ACTA ABP BIOCHIMICA POLONICA

Vol. 62, No 4/2015
721–724
http://dx.doi.org/10.18388/abp.2015_1118

Regular paper

Influence of the silver nanoparticles on microbial community in different environments*

Katarzyna Pietrzak✉ and Beata Gutarowska

Institute of Fermentation Technology and Microbiology, Lodz University of Technology, Łódź, Poland

The aim of this study was to assess the influence of silver nanoparticles (AgNPs) on the number and diversity of microbial community in different environments (soil extract, water, sewage), and to determine whether the environment inhibits or accelerates the influence of AgNPs on moulds. AgNPs (45 ppm) present in the environment decreased bacterial (91%) and fungal (33–85%) numbers, and eliminated some strains, e.g., *Alternaria alternata* and *Cryptococcus laurentii*. Based on the biomass growth of *Aspergillus niger* and *Penicillium chrysogenum* in a medium with AgNPs and the environmental samples, it was noticed that environment can enhance (soil extract) or inhibit (sewage) antifungal activity of AgNPs.

Key words: silver nanoparticles, disinfection, microorganisms, sewage, water, soil

Received: 21 July, 2015; revised: 09 October, 2015; accepted: 30 October, 2015; available on-line: 27 November, 2015

INTRODUCTION

The unique properties of silver nanoparticles (AgNPs) make it possible to use them in various branches of industry (cosmetics, medicine, chemistry, electronics, papermaking, pharmacy, clothing, household appliances, etc.) (Samuel & Guggenbichler, 2004; DiRienzo, 2006; Mishra *et al.*, 2007; Luoma, 2008; Tolaymat *et al.*, 2010).

Although nanoparticles can enter the environment during the manufacturing process and use of the final products, majority of them are released during product disposal or recycling. A large proportion of silver released into the environment remains in the soil (0.3–30 mg/kg dry weight) or wastewater sludge (0.22–21.93 mg/kg dry weight) (Warrington, 1996; Shafer *et al.*, 1998; Shamuyarira & Gumbo, 2014), while the rest is dispersed by air (Ratte, 1999). Studies showed that bacteria can accumulate about 23 mg Ag^+/g dry wt. (Pümpel & Schinner, 1986), while fungi accumulate metals up to 50% of their dry weight (20–50 mg/g dry wt.) (Tobin *et al.*, 1984; Pümpel & Schinner, 1986; Kuyucak & Volesky, 1988). Silver nanoparticles also aggregate in algae, plants, fish, snails, worms and mammals (Campbell, 1994; Eisler, 1996; Ratte, 1999).

A large number of products and waste contains silver nanoparticles. AgNPs accumulate in the environment and their wide antimicrobial effects may potentially cause ecological problems; for example, they can cause damage to the soil by eliminating particular species of microorganisms, or interfering with the phosphorous, sulphur and nitrogen cycles (Ratte, 1999). To date, published studies have solely focused on the influence of AgNPs on the total number of microorganisms in soil and wastewater (Schlich *et al.*, 2013; Patil, 2014). However, there are no detailed studies investigating the influence of the environment (water, soil, sewage) on the microbial activity of AgNPs. The aim of this study was to assess the influence of silver nanoparticles on the number and diversity of microbial communities in different environments (soil extract, water, sewage), and determine whether the environment has a protective effect on microorganisms (particularly moulds) or enhances the antimicrobial effect of AgNPs.

MATERIALS AND METHODS

Environmental samples. Water, soil extract and sewage were used for environmental sample testing (Section "Influence of AgNPs on the number and diversity of the microbial community in water, soil extract and sewage") and studies using model organisms (Section "Influence of the environment (water, soil extract, sewage) on the antifungal properties of AgNPs"). Water was sampled from floodplain ponds of the Sokołówka river in Łódź, Poland (51°48'16.9"N; 19°26'39.3"E). Soil was sampled from farmland (rye) in Syberia, Poland (51°50'26.5"N; 19°45'45.7"E). Sewage was sampled from a grit chamber of the Group Wastewater Treatment Plant, Łódź, Poland (51°43'40.6"N; 19°20'51.6"E). Water and sewage were filtered on cellulose filters (Filtrak, Germany) to separate impurities. Extracts from soil samples were prepared by suspending 10 g of soil in 100 ml of sterile distilled water, shaking (30 min, 200 rpm) and filtering on cellulose filters (Filtrak, Germany).

