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Regular paper

Influence of silver nanoparticles on metabolism and toxicity of moulds*

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The unique antimicrobial features of silver nanoparticles (AgNPs) are commonly applied in innumerable products. The lack of published studies on the mechanisms of AgNPs action on fungi resulted in identification of the aim of this study, which was: the determination of the influence of AgNPs on the mould cytotoxicity for swine kidney cells (MTT test) and the production of selected mycotoxins, organic acids, extracellular enzymes by moulds. The conducted study had shown that silver nanoparticles can change the metabolism and toxicity of moulds. AgNPs decrease the mycotoxin production of *Aspergillus* sp. (81–96%) and reduce mould cytotoxicity (50–75%). AgNPs influence the organic acid production of *A. niger* and *P. chrysogenum* by decreasing their concentration (especially of the oxalic and citric acid). Also, a change in the extracellular enzyme profile of *A. niger* and *P. chrysogenum* was observed, however, the total enzymatic activity was increased.

Key words: silver nanoparticles, moulds, mycotoxins, cytotoxicity, MTT, organic acids, extracellular enzymes

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INTRODUCTION

The unique antimicrobial features of silver nanoparticles (AgNPs) are commonly applied in innumerable products. They are used in cosmetology, pharmacy, medicine, packaging, chemistry, disinfection, electronics and others (DiRienzo, 2006; Huo *et al.*, 2006; Wiley *et al.*, 2005; Tolaymat *et al.*, 2010; Gutarowska *et al.*, 2014a). Antimicrobial features of silver nanoparticles are confirmed by many scientific studies on the mechanisms of action in bacteria. The bactericidal mechanism of action of silver nanoparticles is well known and multidirectional. The first target is the bacterial cell wall, where silver ions can bind to the bacterial cell wall, perforate it and aggregate in the cytoplasm (Feng *et al.*, 2000). They also cause irregular "pits" in the bacterial cell wall (Sondi & Salopek-Sondi, 2004). The cell wall abnormalities appear due to the interactions of silver ions with a number of electron donor functional groups like thiols, phosphates, hydroxyls, imidazoles, indoles, and amines. Studies on bacterial cells also show that due to the different cell wall structure of Gram-positive bacteria (thicker and negatively charged), they are more resistant to the AgNPs activity than Gram-negative species (Egger *et al.*, 2009). The accumulation of AgNPs inside the cell membrane leads to the increased permeability disorder of the respiratory chain (collapse of the proton gradient) (Feng *et al.*, 2000; Sondi & Salopek-Sondi, 2004; Holt & Bard, 2005). Also, the cell enzymes: NADH dehydrogenase and the cytochrome oxidase are potential targets for silver activity (Bragg, Rainnie, 1974; Dallas *et al.*, 2011). The gradual release of free silver ions from AgNPs solution inhibits the bacterial cell DNA replication, due to the Ag^+ ability to bind to phosphate residues of DNA molecules (Morones *et al.*, 2005; Dallas *et al.*, 2011). AgNPs also influences expression of genes coding for proteins and enzymes involved in energy reactions (Gogoi *et al.*, 2006).

Fungal susceptibility mechanism to silver nanoparticles is being investigated as well. There are reports that AgNPs are able to bind yeast cell wall and cell membrane, causing the effluence of intracellular components (Gajbhiye *et al.*, 2009; Nasrollahi *et al.*, 2011). They disorder the potential gradient, inhibit the budding process and mycelia growth (Endo *et al.*, 1997; Lee *et al.*, 2010). AgNPs cause inhibition of the mould sporulation process (Pinto *et al.*, 2013).

Previously conducted studies by Gutarowska *et al.* showed that the AgNPs preparation applied on different technical materials (paper, leather, wood, textiles) demonstrated higher effectiveness against fungi than against bacteria and yeasts. The microorganisms' resistance was as follows: *B. subtilis* > *S. aureus* > *E. coli* > *A. niger* (Gutarowska *et al.*, 2014a). Moreover, the disinfection of historical materials (wood, parchment, canvas, paper) eliminated *Aspergillus niger* and *Cladosporium herbarum* by 99.9% and *Penicillium* sp. by 80.9–98.3% (Gutarowska *et al.*, 2012b). The high susceptibility of moulds to silver nanoparticles is surprising, considering their known resistance to various disinfectants.

