SILVER-MODIFIED ZEOLITE IN BIOREMEDIATION OF SOIL CONTAMINATED WITH *ACINETOBACTER BAUMANNII*

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ABSTRACT

Bacterium *Acinetobacter baumannii* is an opportunistic pathogen of great clinical significance. Clinically significant *A. baumannii* isolates have been recorded in wastewaters, natural aquatic habitats and soils contaminated by human waste. The aim of this study was to investigate the potential application of unmodified (NZ) and Ag-modified (AgNZ) natural zeolitized tuff to remove *A. baumannii* from natural soil. Pure culture of pandrug-resistant *A. baumannii* was suspended in spring water and inoculated into 100 g of fresh alkaline red palaeosol, which was supplemented with 1wt.% of NZor AgNZ. The abundance of *A. baumannii* was monitored on CHROMagarAcinetobacter at 36°C/24h, while total heterotrophic bacteria were monitored on Nutrient agar 22°C/72h. *A. baumannii* successfully survived 50 days in soil with the addition of NZ. The AgNZ showed a strong bactericidal activity, after only 1h exposure (removal of 6.8±0.1 log CFU/g). The abundance of native heterotrophic bacteria was reduced by AgNZ for 2.6±0.1 log CFU/g, but a large portion of the population remained viable (4.1±0.2 log CFU/g). AgNZ is a promising material for the bioremediation of soil contaminated with hospital pathogen *A. baumannii*. The application of AgNZ does not influence the population of native heterotrophic bacteria in soil, which makes it acceptable for use in real environment.

Keywords: *Acinetobacter baumannii*, bacteria, soil, natural zeolite, Ag-modified zeolite.

INTRODUCTION

Bacterium *Acinetobacter baumannii* is a Gram-negative, non-spore forming coccobacillus. It is an opportunistic pathogen causing infections in immunosuppressed patients [1]. The resistance of *A. baumannii* to antibiotics and commonly used disinfectants is a growing concern as well as its persistence under adverse environmental conditions [2,3]. *A. baumannii* isolates of clinical significance have been reported in wastewaters [4] and natural aquatic environments [5], as well as in soils contaminated by human solid waste [6,7]. The aim of this study was to assess the effect of natural zeolitized tuff (NZ) and Ag-modified natural zeolitized tuff (AgNZ) on the abundance of *A. baumannii* in natural soil, to examine zeolitesas potential bioremediation agents.

EXPERIMENTAL

A. baumannii isolate (EF7) recovered from effluent of the wastewater treatment plant in Zagreb was used in the experiment. EF7 is resistant to all tested antibiotics and classified as pandrug resistant. Further characterisation of the isolate is given in [8].

The soil sample used in the experiment is a red palaeosol situated on Cretaceous limestone from Istria, Croatia. The pH value of the soil sample was determined after triplicate suspension of soil in distilled water (1:2.5) with WTWSenTix81 electrode. The chemical composition was determined by the commercial Bureau Veritas Mineral Laboratories, Canada. The mineral composition (fraction < 2 mm and fraction < 2 μm) was determined by X-ray powder diffraction (XRD) using a Philips diffractometer (graphite monochromator,

CuKα radiation, proportional counter). The <2 μm fraction (clay fraction) was separated by sedimentation in cylinders and quantitatively obtained after the appropriate settling time. This fraction was analysed in order to define clay mineral phases. The XRD patterns of clay fraction (non-oriented and oriented preparations) were obtained after the following treatments: air drying; Mg-saturation; K-saturation; K-saturation and ethylene glycol solvation; K-saturation and dimethyl sulfoxide solvation; Mg-saturation and ethylene glycol solvation; and heating for two hours at 350 and 550°C after K and Mg saturation. The mineral phases were identified using the Powder Diffraction File (1996) data system and the PanalyticalXPertHighScore (v. 1.0d) program package. The identification of clay minerals was generally based on the methods in [9].

The NZ was obtained from a sedimentary deposit in the Zlatokop mine, Serbia for which mineralogical analysis [10] gives (wt.%): clinoptilolite - 73, plagioclase - 14 and quartz - 13. AgNZ prepared by an ion-exchange procedure [10] contained 53.78 mg Ag^+ per g of dry sample (0.50 mmol Ag^+/g). Both NZ and AgNZ were of particle size 0.063-0.1 mm.

