African Journal of Biotechnology Vol. 11(58), pp. 12206-12212, 19 July, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.506
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Selection of culturable environmental microbial strains for cellular immobilization: Association of phenotypic adhesive characteristics and quantitative cellular retention

Suzana Cláudia Silveira Martins[1]*, Claudia Miranda Martins[1], Ana Vitória de Oliveira[1], Larissa Maria Cidrão Guedes Fiúza[1], Sandra Tédde Santaella[2]

[1]Laboratory of Environmental Microbiology, Department of Biology, Sciences Center, Federal University of Ceará, Fortaleza, CE, Brazil.
[2]Sea Sciences Institute, Federal University of Ceará, Fortaleza, CE, Brazil.

Accepted 29 June, 2012

Environmental pollution by organic compounds is a global problem. Biological treatment methods are used to restore polluted environments. Microbial immobilization on abiotic surfaces is a recent strategy to improve the efficiency of these processes. In this technique, cell adhesion is a fundamental step for subsequent colonization and biofilm formation. Therefore, the use of strains with adhesive properties is a critical factor for successful immobilization. In this work, culturable environmental microbial strains were phenotypically characterized regarding their hydrophobicity, adhesion to polystyrene and production of exopolysaccharides and amyloid fibers. The cell retention was quantified by counting viable cells using polyurethane foam as material support. The degree of hydrophobicity varied from moderately hydrophobic to hydrophilic, while the adhesion to polystyrene and production of exopolysaccharides and amyloid fibers ranged from strong to negative. The results of qualitative tests were transformed into scores and a direct relationship between the qualitative characteristics and number of adhered cells on polyurethane foam was observed. The Gram-negative bacterium *Serratia marcescens* and the yeast *Candida rugosa* showed the best results and were selected for further immobilization tests.

Key words: Hydrophobicity, cell adhesion, cell immobilization, *Serratia marcescens*, *Candida rugosa*.

INTRODUCTION

One of the greatest problems the world is facing today is the increase of chemical pollution of the environment; causing grave and irreparable damage to the earth. Biological methods have been widely studied as practical, environmental friendly and economical alternative for bioremediation of chemical pollutants (Kumar et al., 2011). Microbial immobilization is a promising strategy to enhance the efficiency of these processes (Ahmad et al., 2012). In this technique, toxic and recalcitrant pollutant compounds are adsorbed on solid surfaces and transformed by the immobilized microbial cells metabolism. Physical confinement provides high biomass concentration, production of protective extrapolymeric substances (EPS) and higher stability than systems with suspended cells and also permits the reuse of cells (Cai et al., 2011).

Among the methods used for cell immobilization, natural adsorption is one of the simplest (Zhou et al., 2008). In this technique, the initial adhesion between the microorganism and abiotic solid surface is fundamental

*Corresponding author. E- mail: suzana220@gmail.com. Tel: +55 85-3366 9815. Fax: +55 85 3366 9806.

for the success of cell immobilization. However, the parameters governing this step are complex, not completely understood and vary among microorganisms. Sensitive and standardized techniques such as atomic force microscopy, which determines the behavior of individual cells and the interactions at the molecular level, are still limited, costly and not always available (Goulter et al., 2009). Thus, qualitative tests of hydrophobicity, adhesion on solid surfaces and production of amyloid fibers and exopolysaccharides (EPS), due to their simplicity and quick and easy implementation, are valuable tools for preliminary selection of promising microbial strains for cell immobilization (Ubbink and Schar-Zammaretti, 2007; Oulahal et al., 2008; Goulter et al., 2009; Pagedar et al., 2010).

In this respect, flexible polyurethane foam has gained relevance as a microbial carrier for its good mechanical properties, high porosity, large adsorption surface, resistance to organic solvents and microbial attack, easy handling, regenerability and cost effectiveness (Patil et al., 2006).

Given the above, the aims of the present work were to evaluate the phenotypic adhesion characteristics of culturable environmental microbial strains, to compare these qualitative characteristics with quantitative cell retention on polyurethane foam and to select the most promising strains for further cell immobilization experiments.

MATERIALS AND METHODS

The microbial strains used in this work are from the culture collection of the Environmental Microbiology Laboratory of the Department of Biology, Federal University of Ceará, where they are preserved on high-layer nutrient agar (NA) medium (Merck, Germany) under sterile mineral oil at 4°C.

Considering the differences among cell microbial surfaces, the Gram-positive bacterium *Micrococus luteus*, the Gram-positive spore forming bacterium *Bacillus* spp., the Gram-negative bacterium *Serratia marcescens* and the yeast strain *Candida rugosa* were tested. *Pseudomonas aeruginosa* ATCC 25619 was used as standard strain. The bacterial strains were isolated from the wastewater treatment plant of the Pici Campus of Federal University of Ceará, Brazil. The isolates were identified according to Machado (2002), using traditional microbiological methods. The *C. rugosa* strain was isolated from wastewater treatment of petroleum products and lubricants (LUBNOR refinery, Fortaleza, CE, Brazil) and also identified by traditional methods according to Rocha et al. (2007).

