DOES BACTERIAL SURFACE HYDROPHOBICITY LEVEL INFLUENCE THEIR IMMOBILIZATION ONTO NATURAL ZEOLITE?

Jasna Hrenović, Darko Tibljaš, Svjetlana Dekić Rozman, Tomislav Ivanković
University of Zagreb, Faculty of Science, Zagreb, Croatia
E-mail: jasna.hrenovic@biol.pmf.hr

ABSTRACT

Bacterium *Acinetobacter baumannii* is nowadays an emerging hospital pathogen. From colonised or infected patients, *A. baumannii* is disseminated into the natural water environments. Due to the resistance of this bacterium to the conventional methods of water disinfection, alternative methods of *A. baumannii* removal from water are needed. There is no literature data how the hydrophobicity level of *A. baumannii* isolates influences the immobilization onto hydrophilic surface of natural zeolitized tuff (NZ). The immobilization of hydrophilic and hydrophobic isolate of *A. baumannii* onto NZ was examined. Both hydrophilic and hydrophobic isolate was successfully immobilized onto NZ, whereby hydrophobic isolate in a higher abundance than hydrophilic one. The NZ is a promising material for the capture of isolates of pathogenic bacteria *A. baumannii* from water, and could find application in water treatment technology.

Key words: *Acinetobacter baumannii*, bacteria, hydrophobic, hydrophilic, immobilization, natural zeolite.

INTRODUCTION

Bacterium *Acinetobacter baumannii* during the last 30 years developed the resistance to commonly used antimicrobial agents. Due to ability to persist in abiotic environment for months, this bacterium represents an emerging human pathogen of 21st century [1]. From colonised or infected patients, *A. baumannii* is disseminated into hospital wastewaters, urban wastewaters, wastewater treatment plants and finally into the natural environment [2,3].

A. baumannii is highly durable in the conventional methods of water disinfection such as chlorination [3]. Therefore, there is a need to find alternative methods for removal of *A. baumannii* from water. It was previously shown that *A. baumannii* could be immobilized on the particles of the natural zeolitized tuff (NZ) [4]. However, phenotypic characteristics among *A. baumannii* isolates could differ in high extent [2,3]. There is no literature data how the hydrophobicity level of bacterial surface influences the immobilization onto hydrophilic surface of NZ. When examining this issue, hydrophilic NZ could be made hydrophobic by surface modification with surfactants e.g. hexadecyltrimethylammonium bromide. Cationic surfactants used for modification act bactericidal, diminishing the answer of particle-bacteria interaction [5].

In this study a hydrophilic and hydrophobic isolate of *A. baumannii* were used to elucidate the effect of hydrophobicity level of bacterial surface on the intensity of immobilization onto hydrophilic surface of NZ.

EXPERIMENTAL

A. baumannii isolates named IN41 and IN58 were recovered from influent of the Zagreb wastewater treatment plant [3]. Isolate IN41 was resistant to carbapenems and fluoroquinolones, while isolate IN58 was sensitive to all relevant antibiotics [3]. Two examined isolates also differed in phenotypic characteristics (Table 1). Bacterial hydrophobicity was measured *via* bacterial adhesion to hydrocarbon assay [6]. Pellicle formation was determined at the air-liquid media interface [7]. Surface of colonies was inspected visually after cultivation of isolates on the solid medium.

Table 1. Characteristics of *A. baumannii* isolates used in experiment.

Isolate	Hydrophobicity (%)	Pellicle formation	Colony surface
IN41	0	none	smooth
IN58	93	huge	rough

Fresh bacterial biomass was suspended in 9 mL of autoclaved nutrient-poor commercial spring water. Into each tube, one gram of NZ was added. The NZ was obtained from the quarry located at Donje Jesenje, Croatia. The composition of NZ is: clinoptilolite (50-55%), celadonite, plagioclase feldspars and opal-CT (10-15% each), analcime and quartz in traces [8]. The NZ was crushed, sieved, and the size fraction 0.263-0.5 mm was used. Prior to its usage, dry NZ was sterilized by autoclaving. Tubes were rotated at 3 rpm at 22°C for 24h.

At the beginning of experiment, and after 24h of incubation, the number of bacteria was determined according to described protocol [4,8]. The cultivation of bacteria was performed on CHROMagar Acinetobacter at 37°C/24h. Number of bacterial colonies was expressed as logarithm of colony forming units (log CFU) per one mL of water or one gram of dry NZ.

RESULTS AND DISCUSSION

As shown in Table 1, isolate IN41 has totally hydrophilic surface while isolate IN58 has strongly hydrophobic surface. After 24h of contact with suspension of NZ in water (Fig. 1), one part of bacteria was immobilized onto NZ particles, while the other part left planktonic in water. There was no multiplication of bacteria due to the lack of nutrients in spring water. The only remarkable difference in experiment was the significantly higher immobilization of the isolate IN58 (6.9±0.07 log CFU/g) as compared to the isolate IN41 (5.2±0.17 log CFU/g). In absolute number, this means that 7.65×10^6 more CFU of isolate IN58 was captured per one gram of dry NZ.

