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Acinetobacter baumannii Biofilms: Variations Among Strains and Correlations with Other Cell Properties

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***Acinetobacter baumannii* is an opportunistic pathogen that causes serious infections in humans by colonizing and persisting on surfaces normally found in hospital settings. The capacity of this pathogen to persist in these settings could be due to its ability to form biofilms on inanimate surfaces. This report shows that although the ATCC 19606^T type strain and 8 different clinical isolates form biofilms, there are significant variations in the cell density and microscopic structures of these cell aggregates, with 3 of the isolates forming pellicles floating on the surface of stagnant broth cultures. PCR indicated that, like ATCC 19606^T, all 8 clinical isolates harbor all the genetic components of the CsuA/BABCDE chaperone-usher pili assembly system, which is needed for biofilm formation on plastic. Pili detection in cells of all strains examined supports the presence and function of a pilus assembly system. However, only one of them produced the putative ATCC 19606^T CsuA/B pilin subunit protein. Hydrophobicity tests and motility assays also showed significant variations among all tested strains and did not result in direct correlations between the biofilm phenotype and cell properties that could affect biofilm formation on abiotic surfaces. This lack of correlation among these 3 phenotypes may reflect some of the variations already reported with this pathogen, which may pose a challenge in the treatment of the infections this pathogen causes in humans using biofilm formation on abiotic surfaces as a target.**

***Keywords*:** biofilms, pellicles, hydrophobicity, cell motility, pili, abiotic surfaces

Acinetobacter baumannii is a nosocomial opportunistic pathogen that causes severe infections in compromised patients including bacteremia, urinary tract infections, secondary meningitis, and pneumonia, the most prevalent infection caused by this pathogen among hospitalized patients (Bergogne-Berezin and Towner, 1996; Bergogne-Berezin, 2001; Joly-Guillou, 2005). More recently, *A. baumannii* has emerged as a rising threat because of its ability to resist a wide range of antibiotics and cause devastating infections in wounded civilians and military personnel (Dijkshoorn *et al.*, 2007; Peleg *et al.*, 2008) as well as severe soft tissue infections such as necrotizing fasciitis (Charnot-Katsikas *et al.*, 2009). The multi-drug resistance (MDR) phenotype of *A. baumannii* (Perez *et al.*, 2007) and its remarkable capacity to persist and prosper in medical settings on inanimate surfaces, such as furniture, linens, phones, and computer keyboards (Neely, 2000; Neely and Maley, 2000; Villegas and Hartstein, 2003; Borer *et al.*, 2005), could be due to the ability of this bacterial pathogen to form biofilms on abiotic surfaces (Tomaras *et al.*, 2003).

Bacterial biofilms are highly organized interdependent communities that form through a series of coordinated steps, which include the initial attachment of bacteria to a surface, the formation of microcolonies, and the development of biofilm structures in which bacterial cells are encased in exopolymeric substances (Costerton *et al.*, 1999; Davey and O'Toole, 2000). These multicellular structures render bacteria more resistant to antibiotics than their planktonic counterparts, allow them to evade the host response (Jefferson, 2004) and facilitate their persistence on medically relevant biotic and abiotic surfaces. Accordingly, it is becoming apparent that biofilm formation is a common trait among *A. baumannii* clinical isolates (Cevahir *et al.*, 2008; Rodriguez-Baño *et al.*, 2008; Wroblewska *et al.*, 2008; King *et al.*, 2009). Their biofilm phenotype is influenced by environmental factors, such as the concentration of free calcium and iron, both of which enhance biofilm formation on plastics (Tomaras *et al*., 2003; Lee *et al.*, 2008). The presence and expression of the bla_{PER-1} genetic determinant and the ability to resist human serum are also factors that play a positive role in *A. baumannii* biofilm formation (Lee *et al*., 2008; Rao *et al.*, 2008; King *et al*., 2009). Significant variations have also been detected among strains that attach to abiotic surfaces without an apparent relationship to other bacterial phenotypes, such as strain origin, gelatinase activity, mannose-resistant hemagglutination, carbapenem resistance, and the severity of the infections caused by this pathogen (Cevahir *et al*., 2008; Wroblewska *et al*., 2008). However, it is not known whether or how other cellular properties, such as surface hydrophobicity, pellicle formation, and pilus production relate to biofilm formation by clinical isolates on abiotic surfaces.