The lack of published studies on the mechanisms of AgNPs action on fungi resulted in identification of the aim of this study, which was: the determination of the influence of AgNPs on the mould cytotoxicity for swine kidney cells (MTT test) and the production of selected mycotoxins, organic acids, extracellular enzymes by moulds.

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Abbreviations: AgNPs, silver nanoparticles; DMSO, dimethyl sulfoxide; IC_{50}, half maximal inhibitory concentration; MEA, malt extract agar; MEB, malt extract broth; MIC, minimal inhibitory concentration; MTT, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide; SK, swine kidney

MATERIALS AND METHODS

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

Microorganisms. In these studies, moulds from pure culture collections: Pure Culture Collection of Institute of Food Technology of Plant Origin at Poznań University of Life Sciences (KA), Poland; Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (DMS); Northern Regional Research Laboratory, USDA, Culture Collection Peoria, IL, USA (NRRL); American Type Culture Collection, Manassas, VA, USA (ATCC); Pure Culture Collection at Institute of Fermentation Technology and Microbiology at Lodz University of Technology, Poland (ŁOCK), were used. The mycotoxin profile and cytotoxicity assays were done for *Aspergillus flavus*, *Aspergillus niger* (strain no. 1), *Aspergillus westerdijkiae*. The extracellular enzyme profile and organic acid production analyses were performed for *Aspergillus niger* (strain no. 2) and *Penicillium chrysogenum*. Strains were selected by defined features determined in the previous studies (Table 1) (Gutarowska *et al*., 2010; 2012a).

Prior to each experiment, the mould inoculum was standardized to 10^6 cfu/ml. For all analyses, the same amount of mycelium biomass or post-incubation medium, gathered on the first day of stationary phase, were tested. Mould growth phases were established by a mathematical method developed in the previous studies.

Influence of AgNPs on mycotoxin production. The influence of silver nanoparticles on the production of selected mycotoxins was performed using HPLC-MS. Moulds were cultivated on MEA (Malt Extract Agar, Merck, Germany) with AgNPs (in MIC) and MEA (control) for 7 days at a temperature of 27 ± 2°C. Each mould sample (5 g; mycelium with medium) was homogenised with 20 ml of mixture of acetonitrile (ACN): water (H_2O): acetic acid (AcOH) (79:20:1) for 3 minutes. Filtered samples (4 ml), were evaporated under nitrogen and reconstituted in a mobile phase (1 ml; A: H_2O + 5 mM CH_3COONH_4 + 1% CH_3COOH, B: MeOH + 5 mM CH_3COONH_4 + 1% CH_3COOH). Detection and quantification of mycotoxins were carried out using high performance liquid chromatograph (HPLC) Nexera (Shimadzu, Tokyo, Japan) with a mass detector API 4000 (AB Sciex, Foster City, CA, USA). Mycotoxins were separated on a chromatographic column Gemini C18 (150×4.6 mm, 3 μm) (Phenomenex Inc., Torrance, CA, USA); mobile phase flow rate: 0.5 ml/min, injection volume: 7 μl. The mycotoxin concentration was calculated using external calibration and standard solutions.

Table 1. Mould sensitivity to silver nanoparticles

Mould	Origin	MIC (ppm)
Aspergillus flavus	KA 30	45.0
Aspergillus niger 1	DMS 12634	45.0
Aspergillus niger 2	ATCC 16404	22.5
Aspergillus westerdijkiae	NRRL 3174	45.0
Penicillium chrysogenum	ŁOCK 0531	45.0

KA — Pure Culture Collection of Institute of Food Technology of Plant Origin at Poznań University of Life Sciences, Poland; DMS — Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures; NRRL — Northern Regional Research Laboratory, USDA, Culture Collection Peoria, IL, USA; ATCC — American Type Culture Collection, Manassas, VA, USA; ŁOCK — Pure Culture Collection at Institute of Fermentation Technology and Microbiology at Lodz University of Technology, Poland (Łódzki Ośrodek Czystych Kultur)

Influence of AgNPs on mould cytotoxicity. The influence of silver nanoparticles on the mould cytotoxicity was performed using a MTT test. The MTT test is a quantitative colorimetric assay of toxicity, it is based on yellow tetrazolium salt reduction of MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) to purple formazan occurring in the mitochondria of active living cells.