Silver nanoparticles. Colloidal silver nanoparticles (AgNPs) were obtained by a chemical reduction of $AgNO_3$ with sodium citrate and polyvinylpyrrolidone (PVP) (Mennica Polska S.A, Poland). The stock solution had a concentration of 90 ppm, pH 7, and particle sizes between 10–15 nm (60–70%) and 50–80 nm (30–40%) (Gutarowska *et al.*, 2012).

Microorganisms. We used fungi as the study model. Stocks were obtained from the American Type Culture Collection (ATCC) and Pure Culture Collection at the Institute of Fermentation Technology and Microbiology, Lodz University of Technology (Łódzki Ośrodek Czystych Kultur – ŁOCK). The following organisms were used: *Aspergillus niger* ATCC 16404 (Minimum In-

✉e-mail: khpietrzak@gmail.com

*The results were presented at the 6th International Weigl Conference on Microbiology, Gdańsk, Poland (8–10 July, 2015).

Abbreviations: AgNPs, silver nanoparticles; MEA, malt extract agar; MEB, malt extract broth; MIC, minimum inhibitory concentration; PVP, polyvinylpyrrolidone; TSA, tryptic soy agar

Table 1. Influence of AgNPs on the number of microorganisms in environmental samples

Environment	Number of microorganisms (cfu/100 ml)				R (%)	
	Control (without AgNPs)		with AgNPs			
	fungi	bacteria	fungi	bacteria	fungi	bacteria
soil extract	$5.28\times10^5 \pm 2.99\times10^5$	$4.10\times10^8 \pm 1.48\times10^8$	$3.53\times10^5 \pm 8.13\times10^4$	$4.06\times10^7 \pm 1.03\times10^6$*	33.22	90.09
water	$3.98\times10^5 \pm 3.02\times10^5$	$1.73\times10^8 \pm 3.09\times10^7$	$9.35\times10^4 \pm 7.07\times10^3$*	$1.60\times10^7 \pm 6.05\times10^6$*	79.15	90.75
sewage	$1.80\times10^5 \pm 1.59\times10^5$	$1.37\times10^7 \pm 2.58\times10^6$	$2.70\times10^4 \pm 1.48\times10^4$*	$1.34\times10^6 \pm 3.01\times10^5$*	85.00	90.20

average ± standard deviation; R, reduction calculated according to eqn. (1); *statistically significant differences between control samples (without AgNPs) and samples with AgNPs; one-way ANOVA $p<0.05$; *others* – unidentified strains with the frequency of occurrence < 20%; *statistically significant differences between control samples (without AgNPs) and samples with AgNPs; one-way ANOVA $p<0.05$

hibitory Concentration (MIC) 22.5 ppm) and *Penicillium chrysogenum* ŁOCK 0531 (MIC 45 ppm). Minimum inhibitory concentrations (MIC) for fungi were established in previous studies (Gutarowska *et al.*, 2012). We selected the above strains as they are frequently isolated from the tested environments, and the lack of research regarding the influence of AgNPs on moulds in these environments.

Influence of AgNPs on the number and diversity of the microbial community in water, soil extract and sewage. The effect of silver nanoparticles on the number of microorganisms in water, soil extract and sewage was determined by adding AgNPs (45 ppm – established in previous studies) to the environmental samples and incubating for 24 h at 30±2°C. Microbial numbers in all samples (with and without silver nanoparticles) were determined using serial dilutions in sterile saline (0.85% NaCl), and culturing on microbiological media: MEA (Malt Extract Agar, Merck, Germany) with chloramphenicol for fungi, and TSA (Tryptic Soy Agar, Merck Germany) with nystatin for bacteria. Samples were incubated using the following conditions: bacteria 24 h, 30±2°C, and fungi 72 h, 27±2°C. After incubation, the microbial colonies were counted and expressed as cfu/ml. All samples were done in triplicate.