In this study, we compare the biofilm phenotype of the *A. baumannii* ATCC 19606^T type strain with 8 different clinical isolates classified by their random amplified polymorphic DNA (RAPD) type to determine whether there is a correlation between biofilm formation and particular cell characteristics that could affect this cellular process.

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Material and Methods

Bacterial strains and culture conditions

The *A. baumannii* ATCC 19606^T type strain was purchased from ATCC. The *A. baumannii* clinical strains BM4420, BM4421, BM4422, BM4424, BM4427, BM4430, BM4432, and BM4436, which represent the RAPD A-F, H, and I types, respectively, were previously described (Ploy *et al.*, 2000). These multiresistant strains were isolated between 1992 and 1998 from French hospitals. *Escherichia coli* DH5α, which was used as a negative control in PCR assays, was obtained from Gibco-BRL (USA). Bacterial strains were maintained on Luria-Bertani (LB) agar or broth (Sambrook and Russell, 2001) and grown at 37°C.

Biofilm assays

One milliliter of LB broth in polystyrene (12×75 mm) or glass (10×100 mm) tubes was inoculated with 0.01 ml of an overnight culture (10-12 h) and incubated stagnantly overnight at 37°C. Biofilm formation was assessed by crystal violet staining as described before (Tomaras *et al*., 2003). The OD_{580}/OD_{600} ratio was used to normalize the amount of biofilm formed to the total cell content of each sample tested to avoid biofilm variations due to differences in bacterial growth. All assays were done in triplicate at least twice using fresh samples each time.

Electron microscopy

For transmission electron microscopy (TEM), cells were stab-inoculated into 0.3% and 1.5% agarose swimming plates and incubated overnight at 37°C. Cells were lifted onto formvar-coated copper grids and stained with ammonium molybdate. Images were taken with a Zeiss EM 10C TEM. For scanning electron microscopy (SEM) cells were grown stagnantly in 25 ml of LB broth in square petri dishes overnight at 37°C. The culture medium was decanted and the samples were processed as described before (Tomaras *et al*., 2003). Pellicles formed at the liquid-air interface of stagnant LB broth tube cultures were gently lifted on pieces of plastic coverslips floating on the surface of the broth and processed for SEM as described for biofilms formed on plastic surfaces. The biofilms were viewed with a JEOL JSM-840A or a Zeiss Supra 35 VP SEM.

Hydrophobicity/hydrophilicity assays

Cell hydrophobicity was determined using a standard microbial adhesion to hydrocarbon (MATH) test as described before (Rosenberg *et al.*, 1980). Briefly, bacterial cells were cultured in LB broth at 37°C in a shaking incubator. The cells were collected by centrifugation, washed twice, resuspended with PUM buffer (22.2 g/L $K_2HPO_4 \cdot 3H_2O$, 7.26 g/L KH_2PO_4, 1.8 g/L urea, 0.2 g/L $MgSO_4 \cdot 7H_2O$, adjusted to pH 7.1), and tested with *p*-xylene. All assays were done in duplicate at least twice using fresh samples each time.

Table 1. Primers used to amply the components of the *csuA/BABCDE* gene cluster

Number	Nucleotide sequence	Amplified intergenic region
1035	5′-ACCAGCACACTCGATCTG-3′	*csuA/B-csuA*
1662	5′-TTACTGGTCAGGTTGACG-3′	*csuA/B-csuA*
1397	5′-AAATGCGGGTGAAATCGG-3′	*csuA-csuB*
1819	5′-TGTAGGTGTTGTAGCAGG-3′	*csuA-csuB*
1680	5′-CTCATCTACAATCAGACG-3′	*csuB-csuC*
1893	5′-TATGCAGCAGATCCTCAG-3′	*csuB-csuC*
1619	5′-TTGAACCGCCTTGATAGG-3′	*csuC-csuD*
1894	5′-GAGCAGTCATATCGTCTG-3′	*csuC-csuD*
1037	5′-CGTAAAGCTACTCATGTC-3′	*csuD-csuE*
1375	5′-AAGTGCCTGATGTTCTGG-3′	*csuD-csuE*

Motility assays

Cell motility was examined using twitching, swarming and swimming plates as described before (Rashid and Kornberg, 2000). Positive re-

