seawater, and the suspension was vigorously mixed to allow the magnetite to become detached from the complex. A second magnetic separation resulted in the immobilization and efficient recovery (>90%) of magnetite in a relatively pure state at the poles of the separator (released magnetite, Table 2) and, at the same time, enabled us to collect the newly formed conjugate of bacterium and TiO_2 at the bottom of the separator tube. This fraction contained more than 75% of TiO_2 with the attached bacterial cells and with minimum contamination by other oxides (Table 2, titanium dioxide-rich fraction). Results obtained with individual oxides (data not shown) indicated that TiO_2 is the only oxide immobilized during such short-term biomagnetic separation and that only minor amounts of the other two oxides are adsorbed by the magnetite-biosorbent. Analyses of cell protein in each fraction indicated that about 90% of the bacterial cells could be accounted for in the TiO_2 fraction. The rest of the bacterium was found associated with the other oxides, probably Al_2O_3, since small amounts were associated with Al_2O_3 and negligible amounts were associated with SiO_2 when individual oxides were tested.

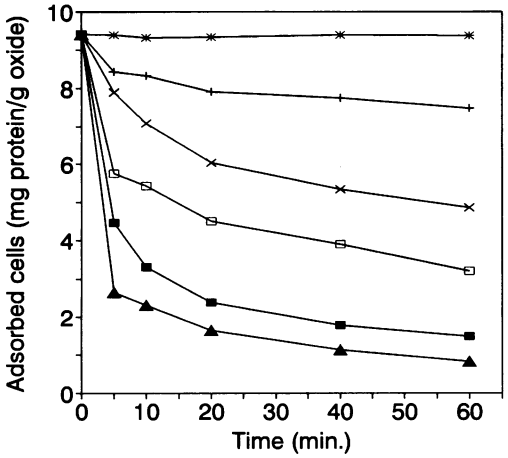

FIG. 9. Desorption of bound *Rhodococcus* strain GIN-1 cells from TiO_2. ▲, 0.1 M NaOH; ■, SDS + urea; □, 8 M urea; ×, 1% SDS; +, 50% 2-propanol; *, 3% NaCl.

DISCUSSION

The isolated bacterium, *Rhodococcus* strain GIN-1, adheres strongly to TiO_2 and CFA particles. This ability to adhere to solid surfaces gives the cells a great advantage under the steady-state conditions in continuous culture, in which the majority of the cells are immobilized on particles and are constantly growing. The bacterium was isolated from a stream

TABLE 1. Unidirectional translocation of *Rhodococcus* strain GIN-1 cells from magnetite to TiO_2

Test[a]	Adsorption of cells (mg of cell protein/g of oxide)[b]			
	Initial[c]		Final[d]	
	Magnetite	TiO_2	Magnetite	TiO_2
I	0.21 (100)	None	<0.005 (2.5)	0.19 (90)
II	None	0.23 (100)	<0.002 (0.9)	0.21 (91)

[a] In test I, the initial adsorption of cells to magnetite is followed by the addition of TiO_2; in test II, the initial adsorption of cells to TiO_2 is followed by the addition of magnetite.
[b] The percent recovery of cells relative to the initial cell input is shown in parentheses.
[c] Initial mixture of cells and adsorbing oxide.
[d] Final mixture of cells and two oxides.

of cooling seawater in the vicinity of a coal-fired power station, where it dominated the bacterial population (unpublished data). This was not surprising, since conditions in the continuous culture in the laboratory resemble those near coal-fired power stations, where warm streams of cooling seawater, relatively rich in nutrients, suspend and flush CFA and sand particles into the open sea. Although the growth medium in the continuous culture was relatively rich in nutrients, only a few other strains of microorganisms were present and their numbers were extremely small. This may have resulted from the selective pressures imposed (adhesion to TiO_2 or CFA in seawater).

The isolated bacterium is highly resistant to deleterious effects that might be caused by binding to the surface of TiO_2 particles. This oxide binds many biomolecules and may damage biological cells and even cause severe health problem in animals (7, 8, 17). In contrast to other bacteria, which eventually died upon adsorption on TiO_2 particles, *Rhodococcus* strain GIN-1 showed excellent survival and its growth was not hindered in presence of TiO_2. This demonstrates its unique ability to cope with the surface energy of these oxide particles. In such surface-to-surface interactions, the extracellular envelope of *Rhodococcus* strain GIN-1 must provide the cells with adhesive properties and at the same time shield them from damage caused during binding to the surface. We found that the overall protein content of the bacterium was very low (26% g of protein/g of cells [dry weight]) compared with 55 to 60% in other bacteria, which suggests that the extracellular envelope of this bacterium might consist of accumulation of other polymers. Analyses carried out to identify the bacterium and preliminary results obtained during cell fractionation indicated a relatively high proportion in the bacterial wall of mycolic acids (lipo-oligosaccharidic compounds) associated with hydrophobic proteins. This is supported by reported findings that extracellular polysaccharides with hydrophobic ligands might play a major role in adhesion and in the protection of oral bacterial flora from TiO_2, as previously observed with oral microflora (15, 23). Recent studies with *Rhodococcus* strains indicate that polysaccharidic capsules, amphiphilic polysaccharides, or mycolic acids are involved in the adhesion of various *Rhodococcus* strains to solid and liquid surfaces (3, 4, 22).