The pigmented bacterial strains *S. marcescens* and *M. luteus* were selected because these substances can give selective advantages which contribute to higher resistance and tolerance to microorganisms facing toxic substances (Arrage et al., 1993).

Inoculum preparation

Each strain was inoculated separately into a Petri dish with Luria-Bertani (LB) medium containing (per liter): 10 g tryptone, 5 g yeast extract, 5 g NaCl and 17 g agar. The plates were incubated at 30°C for 48 h. Then, a colony of each strain was transferred to 100 ml of LB broth and incubated for 18 h at 26°C on a horizontal shaker (150 rpm). After this time, 1 ml of this culture was transferred to 100 ml of LB broth and again incubated under the same conditions (Komlos et al., 2005). The absorbance of this suspension was adjusted to approximately 0.6 at 600 nm, corresponding to concentrations between 10^8 and 10^9 colony-forming units per milliliter (CFU/ml).

Production of amyloid fibers

Qualitative detection of amyloid fibers was performed by cultivation of the microbial strains on Congo red agar (CRA) according to the protocol described by Freeman et al. (1989) and validated by Arciola et al. (2002). The strains were cultured on CRA plates prepared by adding 0.8 g of Congo red and 36 g of saccharose (Sigma) to 1000 ml of brain heart infusion (BHI) agar (Oxoid). The plates were incubated for 24 h at 30°C and subsequently overnight at room temperature (26°C). The colonies were examined for the intensity of the color red. Colorless colonies were classified as negative (0), light red as poor producers (1), dark red as moderately productive (2) and very dark red as strongly productive (3).

Production of exopolysaccharide (EPS)

The standard tube (ST) method described by Christensen et al. (1982) was used. The strains were inoculated in glass tubes containing BHI medium, incubated at 35°C for 24 h. After this time, the contents were decanted and the tubes were then stained with 1% safranin for 7 min. A positive result was indicated by the presence of an adherent film of stained material on the inner surface of the tube. The presence of stained material at the liquid-air interface alone was not regarded as indicative of slime production. EPS production by each isolate was scored as negative (0), weak (1), moderate (2) or strong (3).

Cell surface hydrophobicity (CSH)

Hydrophobicity was determined by an affinity test for xylene. Aliquots of 4 ml of each culture containing 10^8 to 10^9 CFU were added in phosphate buffered saline (PBS), at pH 7.2, mixed with 400 µl of xylene (Merck, Frankfurt, Germany) and incubated in a water bath at 37°C for 10 min. The suspensions were shaken and left to rest for 15 to 20 min at room temperature (26°C). The aqueous lower layer was removed and the absorbance read at 640 nm. The results were used to calculate the hydrophobicity index (HI), using the equation: $HI = (A_{640}-B_{640}) \times 100/A_{640}$, where A_{640} represents the optical density of the microbial suspension before treatment with xylene, and B_{640} represents the optical density of the aqueous phase after treatment of the bacterial suspension with xylene (Serebryakova et al., 2002). Values ≥ 50% represent strains highly hydrophobic (3), values ≥ 20% and < 50% show moderate hydrophobicity (2) and < 20% are considered hydrophilic strains (1) (Mattos-Guaraldi et al., 1999).

Microbial adhesion to polystyrene

Specific interactions between cell and hydrophobic polystyrene surfaces is the basis of this technique, as described by Rosenberg (1981) and adapted as described below: sterile 80-mm diameter disks of polystyrene were pressed on the surface of NA plates measuring 150 x 15 mm containing isolated colonies of each microbial strain. The replica of the colonies on the polystyrene surface was washed for 2 min to remove the cells which were not firmly bound. Translucent areas on the polystyrene surface

corresponding to colonies of adhered cells were indicative of a positive test. To facilitate visualization and comparison with the original colonies, each replica was fixed by dipping it in ethanol P.A 96º GL (Sigma-Aldrich) and was then stained with 2% crystal violet (Sigma). The strains were classified according to the following scale: (0) negative, (1) weak, (2) moderate and (3) strong.

Motility

Since motility is suggestive of the presence of flagella and this structure is considered important for cell adhesion, the bacterial strains were evaluated for this parameter. One drop of inoculum of each culture was deposited on a coverslip, which was inverted on an excavated slide and observed under an Olympus CH 831 optical microscope using 100x objectives (Aygan and Arikan, 2007). The results were reported as (+) positive or (-) negative.

Polyurethane foam

Polyurethane foam of density 23 kg/m^3 was cut into coupons of dimensions of 10 x 10 x 5 mm, corresponding to a surface area of 4.0 cm^2. The coupons were washed with liquid detergent and water, rinsed with distilled water, immersed in 70% ethyl alcohol for 1 h, rinsed with distilled water, dried at 70°C for 4 h, sterilized at 121°C for 15 min and dried overnight at 70°C (Parizzi et al., 2004; Quek et al., 2006; Wang et al., 2009).

