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Effect of host human products on natural transformation in *Acinetobacter baumannii*

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Abstract

Our previous data shows that serum albumin can trigger natural transformation in *A. baumannii*. However, extracellular-matrix/basal membrane components, norepinephrine, and mucin did not have a significant effect on this process. Therefore, the effect of human products appears to be albumin specific, as both BSA and HSA have been identified as inducers of natural competence.

Acinetobacter baumannii, a member of the highly resistant ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species), has emerged over the last few decades as a severe nosocomial pathogen due to its ability to resist desiccation and nutrient starvation, and obtain novel resistance genes (1).

A. baumannii's capacity to incorporate exogenous DNA, via horizontal genetic transfer (HGT), is contributing to its genome plasticity, as well as, to the multidrug resistance phenotype seen in a variety of clinical strains throughout the world [22,21,15]. Recently, the World Health Organization (WHO) classified this species as a priority 1 pathogen, meaning it is considered one of the critical pathogens for antibiotic research and development [28].

Natural transformation, one of the mechanisms of HGT, has been scarcely studied in a few strains among the genus *Acinetobacter* spp [7,16,17,20,29,19,4]. Since 2010, we have studied this process in the naturally competent clinical strain A118, which was isolated from a patient's blood sample and was shown to be susceptible to several antibiotics [20,24], and showed that it can acquire different DNA sources [20,21,25]. Moreover, our recent publication indicates that albumin, the main protein in blood, and Ca^{2+} significantly enhance transformation frequency and increase expression levels of two competence genes (*comEA* and *pilQ*) in *A. baumannii* strains [25].

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With the aim to identify other relevant host products that have an effect on competence during *A. baumannii* colonization/infection, and as it is known that *A. baumannii* can cause a wide variety of serious infections, including wound infections [6,18], and can bind to extracellular matrix/basal membrane (ECM/BM) proteins [2], we tested various human products.

Several kanamycin susceptible *A. baumannii* strains (A118, ATCC 17978, ATCC 19606 and A42) [1,5,8,9,11,12,23,26] were included in the present study and challenged by host human products (collagen IV, collagen I, hyaluronic acid, mucin, and norepinephrine). We decided to include additional *A. baumannii* strains, apart from strain A118, to observe if the effects are strain dependent. The most studied and commonly used ATCC strains were used as well as strain A42, which is a multidrug resistant clinical isolate recovered from endotracheal aspirate and which belongs to the clonal complex I [26]. Transformation assays using a plasmid (pDSredAK, conferring kanamycin resistance (Kan^R) or genomic DNA (from strain *A. baumannii* 144) known to carry a Kan^R determinant, were performed as previously described [20, 24]. Briefly, 50 μl of late stationary-death phase cultures of *A. baumannii* strains were transferred to 50 μl of sterile LB. 100 ng of plasmid DNA and/or gDNA were added and the cultures were incubated for 1 hour at 37°C followed by plating on LB agar with 10 μg/ml Kan. Transformation events were scored by counting Kan^R colonies, while total CFUs were assessed by plating serial dilutions on LB agar plates. Moreover, the acquisition of Kan^R genes was confirmed by PCR as well as by measuring the level of resistance to aminoglycosides by the gradient diffusion method (E-test method) with commercial strips (Biomerieux) [24]. Experiments were repeated at least three times and statistical analysis (Mann-Whitney test) was performed. Statistical data analysis was carried out using GraphPad Prism (GraphPad software, San Diego, CA, USA) and a *P*-value of <0.05 was considered significant. Human serum albumin (HSA) was also tested, as previously described, to see its effect in other *A. baumannii* strains.

The effect of two types of collagen, network-forming collagen (type IV) and fibrillar collagen (type I), were tested. Collagen IV was tested in two physiological concentrations, 115 and 166 ng/ml, which correspond to serum collagen IV levels in healthy and sick patients, respectively [14]. Collagen I was tested at 8 ug/ml which represents approximately 3% of concentration found in adult human skin [27]. Neither collagen IV nor collagen I had a statistically significant effect on transformation frequencies in any of the four strains used (Fig. 1 a, b and c). The effect of both collagen type IV and type I appeared to be both strain and DNA-type dependent and produced varied results. Interestingly, 166 ng/mL of collagen IV decreased both the transformation frequency of strain ATCC 17978 by 10.7-fold, when transformed with plasmid DNA, as well as the transformation frequency of strain A42 by 3.13-fold, when transformed with genomic DNA. In contrast, 115 ng/mL of collagen IV increased the transformation frequency of strain 17978 by 7.74 fold when transformed with genomic DNA and by 3.35-fold when transformed with plasmid DNA. Furthermore, collagen I decreased the transformation frequency of strain A42 by 41-fold when transformed with genomic DNA and increased it by 4.07-fold when transformed with plasmid DNA. In addition, we tested hyaluronic acid, a simple polysaccharide with a relevant role in the organization and maintenance of the extracellular matrix that is also present in blood serum, synovial fluid and thoracic lymph fluid [3]. As *A. baumannii*

frequently leads to skin and blood infections, we choose to examine the effect of hyaluronic acid at concentrations found in those regions. We chose a concentration of 1 mg/mL for our transformation assays to ensure that the experimental concentration was equal to or higher than that found in the human body [3]. Following the same trend we observed for both collagen types, there was no statistically significant effect on transformation frequencies in any of the four strains used (Fig. 1 d).