The reduction in microorganism number (total and particular species) due to AgNPs treatment was calculated using equation (1):

$$R = \frac{N_0 - N}{N_0} \times 100\% \quad (1)$$

where:
N is the number of microorganisms in the sample with silver nanoparticles (cfu/100 ml);
N_0 is the number of microorganisms in the sample without silver nanoparticles (cfu/100 ml).

A one-way ANOVA was performed to assess statistical significance between the number of microorganisms (cfu/100 ml) before and after AgNPs treatment.

The most frequently isolated microorganisms (> 20%) from all environmental samples, before and after silver nanoparticle addition, were identified. Bacteria were identified using macro- and microscopic observations, and API tests: API Coryne and API 20NE (bioMerieux, Germany). Yeasts were identified using macro- and microscopic observations, and API AUX test (bioMerieux, Germany). Moulds were identified using macro- and microscopic observations, and taxonomic keys (Klich, 2002; Pitt & Hocking, 2009; Frisvad & Samson, 2004; Bensch *et al.*, 2010).

Influence of the environment (water, soil extract, sewage) on the antifungal properties of AgNPs. To determine the influence of AgNPs on the growth of the moulds: *A. niger* ATCC 16404 and *P. chrysogenum* ŁOCK 0531, the following media were used: 1) MEB (Malt Extract Broth, Merck, Germany); 2) MEB with AgNPs (22.5-45 ppm depending on the strain); 3) MEB with environmental samples (water, soil extract, sewage); 4) MEB with environmental samples and AgNPs.

MEB and the particular environmental sample were mixed in a ratio of 1:1.

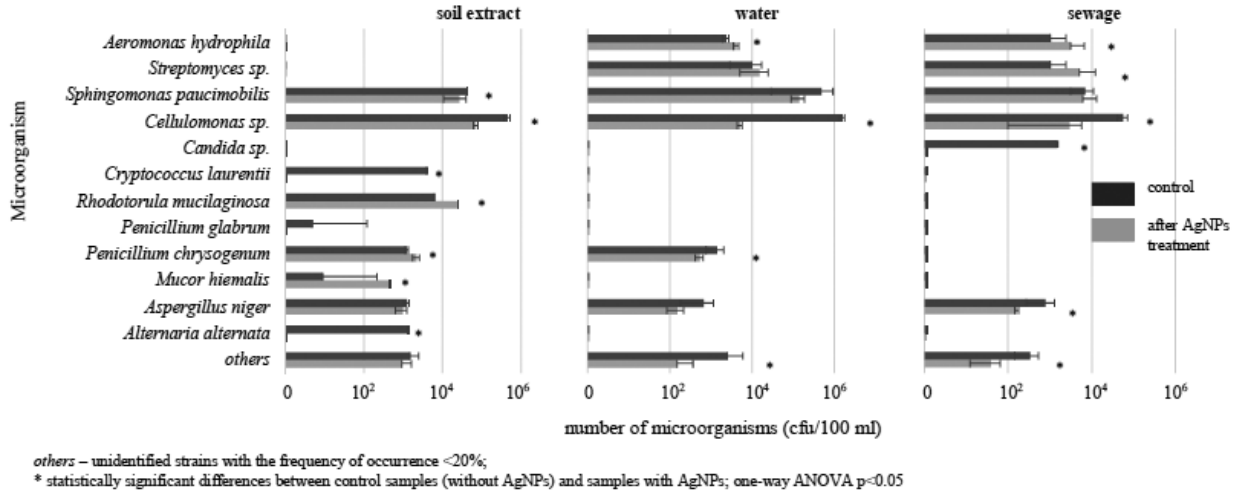
Flasks containing 50 ml of the appropriate medium were inoculated (100 µl) with moulds (inoculum concentration: 10^6 cfu/ml). Samples were incubated for 14 days at 27±2°C. The environmental impact on mould growth was determined by measuring the biomass of the mould after 3, 7 and 14 days. The samples were filtered on cellulose filters (Filtrak, Germany), and the dry matter content was quantified using the gravimetric method (MAC 110/NH, Radwag, Poland). All samples were done in triplicate.