Quantitative adhesion on polyurethane coupons

A volume of 100 ml of inoculum of each culture was transferred to a 500 ml Erlenmeyer flask containing 250 ml of Luria-Bertani broth diluted 10 times with respect to its original concentration (g/l) (tryptone 1.0, yeast extract and NaCl, 0.5). Polyurethane coupons previously cleaned and sterilized were added in each flask. The flasks were incubated at 26°C under horizontal orbital agitation (150 rpm) for 24 h.

After 2, 4, 6, 8, 10 and 24 h of incubation, the coupons were aseptically removed from each flask and added to tubes of 12.0 x 120.0 mm containing 10 ml of 0.1% peptone water (w/v) as diluent. These tubes were swirled in a vortex for 1 min to release loosely adhered cells. The cells that remained on the coupons after this procedure were considered to have adhered. Thus, the first suspension was discarded, the coupons were transferred to tubes with 10 ml of the same diluent and again stirred for 1 min (Parizzi et al., 2004; Vesterlund et al., 2005; Boari et al., 2009; Careli et al., 2009). The bacterial suspensions were diluted and inoculated in triplicate on plates containing the medium agar tryptone glucose yeast extract (ATGE) (Merck, Germany), which were incubated at 37°C for 48 h. The yeast count was performed on potato dextrose agar (PDA) (Merck, Germany) modified by adding tartaric acid to adjust the pH to 3.5 and incubated at 26°C for 72 h. The results were expressed as colony forming unit/cm^2 (CFU/cm^2) by the following transformation:

$$CFU/cm^2 = V (A / a) \times M \times D$$

Where, V = volume of diluent; a = aliquot plated; M = average counts after incubation; D = decimal dilution; A = area of the matrix.

Statistical analysis

All statistical analyses were performed using GraphPad Prism version 5.00 (GraphPad Software, San Diego, CA), at 95% confidence level. The data of the original microbial counts were log transformed. The differences in the quantitative cell retention were examined by the Friedman test, followed by Dunn's multiple comparison. Spearman's correlation coefficient was used to assess the relationship between the contact time and cell adhesion. All experiments were performed in triplicate with at least two repetitions. The reading of the qualitative tests was performed independently by two different observers.

RESULTS

The *M. luteus* strain exhibited colonies with weak red pigmentation on CRA medium. The other microorganisms showed dark red colonies, similar to the control strain *P. aeruginosa* ATCC 25619.

The red viscid slime layer on the glass tubes represented the EPS production. The Gram-negative bacteria *S. marcescens* and control strain *P. aeruginosa* ATCC 25619 presented similar results, followed by *C. rugosa*, *M. luteus* and *Bacillus* spp.

The motility was positive for *P. aeruginosa* ATCC 25619, *S. marcescens* and *Bacillus* spp., suggesting the presence of flagella, while the *M. luteus* was negative for this characteristic. *S. marcescens* showed an intense purple color on polystyrene, similar to *P. aeruginosa* ATCC 25619 and suggestive of cell adhesion on this hydrophobic surface. *C. rugosa* showed a less intense color; followed by *Bacillus* spp. The *M. luteus* strain was negative in this test.

The HI values of *P. aeruginosa* ATCC25619, *S. marcescens*, *C. rugosa, B. cereus* group and *M. luteus* were 25, 28, 26, 9 and 0%, respectively.

The results of the qualitative tests are summarized in Table 1 and the quantitative adhesion of microbial strains on polyurethane foam is shown in Figure 1. The Spearman coefficient (r) of the *P. aeruginosa* ATCC 25619, *S. marcescens* and *C. rugosa* strains showed a significant positive correlation between the concentration of viable cells and the contact time. The number of viable cells of *S. marcescens* and *C. rugosa* strains was not significantly different from the standard strain *P. aeruginosa* ATCC 25619. After 24 h, these strains reached concentrations of about 10^7 CFU/cm^2. The concentration of approximately 10^5 CFU/cm^2 of *M. luteus* was significantly lower than that determined for *P. aeruginosa* ATCC 25619 and was not characteristic of biofilm formation.

The *Bacillus* spp. was detected on polyurethane coupons only after 24 h of contact at concentrations of 10^6 CFU/cm^2.

The adhesion of bacterial vegetative cells and spores of *Bacillus* spp. was evaluated. The strain was immobilized on polyurethane foam coupons and the number of adhered cells was monitored before and after a heat treatment (80°C/10 min) at intervals of 5, 10, 24, 48, 72, 96 and 120 h (Figure 2). After 5 and 10 h, no colony was detected from the polyurethane coupons, but after this period, there was no difference between the cell concentration of adhered cells before and after heat treatment.

Table 1. Adhesive cell qualitative characteristics of culturable environmental microbial strains.