Similarly, and considering *A. baumannii's* role as one of the most important pathogens in ventilator-associated pneumonia (VAP), mucin, a mucopolysaccharide that is the main component of mucus, was tested. A concentration of 0.5% was used, as this concentration had been used in a previous study [13]. The results showed that mucin decreased transformation frequencies in all strains when transformed with plasmid DNA and in all but one strain (ATCC 17978) when transformed with genomic DNA (Fig. 2.a). Strain A42 had a greater than 5-fold decrease in transformation frequencies with both genomic and plasmid DNA. However, none of the effects observed were statistically significant.

Additionally, as it was recently shown that the host stress hormone norepinephrine (NE) upregulates the expression of efflux pump genes and increases biofilm formation in *A. baumannii* [10], we tested this host hormone as a potential inducer of competence. Transformation assays, using 10 uM of NE, produced varied results both within and between strains (Fig. 2.b). Strain ATCC 19606 showed a 1.80-fold increase when transformed with plasmid DNA after growth in NE and a 3.24-fold decrease when transformed with genomic DNA after growth in the same conditions. A similar trend was seen for strain A118. Strain ATCC 17978, however, showed a decrease in transformation frequency (1.9-fold decrease) when transformed with plasmid DNA and an increase (1.51-fold increase) when transformed with genomic DNA. Both genomic and plasmid DNA decreased transformation frequencies in strain A42 by 5.15-fold and 2.04-fold, respectively.

HSA's effect on transformation was also tested in parallel using strain A42, which belong to the widespread clonal complex I, and A118 strain, which is a sporadic clone, to verify its effect. As previously observed, and in agreement with the BSA results, growth in HSA showed a statistically significant transformation frequency increase of 16.4 and 11.8 folds for A118 and A42, respectively.

Overall our results showed that extracellular-matrix/basal membrane components, mucin and the hormone NE do not impose a statistically significant effect in competence in *A. baumannii*. Transformation frequencies varied in each strain and, in some cases, were affected by the type of DNA used. The observed results, taken with our recent discovery that albumin proteins are inducers of natural competence in *A. baumannii*, led us to conclude that not all human proteins contribute to an increase in transformation frequencies and that albumins are exerting a specific effect on transformation in *A. baumannii*.

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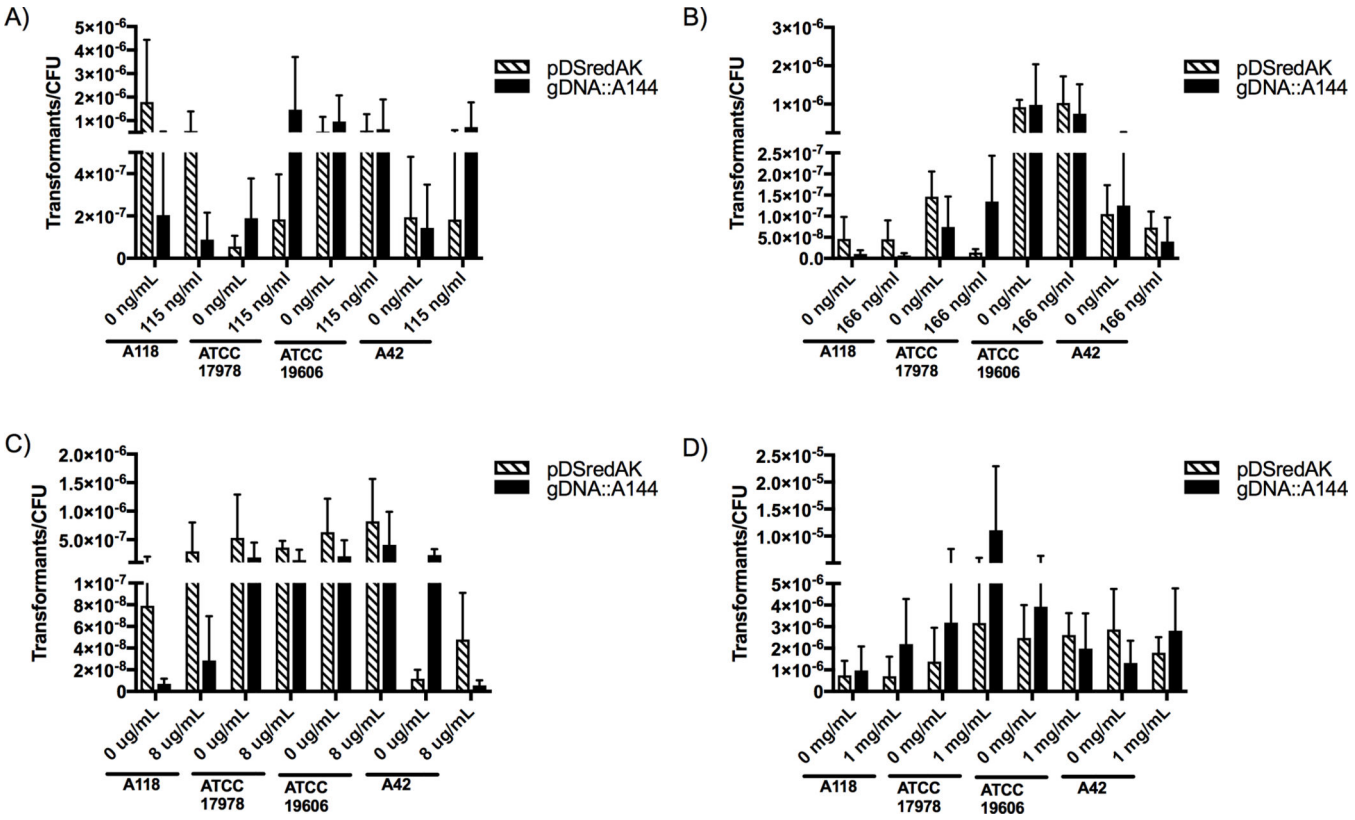