The reduction in mould biomass, due to AgNPs treatment, was calculated using equation (1), where N is the mould biomass. A one-way ANOVA was performed to assess statistical significance between the mould biomass samples (g dry weight) before and after AgNPs treatment.

RESULTS AND DISCUSSION

Bacteria were the predominant group of microorganisms in all environments tested (water, soil extract, sewage). The highest number of bacteria and fungi were in soil extracts, 4.1×10^8 cfu/100 ml and 5.3×10^5 cfu/100 ml, respectively (Table 1). In the environments tested, 11 isolates of microorganisms (4 bacteria, 2 yeasts and 5 moulds) were identified with a frequency of occurrence above 20%. Soil extracts were the most diverse, with 8 isolated strains (Fig. 1). Our results are consistent with other studies on environmental samples (Arvanitidou *et al.*, 2005; Ayansina & Oso, 2006; Chang, 1997; Dunca *et al.*, 2006).

The addition of silver nanoparticles to environmental samples (soil extract, water and sewage) at a concentration of 45 ppm, showed that bacteria are more susceptible to AgNPs than fungi. AgNPs decreased the microorganisms' number by approximately 1 log unit in a statistically significant manner. The reduction of bacterial numbers (R=90.1–90.8%) was similar in all environments tested. The highest reduction in fungal numbers (R=85.0%) was observed with the sewage sample,



others – unidentified strains with the frequency of occurrence <20%;
* statistically significant differences between control samples (without AgNPs) and samples with AgNPs; one-way ANOVA $p<0.05$

Figure 1. Microbial diversity of environments before and after AgNPs application

Table 2. Mycelium biomass in different media containing environmental samples and silver nanoparticles

Day	Biomass in different media (g dry wt.)											
	Control			Soil extract			Water			Sewage		
	M	M+AgNPs	R (%)	M+E	M+E+ AgNPs	R (%)	M+E	M+E+ AgNPs	R (%)	M+E	M+E+ AgNPs	R (%)
P. chrysogenum												
3	0.04±0.01	0.04±0.00*	0.0	0.05±0.01	0.02±0.00*	60.0	0.07±0.03	0.03±0.00*	57.1	0.11±0.01	0.04±0.00*	63.6
7	0.21±0.01	0.15±0.00*	28.6	0.10±0.02	0.04±0.02*	60.0	0.09±0.01	0.06±0.00*	33.3	0.14±0.02	0.05±0.00*	64.3
14	0.25±0.02	0.18±0.01*	28.0	0.11±0.01	0.04±0.02*	63.6	0.12±0.00	0.07±0.01*	41.7	0.14±0.00	0.09±0.01*	35.7
A. niger												
3	0.04±0.01	0.04±0.00	0.0	0.04±0.01	0.02±0.00*	50.0	0.05±0.05	0.02±0.00*	60.0	0.11±0.02	0.03±0.00*	72.7
7	0.30±0.02	0.23±0.02*	23.3	0.16±0.00	0.12±0.00*	25.0	0.14±0.02	0.10±0.00*	28.6	0.15±0.01	0.10±0.02*	33.3
14	0.40±0.11	0.26±0.00*	35.0	0.17±0.03	0.11±0.00*	35.3	0.14±0.00	0.10±0.00*	28.6	0.15±0.01	0.13±0.01*	13.3

average ± standard deviation, M, MEB medium; M+E, MEB + environmental sample; M+E+AgNPs, MEB + environmental sample + MIC AgNPs; R, reduction calculated according to eqn. (1); *statistically significant differences between control samples (without AgNPs) and samples with AgNPs; one-way ANOVA $p<0.05$

while the lowest was with the soil extract (R=33.2%) (Table 1). Schlich *et al.* (2013) tested the influence of silver nanoparticles (NM-300K; <20 nm) on soil microorganisms and obtained a decrease of 39.9–73.2% in microbial biomass after 7 days. Patil (2014) obtained a higher reduction of 92.9–94.6% in bacterial numbers in sewage following AgNPs application, when compared to us; however, they did not conduct studies on fungi, and used silver nanoparticles biosynthesized by *Fusarium semitectum*.