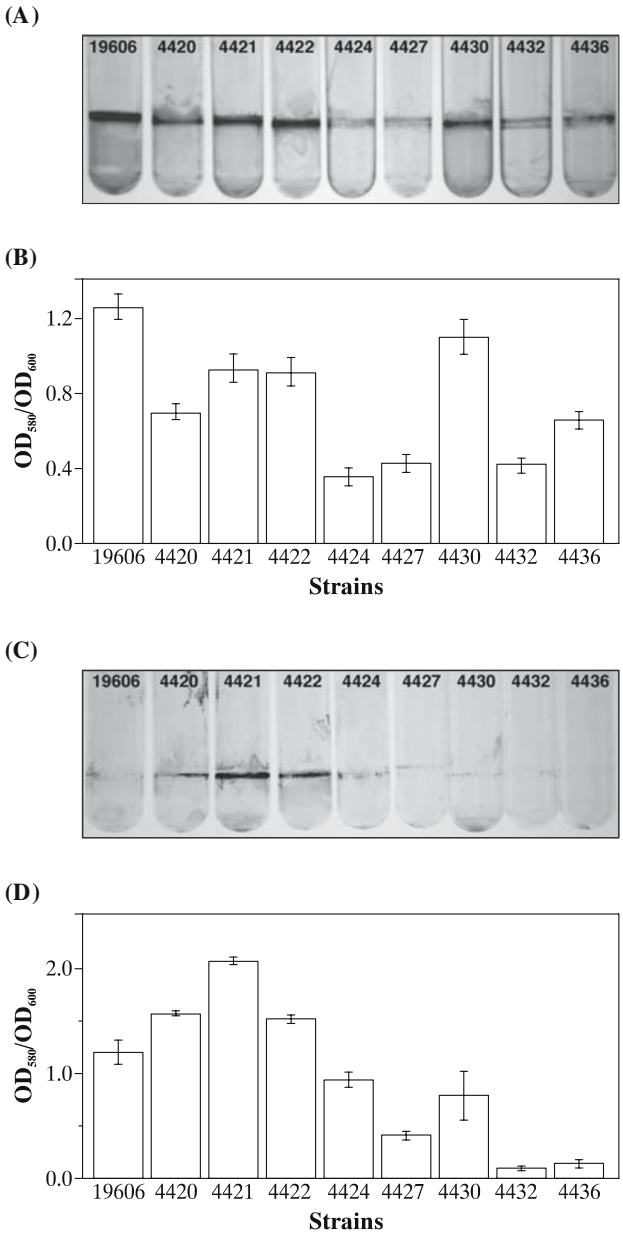

Fig. 1. Biofilm assays on polystyrene and glass tubes. Biofilms formed on polystyrene (A and B) and glass (C and D) tubes were stained with crystal violet and directly visualized (A and C) and quantified (B and D) after the stain was eluted with acetone/ethanol. Error bars represent 1 standard deviation of the mean for 3 separate biological replicates (n=3).

sults with swimming plates do not reflect true swimming motility since it depends on the action of flagellum/flagella, appendages not produced by members of the *Acinetobacter* genus, including *A. baumannii* (Mussi *et al*., 2010). Twitching plates were made with LB broth supplemented with 1% agar. Swarming media consisted of 8 g/L nutrient broth containing 0.5% of each agar and glucose. Swimming plates were made with 10 g/L tryptone, 5 g/L NaCl supplemented with either 0.3% or 1.5% agarose. Nutrient broth, agar and tryptone, from Difco (USA), and agarose, from GIBCO-BRL, were used to prepare these media. Plates were stab-inoculated with a sterile toothpick with bacteria grown on LB agar. Cell motility on the surface of the media was recorded after overnight incubation at 37°C.

DNA analysis procedures

Total DNA from all *A. baumannii* strains and *E. coli* DH5α was isolated using the Qiagen DNeasy Tissue kit (Dorsey *et al.*, 2003). PCR assays were done by using 100 ng of total DNA isolated from each strain as a template, *Taq* DNA polymerase, and the appropriate set of primers (Table 1) to amplify the 3′-5′ junction regions between each gene of the *csuA/BABCDE* operon (Tomaras *et al*., 2003). PCR conditions were as follows: 94°C for 3 min; 30 cycles at 94°C for 45 sec, 50°C for 45 sec, and 72°C for 1 min; and a final extension at 72°C for 10 min. The amplicons were analyzed by ethidium bromide-agarose gel electrophoresis (Sambrook and Russell, 2001).

Detection of the CsuA/B protein

Whole-cell lysate proteins were size-fractionated on 12.5% polyacrylamide gels (Actis *et al.*, 1985), transferred to nitrocellulose and probed with anti-CsuA/B polyclonal antibodies as described before (Tomaras *et al.*, 2008). The immunocomplexes were detected by chemiluminescence using horseradish peroxidase (HRP)-labeled protein A.