The adhesive characteristics of the cell are acquired during balanced growth, regardless of the location of the bacteria (free or adsorbed). Free *Rhodococcus* strain GIN-1 cells in the continuous culture were adhesive. Moreover, the results concerning the growth cycle in a batch culture indicated that the adhesive characteristics of the cells are somehow regulated; the number of adherent cells peaks at the exponential phase of growth and drops when growth is unbalanced at the stationary phase.

The mechanism by which the bacterium adheres to TiO_2 or CFA or regulates its adhesive capacity during a growth cycle has yet to be elucidated. We have not yet clarified which of the described phenomena, e.g., London-van der Waals, electrostatic, and interfacial tension forces (2, 9, 10, 14, 20), are involved in the short- and long-term adhesion of *Rhodococcus* strain GIN-1. In several *Rhodococcus* strains, hydrophobic or amphiphilic molecules (e.g., mycolic acids or amphiphilic polysaccharides) are responsible for the adhesiveness to solid surfaces (3, 4, 22). As in those cases, the extracellular envelope of *Rhodococcus* strain GIN-1 plays a major role in permanent adhesion, possibly through polymeric cross-linking (2, 9, 10). This might explain why mild acids or concentrated alcohols failed to remove the bacterium from the particles.

Previous reports (9, 10) have also indicated that cell surface properties determine the capacity and selectivity of adhesion and that modification of the cell surface might affect their adhesive properties. Changes in hydrophobicity and in structures that establish hydrogen bonding might explain the decrease in adhesion of stationary cells to TiO_2. This notion is supported by the fact that adhesion is strong in the presence of high salt concentrations and that even at low concentrations it is almost irreversible. The measures required for efficient desorption of the cells (SDS plus 8 M urea) also point to the likelihood of hydrogen bonding and strong hydrophobic interactions. It is therefore hypothesized that a certain hydrophobic component(s) on the surface of the cells participates in a hydrophobic interaction with TiO_2 particles. Release of such a component, its masking by hydrophilic moieties, or alteration of its structure might reduce the adhesive capacity of the cells

TABLE 2. Biomagnetic separation of TiO_2 particles from a mixture of oxides

Mixture	Cell protein (μg)[a]	Amt of oxide (mg)[b]			
		Fe_3O_4	Al_2O_3	SiO_2	TiO_2
Oxide mixture			84 (100)[c]	110 (100)	6 (100)
Magnetite-biosorbent	2,230 (100)	19.8 (100)			
Adsorbed oxides[d]	2,100 (94)	19.4 (98)	4.4 (5)	1.2 (1)	5.1 (85)
Nonadsorbed oxides[d]	180 (8)	0.2 (1)	78 (93)	108 (98)	0.7 (12)
Titanium dioxide-rich fraction[e]	1,930 (87)	0.2 (1)	1.9 (2)	0.3 (0.3)	4.7 (78)
Released magnetite[e]	27 (1)	18.6 (94)	1.7 (2)	0.6 (0.5)	0.3 (5)

[a] Protein was measured by the method of Lowry et al. (19) after alkaline pretreatment of samples.
[b] Elemental content was determined with an inductively coupled plasma atomic emission spectrometer and recorded as the content of the corresponding oxide.
[c] Percent recovery of each oxide relative to its input is indicated in parentheses.
[d] First magnetic separation step.
[e] Second magnetic separation step.

or play a major role in their detachment from the binding surfaces. Evaluation of the hydrophobicity of the cells confirmed that cells taken from the stationary stage of the growth cycle, which are less adhesive to TiO_2, are also less hydrophobic than those taken from the exponential phase. This finding agrees with results from other laboratories indicating that the hydrophobicity of the *Rhodococcus* strain plays a major role in its adhesion to solid particles (26, 27).

The specificity of adsorption of *Rhodococcus* strain GIN-1 on solid surfaces appears to be limited, though its capacity for adhesion to TiO_2, ZnO, and CFA particles is considerably high. In view of this, the ability of the bacterium to interact rapidly and strongly with some of the CFA particles was surprising, since these oxides constitute only a very small proportion of the ash. However, the finding that the shell of certain particles in the ash is richer in TiO_2 than the core (as indicated by scanning electron microscopy with X-ray analysis and by unpublished data from acid leaching experiments) may explain the strong binding of the bacterium to a limited fraction of the CFA particle.

The high affinity of the bacterium toward TiO_2 is indicated by its faster selective adhesion to TiO_2 than to Al_2O_3 and by the rapid unidirectional translocation from magnetite to the TiO_2 surface. The extremely rapid adhesion to TiO_2 compared with adhesion to Al_2O_3 was the basis for the separation of these two oxides.

The ability of the bacterium-magnetite conjugate to be adsorbed rapidly and selectively on TiO_2 indicates that the cells serve as multibinding sites for various surfaces. This was observed primarily in the independent binding of the bacterium to different oxides. The brief temporary stage of dual binding of the bacterium to magnetite and TiO_2 enabled us first to separate a triple bacterium-magnetite-TiO_2 complex under the magnetic field. The subsequent detachment of the bacterium from magnetite and its translocation to TiO_2 particles facilitated the isolation of a TiO_2-rich material from a mixture of oxides and the efficient recycling of the magnetite. This biomagnetic separation procedure is the basis of a recently approved patent (25). Biomagnetic separation is currently under investigation on a larger scale, with a view toward establishing a process for recovery of titanium-rich particles from CFA. One aspect of that study involves the localization and isolation of the extracellular biomolecules responsible for selectivity of adhesion to the target particles and for protection of the cells during binding to TiO_2. Use of a purified fraction of the adhesive molecules is likely to enhance selectivity of binding, with consequent improvement of the separation procedure.

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