The addition of silver nanoparticles to environmental samples eliminated 4 fungal species: *Alternaria alternata, Penicillium glabrum, Candida* sp., *Cryptococcus laurentii. Cellulomonas* sp. was the most sensitive species to silver nanoparticles, with a statistically significant reduction above 90% (R=93.2–99.7%), with all environmental samples tested. Some species (*A. niger* and *P. chrysogenum),* exhibited different sensitivities to AgNPs, depending on the environment. *Mucor hiemalis, Rhodotorula mucilaginosa, Aeromonas hydrophila* and *Streptomyces* sp. were insensitive to AgNPs (Fig. 1). Biocide sensitivity is dependent on the microorganism's genus, species, strain, and also on isolates, which might be due to previous exposure to a biocide containing silver (Gutarowska *et al.*, 2012). Based on our findings, we hypothesize that the sensitivity of various microorganisms to AgNPs may be linked to environmental properties (protective or enhancing the effectiveness of AgNPs).

Therefore, we conducted studies using model microorganisms to determine the influence of AgNPs on mould biomass growth in media, with or without the environmental background. These studies showed that the addition of silver nanoparticles (at MIC) to MEB medium caused a statistically significant reduction in the mycelium biomass of *A. niger* and *P. chrysogenum,* starting on the 3rd day and persisting until the end of the incubation period (14 days), when compared to control samples without AgNPs (Table 2). The decrease in mould biomass was 28–35% (14th day).

The supplementation of MEB with soil extract, water and sewage delayed mould growth, and decreased mycelium biomass by 44–63%, when compared to control samples containing only the MEB medium. MEB with AgNPs containing soil extracts showed the highest reduction (R=35–64%). Similar results were obtained for control (MEB) and MEB with water (R=28–42%). MEB containing sewage with AgNPs showed the lowest biomass reduction (R=13–36%). Results suggest that sewage

may inhibit the antimicrobial properties of AgNPs, while soil extract may facilitate them. In future research, environmental components must be analysed focusing on the enhancement and inhibitory properties of AgNPs activity against microorganisms.

Acknowledgements

The authors are grateful to Jowita Polak, Engr., for technical support in the course of this study.

REFERENCES

Arvanitidou M, Kanellou K, Vagiona DG (2005) Diversity of Salmonella spp. and fungi in northern Greek rivers and their correlation to fecal pollution indicators. *Environ Res* **99**: 278–284, http://dx.doi.org/10.1016/j.envres.2005.01.002.

Ayansina ADV, Oso BA (2006) Effect of two commonly used herbicides on soil microflora at two different concentrations. *Afr J Biotechnol* **5**: 129-132.

Bensch K, Groenewald JZ, Dijksterhuis J, Starink-Willemse M, Andersen B, Summerell BA, Shin H-D, Dugan FM, Schroers H-J, Braun U, Crous PW (2010) Species and ecological diversity within the Cladosporium cladosporioides complex (Davidiellaceae, Capnodiales). *Stud Mycol* **67**: 1–94, http://dx.doi.org/10.3114/sim.2010.67.01.

Campbell KR (1994) Silver accumulation in three species of fish (family: Centrarchidae) in stormwater treatment ponds. *Florida Scientist* **57**: 34–42.

Chang AC (1997) Land application of sewage sludge: Pathogen issues. Joint FAO/IAEA Div. of Nuclear Techniques in Food and Agriculture, Vienna (Austria). pp 183–190. International Atomic Energy Agency, Div. of Physical and Chemical Sciences, Austria.

DiRienzo M (2006) New applications for silver. *The LBMA Precious Metals Conference* Montreux.

Dunca S, Tanase C, Stafan M, Cojocariu A (2006) *Characterization of the microbiota from different types of anthropogenic soils*. Scientific Annals of "Aleksandru Ioan Cuza". New Series. Section IIa, Vegetal Biology. pp 53–70. University of Iasi.

Eisler R (1996) *Silver hazards to fish, wildlife and invertebrates: A synoptic review*. Contaminant Hazard Reviews Report 32. National Biological Service, U.S. Department of the Interior, Washington, DC.