Moulds were cultivated on MEA with AgNPs (in MIC) and MEA (control) for 7 days at a temperature of 27 ± 2°C. Swine kidney cells (SK) were grown on the medium with antibiotics (penicillin and streptomycin, Sigma Aldrich, USA) and fetal calf serum (Sigma Aldrich, USA) in a CO_2 Hera Cell incubator (Heraeus, Germany) (5% CO_2, 37°C, RH 98%). The sample (mould + medium) was extracted 2 times with 25 ml of chloroform (Merck, Germany) and evaporated in a vacuum evaporator at 40°C. The residues were dissolved 2 times with 1 ml of chloroform in the ultrasonic cleaner. The solution was evaporated under nitrogen at a temperature of 40°C. The extract was dissolved in a mixture of ethanoldimethylsulfoxide — minimum essential medium with Earle's salts (MEM) (1.7+0.3+98, v/v/v) as described by Hanelt *et al*. (1994). Series of log 2 dilutions of the sample extract were made. All plates were incubated for 48 h at a temperature of 37ºC in a humidified atmosphere with 5% CO_2. A volume of 20 μl of the MTT stock solution was then added to each well and the plates were incubated for another 4 hours. Subsequently, the supernatant was removed using a multichannel micropipette and 100 μl of dimethyl sulfoxide (DMSO) was added to each well and measured spectrophotometrically with an ELISA-Reader. Micro-plate spectrophotometer (Ledetect 96, Labexim Products) and MikroWin 2000 (Mikrotek Laborsysteme GmbH, Germany) were used for quantitative evaluation of cytotoxicity. The absorbance was measured at $\lambda = 510$ nm, the wavelength of maximum absorption of the formazan derivative. All absorption values of the samples were below 50% of the division activity of cell control, thus, all of them were considered as toxic. Therefore, based on the levels of dilution, the maximum acceptable toxic levels were determined, namely the smallest tested sample in (cm^2/ml) which had a toxic effect on the cell (IC_{50}). All samples were done in triplicate.

Influence of AgNPs on mould organic acid production. The influence of silver nanoparticles on the mould organic acid production was determined using HPLC. Moulds were cultivated on MEB (Malt Extract Broth, Merck, Germany) with AgNPs (in MIC and ½ MIC) and MEB (control) in stationary culture for 14 days at 27 ± 2°C. The presence of selected organic acids (oxalic, citric, malic and succinic acids) was established on 3, 7, 10, 14 day of incubation. To separate the biomass from medium, samples were filtered (Filtrak, Germany). The filtrate was filtered again through 0.45 μm syringe filters (Filter-Bio, China). The high performance liquid chromatography analysis was performed with a Surveyor pomp (ThermoScientific, USA), autosampler equipped with a 20 μl loop, detector Surveyor RI Plus and an Aminex HPX 87H, 300 × 7.8 mm column (BioRad, USA). The mobile phase (0.005 M H_2SO_4) was filtered (0.45 μm, Millipore, USA). The separation was made by isocratic elution (flow rate: 0.6 ml/min); column temperature 60°C. Quantitation was made by

Table 2. Influence of AgNPs on mycotoxin production by *Aspergillus* sp.