Results

Formation of biofilms on plastic and glass and pellicles on the surface of liquid cultures

Standard biofilm assays using polystyrene tubes showed that the ATCC 19606^T type strain and all 8 clinical isolates formed biofilms (Fig. 1A). Most of the biofilms were located at the liquid-air interface, with some cells attached to the tubes below the meniscus of the liquid column. However, isolates BM4420, BM4424, BM4427, BM4432, and BM4436 formed reduced amounts of biofilm, as compared with ATCC 19606^T and the isolates BM4421, BM4422, and BM4430. A similar trend was observed when biofilms were quantified after stain elution and normalization to total cell mass (Fig. 1B). Interestingly, the isolates BM4424, BM4427, and BM4432 formed a double ring at the liquid-air interface. Similar assays using glass tubes showed a more pronounced variation in biofilm formation among these strains, with isolates BM4420, BM4421, and BM4422 displaying the most apparent cell aggregates at the liquid-air interface and isolates BM4432 and BM4436 forming the smallest amount of biofilm (Figs. 1C and D).

Although the biofilms formed on plastic were mainly located at the liquid-air interface, strains BM4421, BM4422, and BM4430 formed cell aggregates floating on the surface of the broth forming pellicle-like structures when statically cultured in polystyrene and glass tubes (Figs. 2A and B). In the case of isolate BM4430, the cell clumps were readily visible on the liquid surface, while the liquid column of the broth culture of BM4421 was clear due to the aggregation of most of the cells at the surface of the culture medium. These macroscopic observations were confirmed when the pellicle formed by strain BM4421 was lifted on a piece of plastic coverslip and examined with SEM. Panels C-E of Fig. 2 show the presence of large aggregates in which cell clusters, with cells connected to each other by structures resembling pili, are separated by empty spaces. It is important to note that the biofilm results displayed in Fig. 1 most likely underrepresent the magnitude

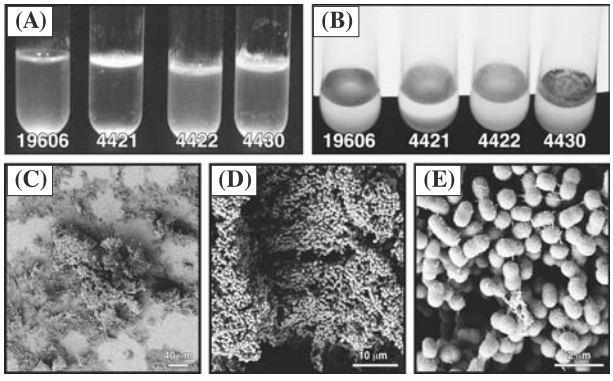

Fig. 2. Detection of bacterial pellicles. Visualization of pellicles formed on the LB broth surface after incubation in polystyrene (A) and glass (B) tubes. The pellicles formed by strain BM4421 were lifted on a piece of plastic coverslip floating on the broth surface and visualized by SEM at a magnification of ×125 (C), ×936 (D), and ×936 (E).

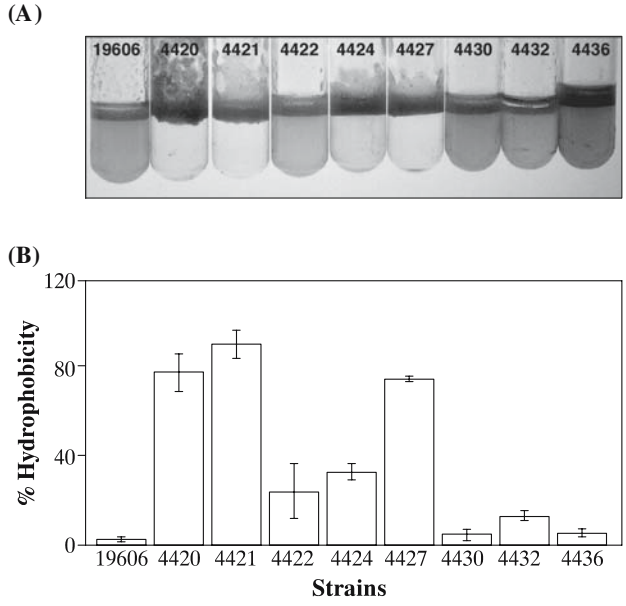

Fig. 3. Cell surface hydrophobicity. Partition of bacterial cells into the organic and aqueous phases was determined visually (A) and spectrophotometrically (B). Error bars represent 1 standard deviation of the mean for 2 biological replicates completed in duplicate (n=4).