Figure 1. Natural transformation frequencies with extracellular matrix/basal membrane proteins.

Transformation assays were performed in LB broth with A) 115 ng/mL collagen IV, B) 166 ng/mL collagen IV, C) 8 μg /mL collagen I or D) 1 mg/mL hyaluronic acid. Cultures were transformed with plasmid DNA (striped) or genomic DNA (black) and plated on LB agar supplemented with 10 μg/mL kanamycin while CFUs were plate on LB agar. Data are presented as the mean and the errors bars represent the standard deviation. At least three independent replicates were performed and $p < 0.05$ was considered significant (Mann Whitney t test, n=3 to 9).

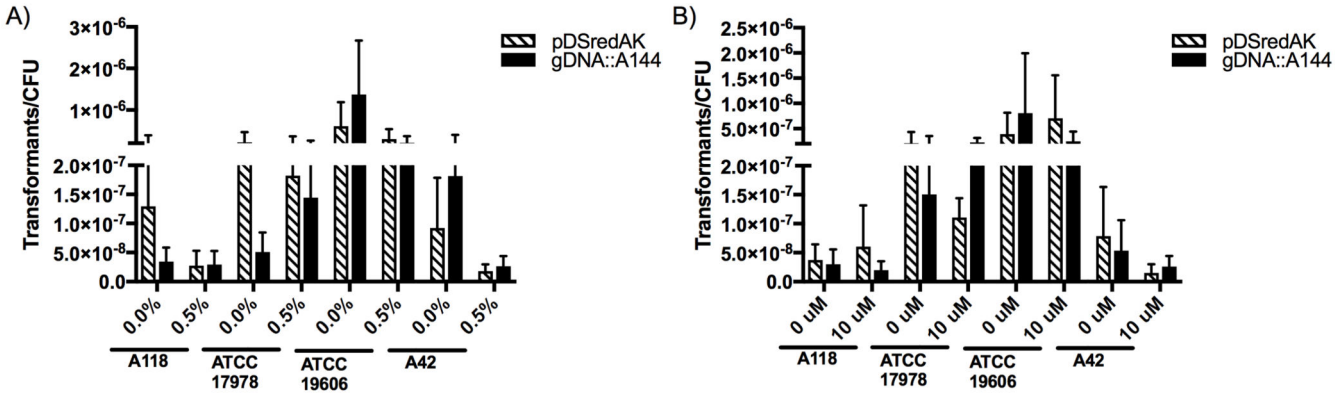

Figure 2. Natural transformation frequencies with additional human proteins.

Transformation assays were performed in LB broth with A) 0.5% (w/v) mucin or B) 10 μM norepinephrine. Cultures were transformed with plasmid DNA (striped) or genomic DNA (black) and plated on LB agar supplemented with 10 μg/mL kanamycin while CFUs were plate on LB agar. Data are presented as the mean and the errors bars represent the standard deviation. At least three independent replicates were performed and $p < 0.05$ was considered significant (Mann Whitney t test, n= 3 to 5).