Overnight culture of EF7 was suspended in autoclaved commercially available spring water. Bacterial suspension was added into 100 g of fresh non-sterilized red palaeosol. This suspension was used to adjust the moisture of soils to maximum water holding capacity (30 wt.%) and simultaneously to supplement the soil with *A. baumannii*. One gram of NZ or AgNZ was then added to soil samples (100 g) and thoroughly mixed. The moisture of the soil was kept constant in both systems with occasional addition of autoclaved spring water. Triplicate subsamples of 1 g soil subjected to experiment were suspended in physiological solution and analysed. During 50 days, the abundance of *A. baumannii* was monitored on CHROMagarAcinetobacter at 36°C/24h, while total aerobic heterotrophic bacteria were monitored on Nutrient agar at 22°C/72h. Bacterial numbers were expressed as log CFU (Colony Forming Unit) per one gram of wet soil. Statistical analysis was carried out using Statistica 13.3 (TIBCO Software, Inc.).

RESULTS AND DISCUSSION

Fresh red palaeosol gave an alkaline reaction (pH=8.43±0.14). Main chemical components were as follows (wt.%): SiO_2- 57.56, Al_2O_3 -15.62, Fe_2O_3- 6.16, CaO-4.62, MgO-1.54, K_2O -1.72. Total content of MnO, Na_2O, TiO_2, P_2O_5 was less than 1 wt.%. The mineralogical analysis revealed the presence of quartz, plagioclase, K feldspar, goethite, haematite, micaceous clay minerals (illitic material and mica), kaolinite, chlorite, mixed-layer clay mineral(s) as well as 14 Å clay mineral, with dolomite and calcite being responsible for the soil alkalinity.

A. baumannii successfully survived in the soil sample supplemented with NZ during 50 days of monitoring (Figure 1). During the experiment, the bacteria did not multiply, probably because of the low content of organic carbon in soil (0.215 wt.%). Statistically significant reduction of *A. baumannii* abundance for 1.5±0.1 log CFU/g at the end of the experiment was recorded (p=0.000). However, the abundance of *A. baumannii* was still high (5.4±0.1 log CFU/g). The reduction of abundance cannot be ascribed to NZ, since NZ does not show antimicrobial activity [10]. By immobilization onto NZ particles, bacteria are protected against unfavourable environmental conditions [11]. The observed reduction could be explained by a prolonged bacterial exposure to starvation. Total heterotrophic bacteria showed less variation in abundance, since they are already adapted to conditions in soil.

Figure 2 shows strong bactericidal activity of the AgNZ toward *A. baumannii* after only 1hexposure (removal of 6.8±0.1 log CFU/g), which is in accordance with a previous study in the water medium [10]. It has been reported that Ag^+ leached from AgNZ are responsible for

the antimicrobial activity [10]. The abundance of total heterotrophic bacteria was reduced significantly for 2.6±0.1 log CFU/g (p=0.000). However, there was still 4.1±0.2 log CFU/g of heterotrophic bacteria. After the microscopic examination, we confirmed that the majority of the remaining heterotrophic bacteria were spore forming, which are common for soil samples (data not shown).

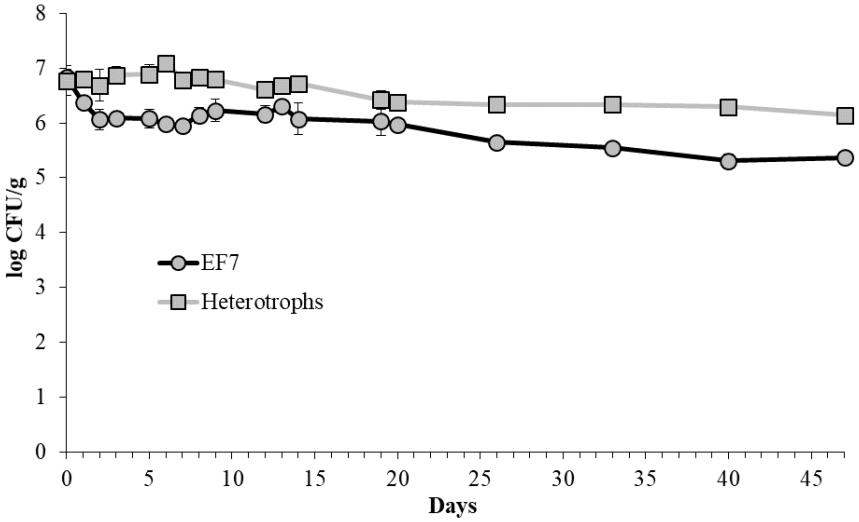

Figure 1. Survival of *A. baumannii* in red palaeosol supplemented with 1 wt.% of unmodified natural zeolitized tuff during 50 days of monitoring. Initial *A. baumannii* abundance was 6.8±0.1 log CFU/g.

