Bactericidal effect of zeolites against Gram-negative bacteria

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Introduction

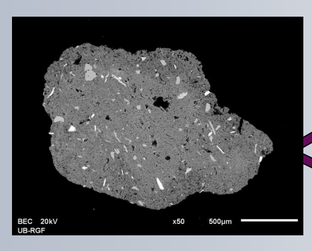
In view of the increasing resistance of bacteria towards available antibiotics, research efforts are focused to alternative disinfectants. *Escherichia coli* and *Acinetobacter baumannii* are one of the most multidrug-resistant bacteria. In this study, the antibacterial activity of natural zeolite – clinoptilolite enriched with metal cations against pathogenic Gram-negative bacteria *E. coli* and *A. baumannii* was tested in different water media.

Aim

Natural zeolite – clinoptilolite (Z) enriched with silver and copper ions was investigated as antibacterial agent toward wildtype of *E. coli* DSM 498, isolates of *E. coli* from a lake water in Serbia, as well as against multi-drug resistant clinical isolate of *A. baumannii* belonging to European clone I and II (EU I and II).

Methods

• Clinoptilolite-rich tuff (Z) from Zlatokop deposit (Serbia) was enriched with Cu(II) and Ag(I) ions by an ion-exchange procedure. The obtained Ag-Z and Cu-Z contained similar amounts of metal ions (~0.3 mmol Cu/Ag per 1 g of Z).



$AgNO_3(aq)$ → Ag-Z (s)

$CuSO_4(aq)$ → Cu-Z (s)

Natural zeolite-Z

• The antibacterial tests were conducted in: phosphate buffer solution (PBS), effluent from a wastewater treatment plant, or water from Sava lake. Solid/liquid ratio was 1:1000.

Ag-Z (s)

Cu-Z (s)

Water medium

E. coli DSM 498, isolates of *E. coli* from lake water, or *A. baumannii* EU I and II

Shaking at 105 rpm during 1 and 24 h at 37±0.1° C

Results

Table 1. Antibacterial activity expressed as percent of reduction of *E. coli* (DSM 498, isolates from lake water 1 and 2) in real water media by Z, Cu- and Ag-Z. Mean values of triplicate measurements and standard deviations are presented. The lowest limit of detection was 10 CFU cm^{-3}. A - significant as compared to positive control without addition of zeolite.

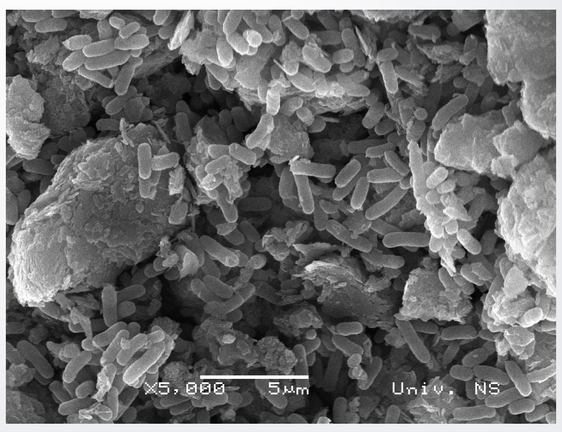
E. coli isolate	Time (h)	Percent of reduction (%)		
		Zeolite		
		Z	Cu-Z	Ag-Z
DSM 498	1	0.3	43.0^{A}	100.0^{A}
	24	-1.1	100.0^{A}	100.0^{A}
Isolate 1	1	-0.2	73.6^{A}	100.0^{A}
	24	-1.4	100.0^{A}	100.0^{A}
Isolate 2	1	-1.5	60.4^{A}	100.0^{A}
	24	1.8	100.0^{A}	100.0^{A}

t_0 (log CFU cm^{-3}) *E. coli* DSM 498 = 7.1±0.3; t_0 (log CFU cm^{-3}) *E. coli* Isolate 1 and 2 = 7.2±0.1.

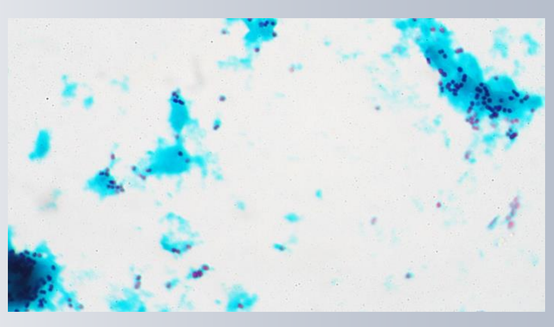
Table 2. Antibacterial activity expressed as percent of reduction of *A. baumannii* EU I and II in PBS buffer by Cu- and Ag-Z. Mean values of triplicate measurements and standard deviations are presented. The lowest limit of detection was 10 CFU cm^{-3}. A - significant as compared to positive control without addition of zeolite.

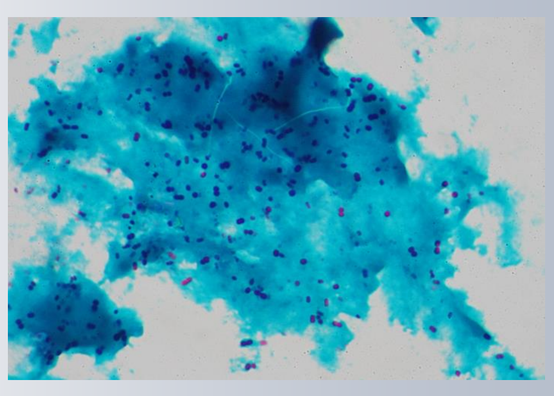
A. baumannii isolate	Time (h)	Percent of reduction (%)	
		Zeolite	
		Cu-Z	Ag-Z
EU I	1	15.80^{A}	13.56^{A}
	24	-1.71	78.61^{A}
EU II	1	21.86^{A}	37.10^{A}
	24	39.83^{A}	100.00^{A}

t_0 (log CFU cm^{-3}) *A. baumannii* EU I = 8.80±0.17; t_0 (log CFU cm^{-3}) *A. baumannii* EU II = 1.40±0.17.



E. coli DSM 498 spontaneously immobilized on the surface of Z.





Thick layer of extracellular substances with embedded cells of *A. baumannii* isolates EU I and II.

Conclusions

- All examined isolates of *E. coli* are more sensitive toward Ag-Z than toward Cu-Z.
- *E. coli* isolate 1 is the more sensitive toward Cu-Z than isolate 2 and DSM 498.
- *A. baumannii* isolates are more resistant than *E. coli* toward Ag-Z and Cu-Z.
- *A. baumannii* belonging to EU II is more sensitive than EU I toward Ag-Z and Cu.
- Concerning the bactericidal effect, Cu-Z and Ag-Z are promising disinfectants.
- The natural unmodified zeolite can be used as carrier of bacteria in bisorption process.

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