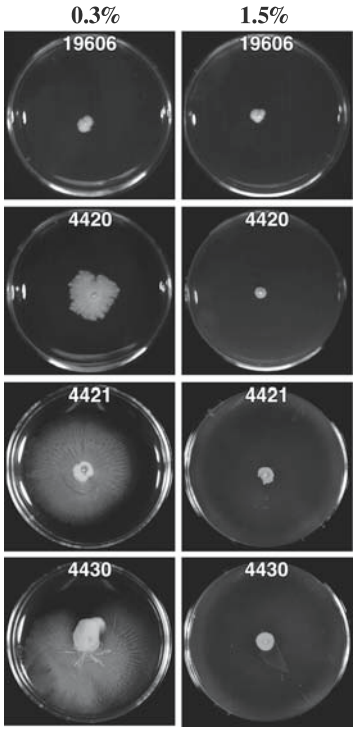

Fig. 4. Cell motility on semisolid swimming plates. Plates containing either 0.3% or 1.5% agarose were stab-inoculated and photographed after overnight incubation at 37°C.

of biofilm formation by isolates BM4421, BM4422, and BM4430 since most of the pellicles were lost when the culture medium was discarded and the plastic and glass tubes were washed before staining with crystal violet.

Cell surface hydrophobicity

The MATH tests using cells cultured in shaken LB broth indicated that strains ATCC 19606^T, BM4430, BM4432, and BM4436 partition mostly into the aqueous phase (Fig. 3A), with hydrophobicity values ranging from 2% for ATCC 19606^T to 12% for isolate BM4432 (Fig. 3B). Isolates BM4422 and BM4424 displayed a more moderate behavior with MATH values of 23% and 32%, respectively. In contrast, strains BM4420, BM4421, and BM4427 displayed a drastically different partitioning pattern with most of the cells adhering to the organic phase (Fig. 3A) and MATH values ranging from 74% for isolate BM4427 to 90% for isolate BM4421 (Fig. 3B).

Cell motility

Our previous work showed that the ATCC 19606^T strain is non-motile when tested on swarming, swimming, or twitching plates (Tomaras *et al*., 2003). When the 8 clinical strains examined in this work were tested using these motility plates, we observed that while none of these isolates were motile on swarming and twitching media, strains BM4420, BM4421, and BM4430 moved on the surface of swimming plates (Fig. 4). Strain BM4421 was the most motile isolate, covering the entire surface of the plate with a fuzzy layer of cells and striations of cell aggregates radiating from the inoculation site after overnight incubation at 37°C. A similar, although lighter, pattern was displayed by the isolate BM4430, whereas strain BM4420 formed a smaller but more dense and compact halo of cells surrounding the inoculation site (Fig. 4). All strains showed an increased reduction in motility when the concentration of agarose was increased stepwise from 0.3% to 1% (data not shown) and no cell motility was observed when the medium contained 1.5% agarose (Fig. 4).

Microscopic analysis of bacterial biofilms

As expected from previous observations (Tomaras *et al*., 2003), the ATCC 19606^T strain formed biofilms above, at, and below the liquid-air interface, which however were different from those formed by strains BM4420, BM4421, BM4422, and BM4430 (Fig. 5). The biofilms formed by the latter 4 strains seemed to lack the exopolymeric substance present in the ATCC 19606^T biofilms, particularly those formed above the liquid-air interface. Strains BM4420 and BM4421 formed similar structures and cell arrangements at the 3 sites examined, although the cell density of the aggregates at and above the meniscus of the liquid culture was reduced when compared with strain ATCC 19606^T. Interestingly, cell projections that attached to the plastic surface were readily visible in the BM4421 biofilms formed above but not at or below the meniscus, where few cells aggregated as discrete isolated clumps. Isolate BM4422 formed the least dense biofilms when compared with the 3 other strains, with mostly isolated cells and very few clumps observed at all 3 sites on the wall of the plastic plates (Fig. 5). Interestingly, in some areas of the biofilms the cells of this strain were deposited on a layer of an exopolymeric substance when located at the meniscus of the liquid column. Isolate BM4430 formed distinct aggregates above and at the meniscus of the liquid column displaying larger and more developed cell aggregates when compared with the structures formed by the 4 other isolates.