Microbial strain	Adhesion phenotypic characteristic					Total score
	AF	EPS	AP	HI	Mot	
P. aeruginosa ATCC 25619	2	3	3	2	+	10
S. marcescens	2	3	3	2	+	10
Bacillus spp.	2	1	1	1	+	5
M. luteus	0	2	0	0	+	2
C. rugosa	2	2	2	2	+	8

AF, Amyloid fibers; EPS, exopolysaccharide; PA, polystyrene adhesion; HI, hydrophobicity index; Mot, motility. 0, No detected characteristic; 1, weak; 2, moderate; 3, strong; +, positive; -, negative. Values are the result of triple observations by two different observers.

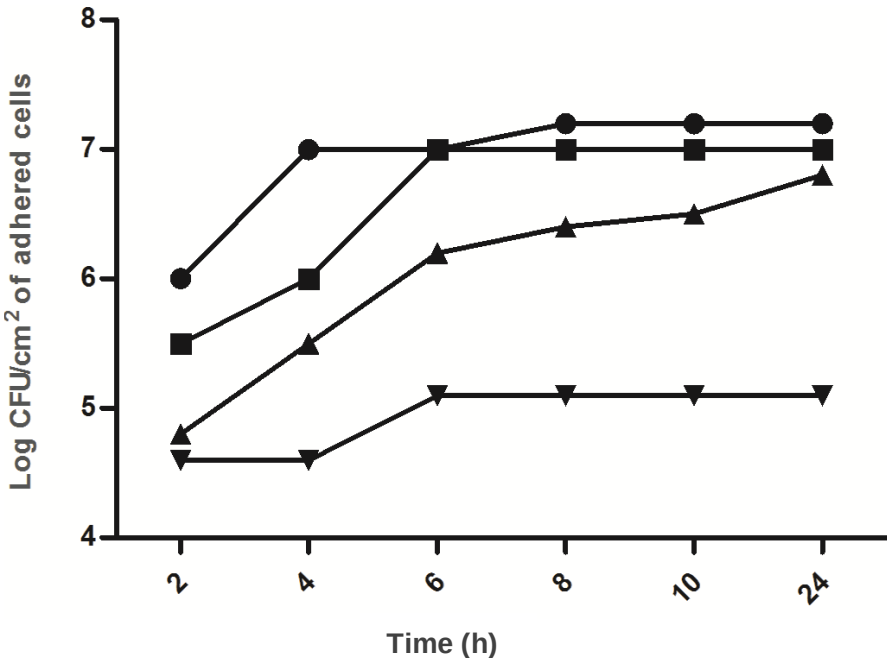

Figure 1. Growth curve of *Pseudomonas aeruginosa* ATCC 25619. (■), *Serratia marcescens;* (●), *Micrococcus luteus;* (▼), *Bacillus* spp; (♦), e *Candida rugosa;* (▲), on polyurethane coupons for 24 h. The results are the average of three different experiments.

DISCUSSION

Amyloid fibers are formed by a group of polymeric proteins about which, little is known. They contribute to the adhesion of microbial cells to each other or with different solid surfaces and play an important role in biofilm formation (Castonguay et al., 2006; Larsen et al., 2007; Dueholm et al., 2010).

Red colonies of *S. marcescens*, *Bacillus* spp. and *C. rugosa* strains on CRA medium suggested binding with amyloid-specific Congo red dye and these strains were classified as producing amyloid fibers. Castonguay et al. (2006) described *Escherichia coli* strains positive for the ARC test, Abdallah et al. (2009) reported positive results for *Vibrio parahaemolyticus* and *V. alginolyticus*, and Romero et al. (2010) reported the same for *Bacillus subtilis*. Garcia et al. (2011) observed amyloid fibers in *Saccharomyces cerevisiae* and *C. albicans* yeast strains.

The development of a mucoid layer, indicating EPS development, was evident in the following sequence: *S. marcescens*, *C. rugosa*, *M. luteus* and *Bacillus* spp. Tsuneda et al. (2003) reported that EPS significantly influenced the microbial adhesion onto solid surfaces, which is recognized as the initial stage in biofilm formation.

In experiments conducted by Ivanova et al. (2008), extracellular polysaccharides were responsible for greatly enhancing cell attachment of *Staleya guttiformis* cells to

