

MODE OF ACINETOBACTER BAUMANNII IMMOBILIZATION ONTO NATURAL ZEOLITE IN NUTRIENT-POOR AND NUTRIENT-RICH WATER

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ABSTRACT

Acinetobacter baumannii is an emerging human pathogen. Antibiotics-resistant *A. baumannii* disseminate via the untreated hospital sewage to urban wastewaters and nature, which represent the public health concern. Here we tested the natural zeolitized tuff (NZ) as a material for the capture of pandrug-resistant *A. baumannii* from nutrient-poor and nutrient-rich water. According to the behaviour of bacteria in two different water media, the mode of *A. baumannii* immobilization onto NZ is proposed. Planktonic cells of *A. baumannii* present in water became quickly attached onto the surface of NZ particles, regardless of the nutrient concentration in water. Immobilized cells excrete the extracellular polymeric substances and form stable biofilm on the particles within 24 h. No further incorporation of planktonic cells occurs in the formed biofilm. In the nutrient-poor water the shortage of nutrients prevents the multiplication of bacteria previously incorporated in the biofilm, and consequently biofilm stays conserved. In the nutrient-rich water the availability of nutrients enables the multiplication of bacteria inside the initially formed biofilm, which results in the increase of bacterial number and further maturation of biofilm. The NZ is efficient in removal of *A. baumannii* from nutrient-poor and nutrient-rich waters.

Keywords: *Acinetobacter baumannii*, biofilm, immobilization, natural zeolite, scanning electron microscopy, water.

INTRODUCTION

Bacterium *Acinetobacter baumannii* is a Gram-negative bacterium with cells of coccobacillus shape (1181 x 996 nm). Although not an obligate pathogen, during the last 30 years *A. Baumannii* developed the resistance to commonly used antimicrobial agents. *A. Baumannii* resistant to last-resort antibiotics is nowadays a leading cause of nosocomial infections worldwide [1]. Recently the occurrence of *A. baumannii* in urban wastewaters and rivers influenced by the untreated hospital sewage have been reported [2,3]. This suggest the water as a potential source of clinically relevant *A. Baumannii* isolates that poses a threat to people that come into contact with water. The goal of this study was to examine the natural zeolitized tuff (NZ) as a material for the capture of pandrug-resistant *A. baumannii* from nutrient-poor and nutrient-rich water.

EXPERIMENTAL

A. baumannii isolate (named EF7) was recovered from effluent of the Zagreb wastewater treatment plant [3]. This isolate was highly related to clinical *A. baumannii* isolates, resistant to all antibiotics, and is classified as a pandrug-resistant “super bacterium”. Overnight biomass was suspended in 100 mL of autoclaved nutrient-poor (commercially available spring water) and nutrient-rich water (commercially available spring water with addition of 1 % of nutrient broth). The physico-chemical properties of used water media are given in [4]. Into each bottle one gram of NZ was added. The NZ was obtained from 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

[5]. The NZ was crushed, sieved, and the size fraction ≤ 0.122 mm was used. Prior to its usage, dry NZ was sterilized by autoclaving. Bottles were incubated at 35 °C and aerated with sterile air.

At the beginning of the experiment, and after 24 h and 72 h of incubation, the number of bacteria was determined according to described protocol [5]. The cultivation of bacteria was performed on CHROMagarAcinetobacter at 42 °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. To confirm the immobilization of bacteria onto NZ, particles of NZ were taken after 24 h and 72 h of contact, fixed in 2 % of glutaraldehyde in phosphate buffer, prepared using standard techniques for low voltage (0.5 kV) scanning electron microscopy (SEM), and viewed by Zeiss Ultra PLUS FEG SEM.

RESULTS AND DISCUSSION

The numbers of *A. baumannii* that left planktonic or were immobilized onto particles of NZ, as well as the total number of bacteria in the systems containing nutrient-poor or nutrient-rich water are shown in Table 1. In the system with nutrient-poor water, bacteria did not multiply, and consequently there was no significant change in the number of planktonic or total bacteria as compared to the initial bacterial number. *A. baumannii* was immobilized onto NZ in high extent (8.0 log CFU/g) within 24h of contact, and this number stayed constant within 72 h of monitoring. In the system with nutrient-rich water, high multiplication of bacteria occurred up to 72 h of monitoring, which resulted in the increase of planktonic, immobilized and total bacteria as compared to initial number. High number of *A. baumannii* immobilized onto NZ within 24 h of contact (9.1 log CFU/g), increased further up to 72 h of contact (9.5 log CFU/g). The SEM analysis (Figure 1) revealed the formation of biofilm on the rough surface of NZ particles within 24 h of contact in both nutrient-poor and nutrient-rich water. Bacteria stayed tightly attached onto NZ and covered by extracellular polymeric substances up to 72 h of monitoring.

According to the results of monitoring of bacteria in two different water media, the mode of *A. baumannii* immobilization onto NZ could be proposed. Planktonic cells of *A. baumannii* present in water became quickly attached onto the surface of NZ particles, regardless of the nutrient concentration in water. Immobilized cells excrete the extracellular polymeric substances and form stable biofilm on the particles within 24h. No further incorporation of planktonic cells occurs in the formed biofilm. In the nutrient-poor water the shortage of nutrients prevents the multiplication of bacteria previously incorporated in the biofilm, and consequently biofilm stays conserved. In the nutrient-rich water the availability of nutrients enables the multiplication of bacteria inside the initially formed biofilm, which result in the increase of bacterial number and further maturation of biofilm.