TEM analysis of the ATCC 19606^T strain and the motile isolates BM4420, BM4421, and BM4430 showed that all of them produce pili located around the cell surface (Fig. 6). However, the pili produced by isolates BM4420 and particularly BM4430 appear thinner and shorter than those produced by the ATCC 19606^T and BM4421 strains.

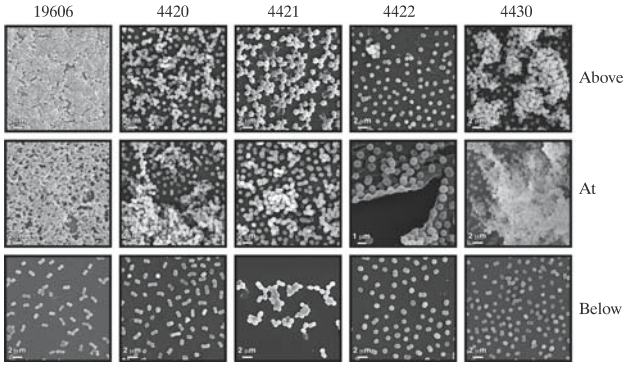

Fig. 5. Representative scanning electron micrographs of bacterial biofilms formed on plastic square petri dishes below, at, and above the meniscus of the liquid phase. Scale bars are 2 μm with the exception of BM4422 at the meniscus in which the scale bar is 1 μm.

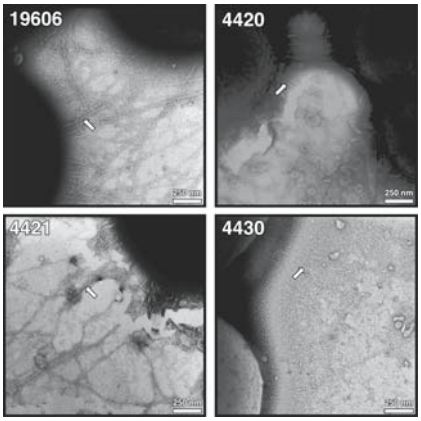

Fig. 6. Representative transmission electron micrographs of bacterial cells lifted from 0.3% agarose swimming plates. White arrows indicate pili.

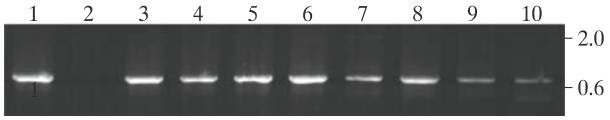

Fig. 7. Detection of *csu* genes. Amplicons of the *csuD-csuE* intergenic region produced by PCR amplification of total DNA isolated from *A. baumannii* ATCC 19606^T (lane 1), BM4420 (lane 3), BM4421 (lane 4), BM4422 (lane 5), BM4423 (lane 6), BM4427 (lane 7), BM4430 (lane 8), BM4432 (lane 9), and BM4436 (lane 10). PCR of total DNA isolated from *E. coli* DH5α was used as negative control (lane 2). The position of the 2.0- and 0.6-kb λ-*Hin*dIII markers is shown on the right.

Presence and expression of the *csu* genes

PCR analysis of total DNA isolated from all clinical isolates with primers 1037 and 1375, which anneal to the 3′-end of *csuD* and the 5′-end of *csuE* (Table 1), respectively, produced the same amplicon detected when DNA from the ATCC 19606^T strain was used as a template (Fig. 7). These 2 genes are the last 2 components of the *csuA/BABCDE* pilus assembly polycistronic operon, with *csuE* coding for the predicted tip adhesin of the pili assembled by this chaperone-usher assembly system (Tomaras *et al*., 2003) and used for sequence-based typing of *A. baumannii* clinical isolates (Turton *et al.*, 2007). No detectable amplicons were produced when *E. coli* DH5α total DNA was used as a negative control. Amplicons of comparable size were obtained when total DNA isolated from all *A. baumannii* strains were PCR-amplified using primers annealing to the 3′-end and 5′-end of the *csuA/B-A* (1035 and 1662), *csuA-B* (1397 and 1819), *csuB-C* (1680 and 1893), and *csuC-D* (1619 and 1894) (Table 1) intergenic regions (data not shown).