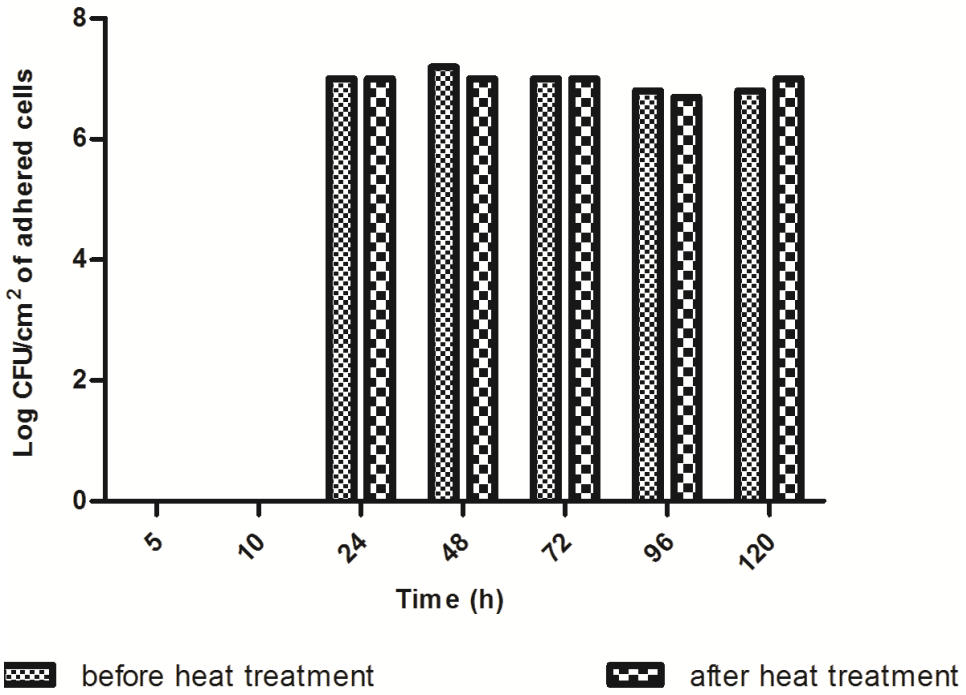

Figure 2. Log CFU/cm^2 of *Bacillus* spp. released from polyurethane coupons at different contact times before and after heat treatment. The results are the average of two different experiments.

poly(tert-butyl methacrylate) (P(tBMA) polymer surfaces. *S. marcescens* and *Bacillus* spp. strains exhibited motility, while *M. luteus* was negative for this characteristic. These results corroborate those of Garrity (2005), who observed the presence of flagella in most Gram-negative bacteria studied, like with the strains *P. aeruginosa* and *S. marcescens.* According to the same author, this structure, although rare, can be found in Gram-positive bacteria, as in some species of the *Bacillus* genus. The negative response of the *M. luteus* strain confirms the description of Garrity (2005) for this species. According to Klausen et al. (2003), this structure, while not indispensable, does facilitate cell adhesion. The *S. marcescens* strain showed similar ability of adhesion on polystyrene as the control strain *P. aeruginosa* ATCC 25619, followed by *C. rugosa* and *Bacillus* spp. *M. luteus* was negative for this characteristic. These results are in accordance with those of Boari et al. (2009), who reported that Gram-negative bacteria, due to their lipid-rich outer membrane, tend to be more hydrophobic than the Gram-positive bacteria. Zeraik and Nitschke (2010) reported that when cultured in a medium with peptone, *M. luteus* strains showed reduced adhesion on polystyrene. Since NA contains peptone in its composition, it is possible to consider this effect on the referred strain.

The literature indicates that for polymeric surfaces, the hydrophobicity varies among *Candida* species. *C. tropicalis*, *C. glabrata* and *C. krusei* are described as more hydrophobic than *C. albicans*, *C. parapsilosis* and *C. stellatoidea* (Luo and Samaranayake, 2002). No reports on hydrophobicity of the *C. rugosa* species were found.

The HI values classified *S. marcescens* and *C. rugosa* as moderately hydrophobic, *Bacillus* spp. as weakly hydrophobic, while *M. luteus* was hydrophilic (Mattos-Guaraldi et al., 1999). These results were coherent with microbial adhesion on the hydrophobic polystyrene surface.

It is believed that microorganisms that can live in aqueous media containing a dispersed hydrophobic carbon source have evolved mechanisms to facilitate access to the substrate, since there is little likelihood of contact between the microorganisms and hydrophobic droplets in constant motion (Fickers et al., 2005). Since the *C. rugosa* strain was isolated from wastewater from an oil refinery, the result of adherence to xylene confirms that this strain is adapted to hydrophobic surfaces.

Serebryakova et al. (2002) described similar hydrophobicity results for *S. marcescens* strains and Ryazantseva et al. (2012) reported that the cell surface components responsible for mediating the hydrophobicity of *S. marcescens* may include prodigiosin pigment, which is associated with a hydrophobic protein.

The results of the qualitative tests (Table 1) show that *S. marcescens* and *C. rugosa* were the most promising strains for cell immobilization. The number of viable cells of *S. marcescens* and *C. rugosa* strains retained on polyurethane was not significantly different from that of

the standard strain *P. aeruginosa* ATCC 25619. After 24 h, both strains reached concentrations of about 10^7 CFU/cm^2, characteristic of biofilm formation (Quek et al., 2006; Carelli et al., 2009).