Mould	Mycotoxin	Concentration (ppb)		Change (%)
		MEA	MEA+AgNPs	
A. niger 1	Fumonisin B_1	275.00	52.10	81.1 ↓
A. flavus	Aflatoxin B_1	750.00	80.20	89.3 ↓
	Aflatoxin B_2	54.20	3.71	93.2 ↓
	Aflatoxin G_1	1210.00	167.00	86.2 ↓
	Aflatoxin G_2	70.90	3.55	95.0 ↓
A. westerdijkiae	Ochratoxin A	23.10	1.04	95.5 ↓

↓ decrease in the mycotoxin concentration

peak area measurement. Standard solutions of organic acids (Supelco, USA) were chromatographically separated to determine the retention time of each acid. All samples were done in triplicate.

The pH measurement of culture medium was made in the same samples using pH meter CP-411 (Elmetron, Poland).

Influence of AgNPs on mould extracellular enzyme activity. The influence of silver nanoparticles on the 19 selected extracellular enzymes' activity of moulds was determined using an API-Zym test (Biomerieux, Germany). Moulds were cultivated on MEB (Malt Extract Broth, Merck, Germany) with AgNPs (at MIC and ½ MIC) and MEB (control) in stationary culture for 7 days at a temperature of 27 ± 2°C. To separate the biomass from medium, samples were filtered (Filtrak, Germany) and the activity of enzymes was established in the filtrate. The quantitation was made on the base of increase in the colour intensity of the samples (0–5 scale). The approximate number of free nmol hydrolysed substrate may be obtained from the colour strength: 0 - no activity; 1 — liberation of 5 nmol; 2 — 10 nmol; 3 — 20 nmol; 4 — 30 nmol; and 5 — ≥40 nmol (Papamanoli *et al.,* 2003; Nowak & Piotrowska, 2012).

Statistical analysis. The results obtained for extracellular enzyme activity were analysed with Statistica 10 using Two-Way Joining Analysis.

RESULTS AND DISCUSSION

The mycotoxin production was established for 3 mould strains from the *Aspergillus* genus (Table 2). Six mycotoxins were identified: Fumonisin B_1 (275 ppb) for *A. niger,* Aflatoxins B_1 (750 ppb), B_2 (54 ppb), G_1 (1210 ppb) and G_2 (71 ppb) for *A. flavus* and Ochratoxin A (23 ppb) for *A. westerdijikiae.*

The ability to produce mycotoxins, as well as the amounts produced, are a strain specific feature. It also depends on the composition of the medium used for mould growth (Muñoz *et al.*, 2011). *A. niger* is known for production of mycotoxins (fumonisins, ochratoxins, oxalic acid). However, researchers report the production of Fumonisin B_2 (0.1–26.2 ppm) and the absence of Fumonisin B_1 (Blumenthal, 2004; Frisvad *et al.*, 2007; Susca *et al.*, 2010; Frisvad *et al.*, 2011; Soares *et al.*, 2013). Fumonisin B1 is mostly produced by mould from the *Fusarium* genera, e.g., *F. moniliforme, F. proliferatum, F. nygamai* (170–3976 ppm) (Nelson *et al.*, 1992; Rheeder *et al.*, 2002). Aflatoxins are mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus.* Studies show that *A. flavus* is able to produce aflatoxins (AF) at higher concentrations: AFB_1: 18.6–740000 ppm (Aziz *et al.*, 2000; Al-Othman *et al.*, 2014); AFB_2: 4.5–10 329 ppm (Lai *et al.*, 2015; Fakruddin *et al.*, 2015); AFG_1: 20.6–16000 ppm (Davis *et al.*, 1966; Bokhari & Mohammad Aly, 2009); AFG_2: 22–62 ppm (Ravi Babu *et al.*, 2011). *A. westerdijkie* (a fungus that was dismembered from *Aspergillus ochraceus* taxon) is a known producer of Ochratoxin A: 0.001–8 ppm (Marino *et al.*, 2009; Gil-Serena *et al.*, 2011).