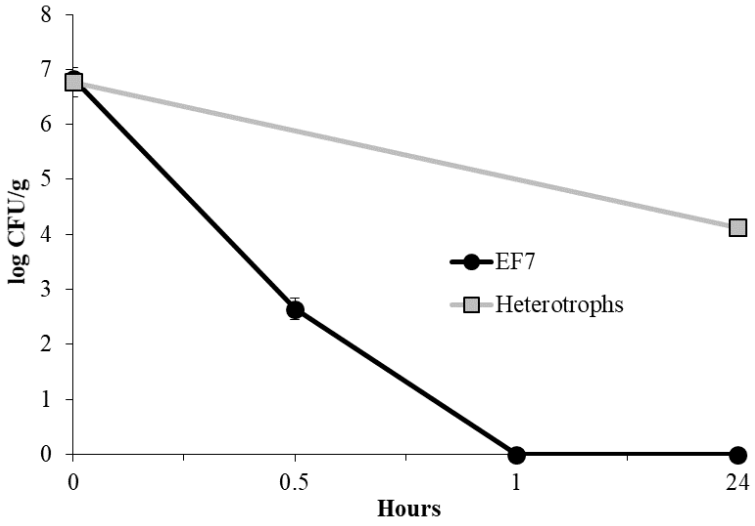

Figure 2. Survival of *A. baumannii* in red palaeosol supplemented with 1 wt.% of Ag-modified zeolite during 24h of monitoring. Initial *A. baumannii* abundance was 6.8±0.1 log CFU/g.

CONCLUSION

The addition of NZ supports the long-term survival of *A. baumannii* in the studied red palaeosol. In contrast, AgNZ shows remarkable bactericidal activity against *A. baumannii* after only 1h exposure, while the abundance of total native heterotrophic bacteria remains high. AgNZ is a promising material for the bioremediation of soils contaminated with hospital pathogens.

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REFERENCES

[1] K. J. Towner. *J. Hosp. Infect.*, 2009,**73**, 355-363.

[2] C.-R. Lee, J.H. Lee, M. Park, K.S. Park, I.K. Bae, Y.B. Kim, C.-J. Cha, B.C. Jeong and S.H. Lee, *Front. Cell. Infect. Microbiol.*, 2017, **7**, 1-35.

[3] Z. Bravo, M. Orruño, C. Parada, V.R. Kaberdin, I. Barcina and I. Arana, *Arch. Microbiol.*, 2016,**198**, 399-407.

[4] P. G. Higgins, J. Hrenović, H. Seifert and S. Dekić, *Water Res.*, 2018, **140**, 261-267.

[5] M. Šeruga Musić, J. Hrenović, I. Goić-Barišić, B. Hunjak, D. Škorić and T. Ivanković, *J. Hosp. Infect.*, 2017, **96**, 323-327.

[6] J. Hrenović, G. Durn, I. Goić-Barišić and A. Kovačić, *Appl. Environ. Microbiol.*, 2014, **80,** 2860-2866.

[7] J. Hrenović, G. Durn, M. Šeruga Musić, S. Dekić, T. Troskot-Čorbić and D. Škorić, *Sci. Total Environ.*, 2017, **607–608**, 1049-1055.

[8] I. Goić-Barišić, M. Šeruga Musić, A. Kovačić, M. Tonkić and J. Hrenovic, *Microb. Drug Resist.*, 2017, **23**, 494-496.

[9] D.M. Moore and R.C. Reynolds, 1989, Oxford University Press, Oxford, pp. 378.

[10] J. Hrenović, J. Milenković, I. Goić-Barišić and N. Rajić, *Micropor. Mesopor. Mater.*, 2013, **169**, 148-152.

[11] T. Ivanković and J. Hrenović, Proceedings of the 4th Slovenian-Croatian Symposium on Zeolites, 2011, pp. 73-76.