The results shown in Figure 1 are in agreement with that of Kumamoto and Vince (2005) and Dusane et al. (2008), who reported that although yeasts grow slower than bacteria, they are also able to adhere and grow on inert surfaces to form biofilms. The concentration of approximately 10^5 CFU/cm^2 of *M. luteus* was significantly lower than that of *P. aeruginosa* ATCC 25619 and was not characteristic of biofilm formation, confirming the results of the qualitative adhesion tests.

Pompermayer and Gaylarde (2000), in an experiment for cell immobilization of a Gram-negative bacterium, *P. fluorescens* and a Gram-positive one, *Staphylococcus aureus* on stainless steel observed that while the first strain reached final values of about 10^7 CFU/cm^2, the *S. aureus* strain reached values of 10^5 CFU/cm^2. Although, the strains and support material used in the present study and those employed by the above mentioned authors are different, it is possible to compare these results with those shown in Figure 1, in which the Gram-negative bacteria *S. marcescens* and the control strain *P. aeruginosa* reached concentrations of 10^7 CFU/cm^2 and Gram positive, *M. luteus* reached about 10^5 CFU/cm^2.

As seen in Figure 1, cells of *Bacillus* spp. only were detected on polyurethane coupons after 24 h of contact at concentrations of 10^6 CFU/cm^2. The absence of *Bacillus* cells on the coupons before and after heat treatment (80°C/10 min) in the first 10 h of contact shows the inability or reduced ability of vegetative cells to adhere to this surface. The detection of cells on polyurethane only after 24 h suggests that the depletion of nutrients contributed to the sporulation process. The spores survive the heat treatment and the cell counts showed no significant differences. In this respect, Elhariry (2008) reported that the hydrophobicity of spores of *Bacillus* was significantly higher than that of vegetative cells, concluding that spores play an important role in adherence to inert surfaces, especially in oligotrophic environments.

Even considering that concentrations of 10^6 CFU/cm^2 are characteristic of biofilm formation, it is important to note that spores are metabolically inactive under limiting conditions, which would hinder the transformation of recalcitrant compounds by these organisms in immobilized form.

The cell retention on polyurethane confirmed the results of phenotypic characteristics, showing that the qualitative tests presented here can be used for preliminary selection of strains with potential for immobilization. Although, the search for strains producing biosurfactants was not the objective of this study, it was observed that the culture of *S. marcescens* after 5 days in contact with the polyurethane coupons showed the formation of intensely white foam, strongly suggestive of a producing strain.

This was also noted by Nitschke and Pastore (2002), who reported a biosurfactant called serravetina produced by strains of *S. marcescens*. Anyanwu et al. (2011) also described a lipopeptide biosurfactant produced by a *S. marcescens* NSK-1 strain isolated from petroleum-contaminated soil. Since biosurfactants, also called bioemulsifiers, reduce the surface tension of the medium and can facilitate the solubilization and utilization of complex substrates, strains producing these substances are of great interest for bioremediation processes (Ward, 2010).

In conclusion, the culturable environmental microbial strains tested here had different potentials for cell adhesion. A correlation between qualitative adhesive characteristics and quantitative cell retention was observed and Gram-negative bacteria were more promising than Gram-positive for immobilization. Bacterial spores showed better adhesion ability than the corresponding vegetative form. Yeasts can also be used in processes of cell immobilization. Among the microbial strains evaluated, *S. marcecens* and *C. rugosa* showed better qualitative and quantitative results for cell adhesion and were selected for future experiments of immobilization in mixed and pure cultures.

ACKNOWLEDGEMENTS

The authors thank the Brazilian agencies FINEP (Financiadora de Estudos e Projetos), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and the company Petrobras for their financial support of this work.

REFERENCES

Abdallah FB, Chaieb K, Zmantar T, Kallel H, Bakhrouf A (2009). Adherence assays and slime production of *Vibrio alginolyticus* and *Vibrio parahaemolyticus*. Braz. J. Microbiol. 40(2):394-398.

Ahmad SA, Shamaan NA, Noorliza MA, Koon GB, Shuko MYA, Syed MA (2012). Enhanced phenol degradation by immobilized *Acinetobacte*r sp. strain AQ5NOL 1. World J. Microbiol. Biotechnol. 28(1):347-352.

Anyanwu CU, Obi SKC, Okolo BN (2011). Lipopeptide biosurfactant production by *Serratia marcescens* NSK-1 strain isolated from petroleum-contaminated soil. J. Appl. Sci. Res. 7(1):79-87.

Arciola CR, Campoccia D, Gamberi S (2002). Detection of slime production by means of an optimized Congo red agar plate test based on a colourimetric scale in *Staphylococcus epidermidis* clinical isolates genotyped for *ica* locus. Biomaterials 23(21):4233-4239.

Arrage AA, Phelps TJ, Benoit RE, White DC (1993). Survival of subsurface microorganisms exposed to UV radiation and hydrogen peroxide. Appl. Environ. Microbiol. 59(11):3545-3550.