Although the data described above indicate the presence of the ATCC 19606^T *csuA/BABCDE* pili assembly polycistronic operon in all strains examined, only the BM4422 isolate tested positive for the production of CsuA/B, the putative pilin subunit of the appendages assembled via this system, when total cell lysate proteins were size-fractionated by SDS-PAGE, electrotransferred to nitrocellulose and probed with anti-CsuA/B polyclonal antibodies (data not shown).

Discussion

Table 2 summarizes the observations collected during this study. The interaction of *A. baumannii* with 2 types of surfaces having opposite properties, with glass and plastic representing extreme hydrophilic and hydrophobic surfaces, respectively, is reasonable considering the ability of this pathogen to persist in medical environments where these surfaces are normally found. However, these observations also show significant variations in the amount of biofilms these strains form on glass, for which there are no reports describing the type of study we present in this work, and plastic surfaces, an outcome that is in agreement with previous reports describing significant variability in the biofilms this pathogen forms on this type of surface (Cevahir *et al*., 2008; Lee *et al*., 2008; Rodriguez-Baño *et al*., 2008; Wroblewska *et al*., 2008; King *et al*., 2009). This variability seems to have a positive correlation with the serum resistance phenotype of the clinical isolates (King *et al*., 2009) and appears to be associated with the presence and expression of bla_{PER-1} (Lee *et al*., 2008; Rao *et al*., 2008). However, this conclusion is questionable considering the report by Sechi *et al.* (2004), who demonstrated that this antibiotic resistance gene plays a role in bacterial adherence to Caco-2 cells, but not in biofilm formation on abiotic surfaces.

Our report also indicates that there is significant variation in biofilm formation among strains when the interaction of

Table 2. Cellular properties of *A. baumannii* ATCC 19606^T and eight clinical isolates

Strain[a]	Biofilm[b] Polystyrene	Biofilm[b] Glass	Pellicle %	Hydrophobicity	Motility	*csuD-csuE*[c]	CsuA/B[d]
19606^T	1.2	1.2	Neg[e]	2	Neg	Pos[f]	Pos
4420	0.7	1.6	Neg	77	Pos	Pos	Neg
4421	0.9	2.1	Pos	90	Pos	Pos	Neg
4422	0.9	1.5	Pos	23	Neg	Pos	Pos
4424	0.3	0.9	Neg	32	Neg	Pos	Neg
4427	0.4	0.4	Neg	74	Neg	Pos	Neg
4430	1.1	0.7	Pos	4	Pos	Pos	Neg
4432	0.4	0.05	Neg	12	Neg	Pos	Neg
4436	0.6	0.1	Neg	6	Neg	Pos	Neg

[a] ATCC or BM clinical strain; [b] OD_{580}/OD_{600}; [c] PCR product; [d] immunoblot detection with anti-CsuA/B serum; [e] negative; [f] positive

bacterial cells with plastic and glass is compared, with the latter type of surface being the less favorable for cell-substratum interactions. Such observation suggests the possibility that cell properties, such as high hydrophobicity and hydrophilicity favoring interactions with plastic and glass surfaces, respectively, could determine the outcome of biofilm formation on abiotic surfaces. However, comparable amounts of biofilms were formed on plastic by BM4421, the most hydrophobic isolate (MATH value of 90%), and BM4430, a hydrophilic isolate (MATH value of 4%). Similarly, there is no apparent correlation between cell hydrophobicity and biofilm formation on glass, since the most hydrophobic strain, BM4421, formed the largest amount of biofilm, whereas one of the most hydrophilic strains, BM4436, formed almost no detectable biofilms on this abiotic hydrophilic surface (panels C and D of Fig. 1 and panels A and B of Fig. 3). These observations indicate that cell hydrophobicity/ hydrophilicity is not a good predictor of the biofilm properties of this bacterium, in contrast to other bacterial pathogens such as *Neisseria meningitidis* and *Listeria monocytogenes*, both of which display a direct correlation between surface hydrophobicity and biofilm formation on glass (Yi *et al.*, 2004; Di Bonaventura *et al.*, 2008). Direct correlation was also observed between cell adhesion and biofilm formation on plastic and the hydrophobicity of *Stenotrophomonas maltophilia* cells, although strains showing different hydrophobicity properties could form similar biofilms (Di Bonaventura *et al*., 2008). These reports together with our results indicate that although cell hydrophobicity could play a role, other cellular factors contribute to biofilm formation on abiotic surfaces by bacterial pathogens. This conclusion also applies to pellicle formation since there is no correlation between cell hydrophobicity/ hydrophilicity and the formation of biofilms on the surface of static liquid cultures. Strains displaying high (BM4421, MATH value of 90%), medium (BM4422, MATH value of 23%), and low (4430, MATH value of 4%) cell hydrophobicity produced pellicles on the surface of the LB broth static cultures either in plastic or glass tubes (Fig. 2). Microscopic analysis of selected isolates suggests that the variations in biofilms among the tested strains are quantitative as well as qualitative (Fig. 5). However, cell hydrophobicity could have an effect on the cell arrangements formed on polystyrene, with the 2 most hydrophobic strains, BM4420 and BM4421, forming less dense biofilms than those formed by ATCC 19606^T and BM4430, 2 of the most hydrophilic isolates. Hydrophilic isolates could more readily attach to each other than hydrophobic strains, a possibility that remains to be confirmed experimentally.