The possible explanation for the more abundant immobilization of hydrophobic isolate IN58 onto NZ as compared to hydrophilic isolate IN41 is as follows. Hydrophobic isolate prefers the immobilization onto NZ because in this way it reduces its surface in direct contact with water. This behaviour is supported by the huge pellicle formation at the water-air contact (Table 1). Obviously, hydrophobic isolate IN58 has a tendency to escape from water. The water medium is preferable surrounding for hydrophilic isolate IN4, which therefore showed less abundant immobilization onto NZ.

The intensity of immobilization of hydrophilic isolate IN41 in this work (5.2 log CFU/g) is lower than previously reported immobilization of hydrophilic isolate of *A. baumannii* EF7 (8.0 log CFU/g) on the same NZ in the same water medium [4]. Since the immobilization of isolate EF7 was tested on the NZ with the particle size ≤ 0.122 mm, while in this study 0.263-0.5 mm fraction was used, lower intensity of immobilization of IN41 may be explained by the fact that the number of immobilized bacteria increase with the decrease of particle size [7].

In the process of preparation of biosolids it is necessary to pay attention to the particle size of chosen material and water medium used for the growth of bacteria, two parameters that mostly influence the final number of bacteria on biosolids [4,8]. However, when preparing biosolids, the hydrophilic surface of bacterial isolate would not be a guaranty for better immobilization of bacteria onto hydrophilic material.

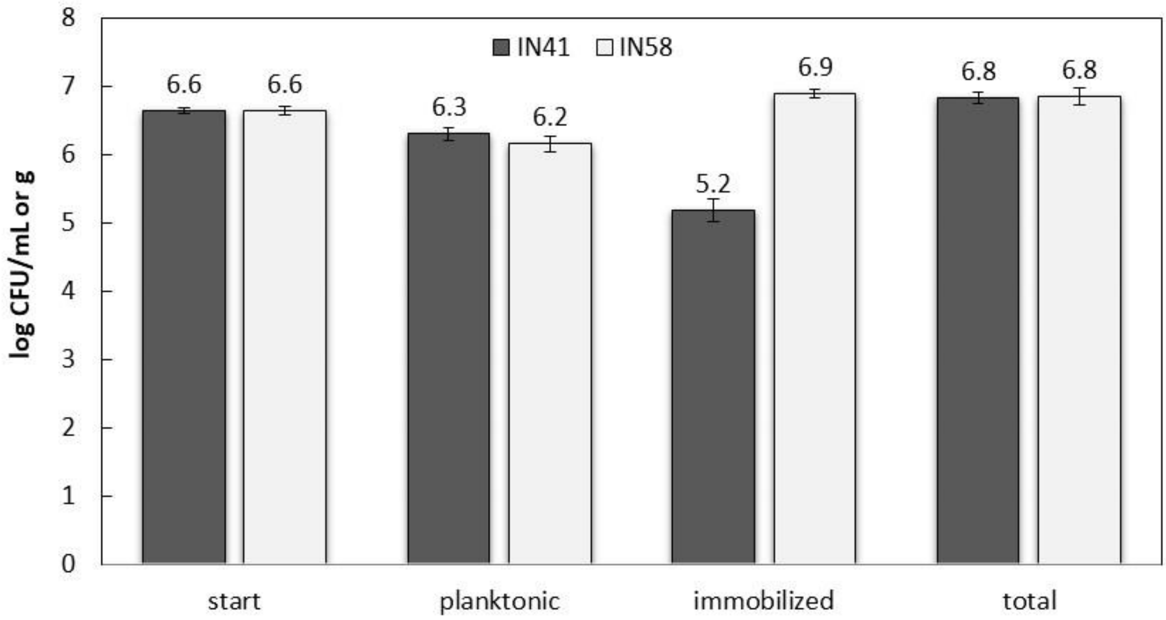

Figure 1. Numbers of hydrophilic (IN41) and hydrophobic (IN58) bacteria *A. baumannii* at the start of experiment and after 24h of contact with suspension of NZ in water (planktonic in water, immobilized onto NZ and total number in experiment).

CONCLUSION

The hydrophobicity level of bacterial surface is not a crucial factor which determines its immobilization onto hydrophilic surface of NZ. Both hydrophilic and hydrophobic bacteria could be successfully immobilized onto NZ, whereby hydrophobic bacteria in higher abundance than hydrophilic ones. The NZ is a promising material for the capture of pathogenic bacteria *A. baumannii* from water, and could find application in water treatment technology.

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