Aygan A, Arikan B (2007). An overview on bacterial motility detection. Int. J. Agric. Biol. 9(1):193-196.

Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti, MG, Fracchia L, Smyth TJ, Marchant R (2010). Microbial biosurfactants production, applications and future potential. Appl. Microbiol. Biotechnol. 87(2): 427-444.

Boari CA, Alves MP, Tebaldi VMR, Savian TV, Piccoli RH (2009). Biofilm formation by *Aeromonas hydrophila* and *Staphylococcus aureus* on stainless steel using milk and different conditions of cultivation. Ciênc. Tecnol. Aliment. 29(4):886-889.

Cai T, Chen L, Ren Q, Cai S, Zhang J (2011). The biodegradation pathway of triethylamine and its biodegradation by immobilized *Arthrobacter protophormiae* cells. J. Hazard Mater. 186(1):59-66.
Careli RT, Andrade NJ, Soares NF, Ribeiro Júnior JI, Rosado MS, Bernardes PC (2009). The adherence of *Pseudomonas fluorescens* to marble, granite, synthetic polymers, and stainless steel. Ciênc. Tecnol. Aliment 29(1):171-176.
Castonguay MH, Schaaf SVD, Koester W, Krooneman W, Meer WVD, Harmsen H, Landini P (2006). Biofilm formation by *Escherichia coli* is stimulated by synergistic interactions and co-adhesion mechanisms with adherence-proficient bacteria. Res. Microbiol. 157(5):471-478.
Christensen GD, Simpson WA, Bisno AL, Beachey EH (1982). Adherence of slime producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect. Immun. 37(1):318-326.
Dueholm MS, Petersen SV, Sonderkær M, Larsen P, Christiansen G, Hein KL, Jan J. Enghild JJ, Nielsen JL, Nielsen KL, Nielsen PH, Otzen DE (2010). Functional amyloid in *Pseudomonas*. Mol. Microbiol. 77(4):1009-1020.
Dusane D, Nancharaiah YV, Venugopalan VP, Kumar AR, Zinjarde SS (2008). Biofilm formation by a biotechnologically important tropical marine yeast isolate, *Yarrowia lipolytica* NCIM 3589. Water Sci. Technol. 58(6):2467-2475.
Elhariry HM (2008). Biofilm formation by endospore-forming *Bacilli* on plastic surface under some food related and environmental stress conditions. Global J. Biotechnol. Biochem. 3(2):69-78.
Fickers P, Benetti PH, Waché Y, Marty A, Mauersberger S, Smit MS, Nicaud JM (2005). Hydrophobic substrate utilisation by the yeast *Yarrowia lipolytica*, and its potential applications. FEMS Yeast Res. 5(6-7):527-543.
Freeman DJ, Falkiner FR, Keane CT (1989). New method for detecting slime production by coagulase negative staphylococci. J. Clin. Pathol. 42(8):872-874.
Garcia MC, Lee JT, Ramsook CB, Alsteens D, Dufrêne YF, Lipke PN (2011). A role for amyloid in cell aggregation and biofilm formation. PLoS ONE 6(3):e17632.
Garrity GM (Ed.) (2005). Bergey's Manual of Systematic Bacteriology, 2ed, Parts B; C The Williams and Wilkins Co., Baltimore 2:1203
Goulter RM, Gentle IR, Dykes GA (2009). Issues in determining factors influencing bacterial attachment: a review using the attachment of *Escherichia coli* to abiotic surfaces as an example. Lett. Appl. Microbiol. 49(1):1-7.
Ivanova EP, Mitik-Dineva N, Wang J, Pham DK, Wright JP, Nicolau DV, Mocanasu RC, Crawford RJ (2008). *Staleya guttiformis* attachment on poly(tert-butylmethacrylate) polymeric surfaces. Micron 39(8):1197-1204.
Klausen M, Heydorn A, Ragas P, Lambertsen L, Jorgensen A, Molin S, Nielsen T (2003). Biofilm formation by *Pseudomonas aeruginosa* wild type, flagella and type IV pili mutants. Mol. Microbiol. 48(6):1511-1524.
Komlos J, Cunningham AB, Camper AK, Sharp RR (2005). Interaction of *Klebsiella oxytoca* and *Burkholderia cepacia* in dual-species batch cultures and biofilms as a function of growth rate and substrate concentration. Microb. Ecol. 49(1):114-125.
Kumamoto CA, Vinces MD (2005). Alternative *Candida albicans* lifestyles: growth on surfaces. Annu. Rev. Microbiol. 59:113-133.
Kumar A, Bisht BS, Joshi VD, Dhewa T (2011). Review on bioremediation of polluted environment: A management tool. Int. J. Environ. Sci. 1(6): 1079-1093.
Larsen P, Nielsen JL, Dueholm MS, Wetzel R, Otzen D, Nielsen PH (2007). Amyloid adhesins are abundant in natural biofilms. Environ. Microbiol. 9(12):3077-3090.