From the above-mentioned, the capacity of the examined NZ for the immobilization of bacterium *A. baumannii* could be set at 8.0 log CFU per one gram of dry weight. Higher number of immobilized bacteria could be obtained as a result of bacterial multiplication inside the formed biofilm. The NZ was previously shown to be a good material for the immobilization of bacteria. The clinoptilolite content in NZ was shown to be irrelevant factor for bacterial immobilization [6], but the number of immobilized bacteria increase with the decrease of particle size [7]. Here, it is shown that the number of bacteria immobilized onto NZ will depend on the nutrient availability in surrounding water. The number of immobilized *A. baumannii* after 24 h of contact in nutrient-rich water containing 1 % of nutrient broth (9.1 log CFU/g) were lower than the number of related bacterium *A. junii* (10.1 log CFU/g) immobilized onto NZ of larger particle size (0.122-0.263 mm) in a concentrated nutrient broth [5]. In the synthetic wastewater containing lower nutrient availability than nutrient broth, *A.*

junii was immobilized onto NZ of the same particle size used in this study ($\leq 0.122\text{mm}$) at 9.7 CFU/g [7]. These differences confirm that the number of immobilized bacteria onto NZ are dependable on the nutrients available for bacterial multiplication inside biofilm.

Table 1. Numbers of *A. Baumannii* after 24 h and 72 h of contact with NZ. $c_0(\log\text{ CFU/mL})=7.3\pm 0.0$.

	<u>Nutrient-poor water</u>	<u>Nutrient-rich water</u>
24h of contact		
Planktonic cells (log CFU/mL)	6.9 ± 0.2	9.7 ± 0.0
Immobilized cells (log CFU/g)	8.0 ± 0.1	9.1 ± 0.1
Total cells (log CFU/mL)	7.1 ± 0.2	9.8 ± 0.1
72h of contact		
Planktonic cells (log CFU/mL)	7.2 ± 0.2	9.9 ± 0.0
Immobilized cells (log CFU/g)	8.0 ± 0.1	9.5 ± 0.2
Total cells (log CFU/mL)	7.3 ± 0.2	10.1 ± 0.0

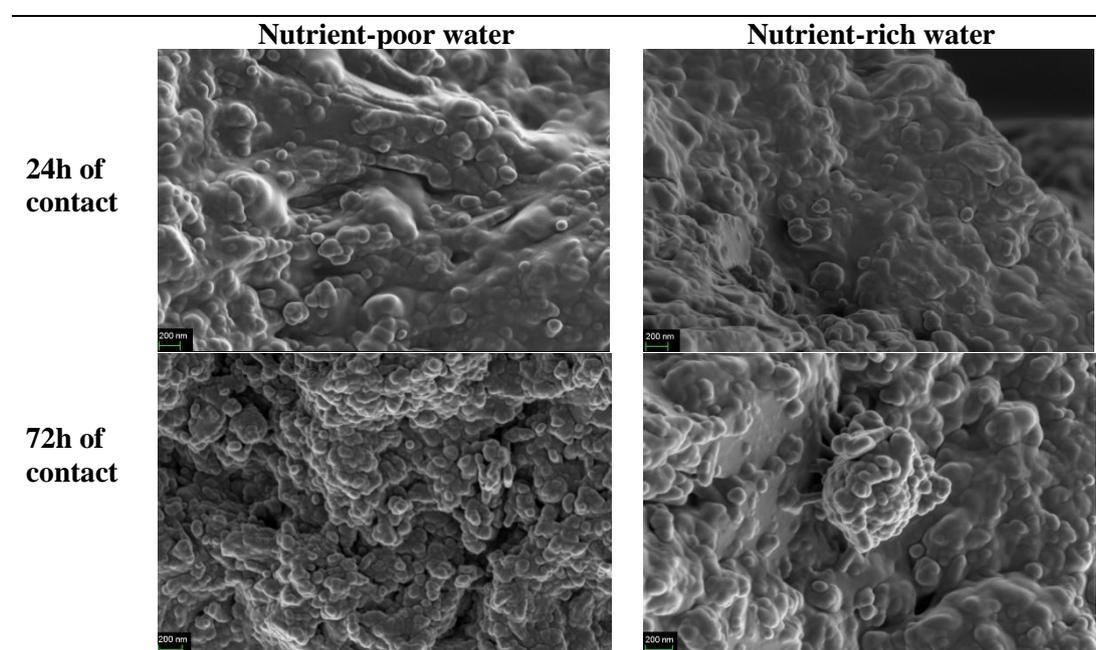


Figure 1. Cells of *A. baumannii* immobilized onto NZ particles.

CONCLUSION

The NZ is a promising material for the immobilization of super-bacterium *A. Baumannii* in both nutrient-poor and nutrient-rich water. This feature could find application in the removal of *A. Baumannii* from contaminated water, in order to mitigate the propagation of this emerging human pathogen in nature and to avoid the consequent public health risk.

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