Motility is another cell property that could affect biofilm formation, as was described for *Pseudomonas aeruginosa* (Shrout *et al.*, 2006), in which flat biofilms are the result of high rates of swarming motility. Although this type of motility cannot be ascribed to *Acinetobacter*, because of the lack of flagellum formation by members of the species, the motility of some members of this bacterial family, which was defined as twitching motility, is the result of polar piliation during the exponential growth phase (Henrichsen, 1975a, 1975b, 1984). Accordingly, the analysis of the *A. baumannii* strains used in this work indicated that 3 of the clinical isolates move on the surface of semisolid agarose plates, a response that is impaired as the concentration of agarose was increased to 1.5% (Fig. 4). Interestingly, the isolate BM4420 displayed a motility phenotype different from that of the isolates BM4421 and BM4430. As was observed with the other cell properties analyzed in this study, cell motility does not seem to be a major factor influencing pellicle formation, which was detected in the nonmotile BM442 isolate as well as in the motile isolates BM4421 and BM4430. A lack of correlation between cell motility and biofilm formation was also detected when cells were cultured in plastic or glass tubes; motile strains formed more, equal, or less amounts of biofilms than non-motile strains. This observation is similar to that made during the analysis of factors affecting biofilm formation by *L. monocytogenes* (Di Bonaventura *et al*., 2008).

One factor that seems to be common among all isolates studied in this work is the presence of the *csuA/BABCDE* chaperone-usher pilus assembly operon and the detection of pili around the cell surface by electron microscopy. *csuE* has been used as a phylogenetic tool for typing *A. baumannii* clinical isolates (Turton *et al*., 2007) and this gene cluster is present in the genomes of all sequenced *A. baumannii* strains (Adams *et al.*, 2008). Although the formation of pili via this assembly system is essential for biofilm formation by the ATCC 19606^T strain (Tomaras *et al*., 2003), the production of this type of cell appendage does not correlate with the amount and type of biofilms formed by different *A. baumannii* clinical isolates on plastic and glass surfaces as well as the motility and hydrophobicity properties of the analyzed isolates. It is possible that these variations could be due to the production of more than one type of pilus with different properties. This possibility is supported by the data presented in Fig. 6 showing the presence of structurally different pili in different strains and the observation that ATCC 19606^T and BM4422 were the only strains that produce the CsuA/B pilin. Our recent observation that the ATCC 19606^T strain produces CsuA/BABCDE-dependent and -independent pili (de Breij *et al.*, 2009) lends further support to this possibility. It is also possible that there could be significant variations in the *csuA/BABCDE* genes among different strains as we have observed in the strain ATCC 17978. This piliated motile strain, which forms biofilms and pellicles (Mussi *et al*., 2010), produces longer and thinner pili than those detected in the ATCC 19606^T strain. *In silico* genomic and western blot analyses using anti-CsuA/B serum showed that there is a deletion in the 5′-end of the ATCC 17978 *csuA/B* gene, which results in the abolishment of CsuA/B production. These observations together with the presence of ATCC 17978 gene clusters coding for predicted pili assembly functions different from those mediated by the ATCC 19606^T CsuA/BABCDE system (McQueary and Actis, manuscript in preparation) support our hypothesis that different *A. baumannii* clinical isolates could produce different pili with different physicochemical and functional properties.

In summary, our results indicate that although all *A. baumannii* clinical isolates analyzed in this work interact with abiotic surfaces normally found in medical environments, different cell properties and structures affect this process differently (Table 2). Such variations and differences could pose a challenge in the development of strategies that could use biofilm formation as a convenient and general therapeutic target to treat *A. baumannii* infections in humans.

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