Luo G, Samaranayake LP (2002). *Candida glabrata*, an emerging fungal pathogen, exhibits superior relative cell surface hydrophobicity and adhesion to denture acrylic surfaces compared with *Candida albicans*. APMIS 110(9):601-610.
Machado BM. Extremophile bacteria of the final effluent from sewage treatment plant of the Pici Campus: Risks and biotechnological potential (2002). Monography. Federal University of Ceará, Brazil.
Mattos-Guaraldi AL, Formiga LCD, Andrade AFB (1999). Cell surface hydrophobicity of sucrose fermenting and non-fermenting *Corynebacterium diphtheriae* strains evaluated by different methods. Curr. Microbiol. 38(1):37-42.
Nitschke M, Pastore GM (2002). Biosurfactants: Properties and Applications. Quím. Nova. 25(5):772-776.
Oulahal N, Brice W, Martial A, Degraeve P (2008). Quantitative analysis of survival *Staphylococcus aureus* or *Listeria innocua* on two types of surfaces: propylene and stainless steel in contact with three different dairy products. Food Control 19(2):178-185.
Pagedar A, Singh J, Virender K. Batish VK (2010). Surface hydrophobicity, nutritional contents affect *Staphylococcus aureus* biofilms and temperature influences its survival in preformed biofilms. J. Basic Microbiol. 50(1):96-108.
Parizzi SQFF, Andrade NJ, Silva CAS, Soares NFF, Silva EAM (2004). Bacterial adherence to different inert surfaces evaluated by epifluorescence microscopy and plate count method. Braz. Arch. Biol. Technol. 47(1):77-83.
Patil NK, Veeranagouda Y, Vijaykumar M, Nayak S, Karegoudar T (2006). Enhanced and potential degradation of o-phthalate by *Bacillus* sp. immobilized cells in alginate and polyurethane. Int. Biodeter. Biodegr. 57(2):82-87.
Pompermayer DMC, Gaylarde CC (2000). The influence of temperature on the adhesion of mixed cultures of *Staphylococcus aureus* and *Escherichia coli* to polypropylene. Food Microbiol. 17(4):361-365.
Quek E, Ting YP, Tan HM (2006). *Rhodococcus* sp. F92 immobilized on polyurethane foam shows ability to degrade various petroleum products. Bioresour. Technol. 97(1):32-38.
Rocha LL, Cordeiro RA, Cavalcante RM, Nascimento RF, Martins SCS, Santaella ST, Melo VMM (2007). Isolation and characterization of phenol-degrading yeasts from an oil refinery wastewater in Brazil. Mycopathologia 164(4):183-188.
Romero D, Aguilar C, Losick R, Kolte R (2010). Amyloid fibers provide structural integrity to *Bacillus subtilis* biofilms. Proc. Natl. Acad. Sci. USA 107(5):2230-2234.
Rosenberg M (1981). Bacterial adherence to polystyrene: a replica method of screening for bacterial hydrophobicity. Appl. Environ. Microbiol. 42(2):375-377.
Ryazantseva IN, Saakov VS, Andreyeva IN, Ogorodnikova TI, Zuev YF (2012). Response of pigmented *Serratia marcescens* to the illumination. J. Photochem. Photobiol. B 106(5):18-23.
Serebryakova EV, Darmov IV, Medvedev NP, Alekseev SM, Rybak SI (2002). Evaluation of the hydrophobicity of bacterial cells by measuring their adherence to chloroform drops. Microbiology 71(2):237-239.
Tsuneda S, Aikawa H, Hayashi H, Yuasa A, Hirata A (2003). Extracellular polymeric substances responsible for bacterial adhesion onto solid surface. FEMS Microbiol. Lett. 223(2):287-292.
Ubbink J, Schar-Zammaretti P (2007). Colloidal properties and specific interactions of bacterial surfaces. Curr. Opin. Colloid Interface Sci. 12(4-5):263-270.
Vesterlund SA, Matti JK, Ouwehand AC (2005). Measurement of bacterial adhesion in vitro evaluation of different methods. J. Microbiol. Methods 60(2):225-233.
Ward OP (2010). Microbial biosurfactants and biodegradation. Adv. Exp. Med. Biol. 672:65-74.
Wang J, Yang H, Lu H, Zhou J, Wang J, Zheng C (2009). Aerobic biodegradation of nitrobenzene by a defined microbial consortium immobilized in polyurethane foam. World J. Microbiol. Biotechnol. 25(5):875-881.
Zeraik AE, Nitschke M (2010). Biosurfactants as agents to reduce adhesion of pathogenic bacteria to polystyrene surfaces: Effect of temperature and hydrophobicity. Curr. Microbiol. 61(6):554-559.
Zhou L, Guiying L, Taicheng A, Jiamo F, Guoying S (2008). Recent patents on immobilized microorganism technology and its engineering application in wastewater treatment. Recent Pat. Eng. 2:28-35.