Frisvad J.C., Samson R.A. (2004) Polyphasic taxonomy of Penicillium subgenus Penicillium. A guide to identification of food and airborne terverticillate penicillia and their mycotoxins. *Stud Mycol* **49**: 1–174.

Gutarowska B, Skóra J, Zduniak K, Rembisz D (2012) Analysis of the sensitivity of microorganisms contaminating museums and archives to silver nanoparticles. *Int Biodeter Biodegrad* **68**: 7–17, http://dx.doi.org/10.1016/j.ibiod.2011.12.002.

Klich MA (2002) *Identification of common Aspergillus species*. Utrecht: Centraalbureau voor Schimmelcultures.

Kuyucak N, Volesky B (1988) Biosorbents for the recovery of metals from industrial solutions. *Biotechnol Lett* **10**: 137–142.

Luoma SN (2008) *Silver nanotechnologies and the environment: old problems or new challenges? Project on Emerging Nanotechnologies*. The Pew Charitable Trusts.

Mishra YK, Mohapatra S, Kabiraj D, Mohanta B, Lalla NP, Pivin JC, Avasthi DK (2007) Synthesis and characterization of Ag nanoparticles in silica matrix by atom beam sputtering. *Scripta Materialia* **56**: 629–632, http://dx.doi.org/10.1016/j.scriptamat.2006.12.008

Patil SR (2014) Antibacterial activity of silver nanoparticles synthesized from *Fusarium semitectum* and green extracts. *Int J Sci Eng Res* **2**: 140–145.

Pettibone GW, Sullivan SA, Shiaris MP (1987) Comparative survival of antibiotic-resistant and -sensitive fecal indicator bacteria in estuarine water. *Appl Environ Microbiol* **53**: 1241–1245.

Pitt JI, Hocking AD (2009) *Fungi and food Spoilage*. North Ryde. Springer.

Pümpel T, Schinner F (1986) Silver tolerance and silver accumulation of microorganisms from soil materials of a silver mine. *Appl Microbiol Biotechnol* **24**: 244 -247, http://dx.doi.org/10.1007/BF00261545.

Ratte HT (1999) Bioaccumulation and toxicity of silver compounds: a review. *Environ Toxicol Chem* **18**: 89–108, http://dx.doi.org/10.1002/etc.5620180112.

Samuel U, Guggenbichler JP (2004) Prevention of catheter-related infections: the potential of a new nano-silver impregnated catheter. *Int J Antimicrob Ag* **23S1**: S75–S78, http://dx.doi.org/10.1016/j.ijantimicag.2003.12.004.

Shafer MM, Overdier JT, Armstrong DE (1998) Removal, partitioning, and fate of silver and other metals in wastewater treatment plants and effluent-receiving streams. *Environ Toxicol Chem* **17**: 630–641, http://dx.doi.org/10.1002/etc.5620170416.

Shamuyarira KK, Gumbo JR (2014) Assessment of Heavy Metals in Municipal Sewage Sludge: A Case Study of Limpopo Province, South Africa. *Int J Environ Res Public Health* **11**: 2569–2579, http://dx.doi.org/10.3390/ijerph110302569.

Schlich K, Klawonn T, Terytze K, Hund-Rinke K (2013) Hazard assessment of a silver nanoparticle in soil applied via sewage sludge. *Environ Sci Eur* **25**: 17, http://dx.doi.org/10.1186/2190-4715-25-17.

Tobin JM, Cooper DG, Neufeld RJ (1984) Uptake of metal ions by Rhizopus arrhizus biomass. *Appl Environ Microbiol* **47**: 821–824.

Tolaymat T, El Badawy A, Genaidy A, Scheckel K, Luxton T, Suidan M (2010) An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: A systematic review and critical appraisal of peer reviewed scientific papers. *Sci Total Environ* **408**: 999–1006, http://dx.doi.org/10.1016/j.scitotenv.2009.11.003.

Warrington PD (1996) *Ambient water quality criteria for silver*. Ministry of Environment. Lands and Parks Province of British Columbia. Environmental Protection Department. Water Quality Branch. Victoria, BC.