The addition of silver nanoparticles to the medium decreased the produced mycotoxins by 81.1–95.5%. The highest decrease of mycotoxin amount was noticed for Ochratoxin A (*A. westerdijikiae* — 95.5%). Other studies show that silver nanoparticles are able to inhibit the Aflatoxin B1 production by *A. flavus* up to 86% (50 ppm: R=8.9–17.4%; 100 ppm: R=43.3–54.8%; 150 ppm: R=86.3%) (Al-Othman *et al.*, 2014). Other nanoparticles (2–10 ppm ZnNPs) are also very efficient, decreasing the mycotoxin concentration (Fumonisin B_1, Ochratoxin A, Aflatoxin B_1 and M_1) by 20–100% (Hassan *et al.*, 2013). Researchers report that ozone is also able to reduce mycotoxin formation (reduction of Aflatoxin B1 by 55–77%) (El-Desouky *et al.*, 2012). Also, plants and spice extracts (saffron, ginger, cinnamon, cloves, cardamom) decrease the mycotoxigenicity by 12.5–37.5% (Bokhari & Mohammad Aly, 2009).

On the contrary, exposure of *A. flavus* to gamma irradiation (1.5–3 kGy) induced the Aflatoxin G1 production (Applegate & Chipley, 1973). Fungicides (epoxiconazole, propiconazole) are able to increase or decrease the *F. culmorum* mycotoxin concentration, depending on the level of water activity (a_w) (Ramirez *et al.*, 2004).

The cytotoxicity analysis revealed that the most cytotoxic mould was *A. westerdijikiae* (Ochratoxin A), then *A. niger* 1 (Fumonisin B_1) (Table 3). Low cytotoxicity characterized *A. flavus* (Aflatoxins B_1, B_2, G_1, G_2).

The cytotoxicity of moulds, likewise for mycotoxins, depends on the strain. Moulds isolated from hospitals were characterized by different cytotoxicity: *A. niger* (2 from 12 isolated strains had high cytotoxicity; 6/12 — none), *A. flavus* (1/5 — high; 0/5 — none), *A. ochraceus* (7/13 — high; 1/13 — none) (Gniadek *et al.*, 2011). Other moulds from the *Aspergillus* genus are highly cytotoxic e.g. *A. fumigatus* (Gutarowska *et al.*, 2014b).

The low cytotoxicity of AgNPs was also confirmed. The IC_{50} of MEA medium with the addition of AgNPs decreased, meaning that the medium became more cytotoxic. Silver nanoparticles are 30 times more cytotoxic than silver ions (Kvitek *et al.*, 2011). The smaller the size of nanoparticles, the higher the chance that they could cause cell apoptosis (Braydich-Stolle *et al.*, 2010).

AgNPs increased the IC_{50} of *A. niger* 1 (from 1.953 to 7.813) and *A. westerdijikiae* (from 0.244 to 0.488), which

Table 3. Influence of AgNPs on cytotoxicity of moulds

Mould	IC_{50} (mg/ml)		
	MEA	MEA+AgNPs	Change (%)
A. niger 1	1.953	7.813	75 ↓
A. flavus	7.813	7.813	0 –
A. westerdijkiae	0.244	0.488	50 ↓
control*	31.250	15.625	50 ↑

*control medium without mould; ↓ decrease in the mould cytotoxicity; ↑ increase in the mould cytotoxicity

means that both moulds became less cytotoxic. No effect was noticed for *A. flavus*. For the mould cytotoxicity, not only mycotoxins are responsible, but also structural components (β-D-glucan). β-D-glucan can inhibit cancer cell proliferation (Zhang *et al.*, 2006; Jafaar *et al.*, 2014).

The presence of oxalic, citric, malic and succinic acids was detected in the medium for *P. chrysogenum* and *A. niger* (Table 4) with the highest concentration on the 3rd day of incubation. The amount of a particular acid decreased during 14-day incubation. The highest amounts of organic acids were noticed on the 3rd day of incubation. Moulds produced oxalic acid with the highest yield (2.764–2.846 g/100 ml), as well as the citric acid (0.708–0.712 g/100 ml). The lowest concentration was found for succinic acid (0.010–0.013 g/100 ml). The pH of culture medium decreased during the incubation time from 4.73–4.86 to 2.25–3.94. The pH decreased more for *Aspergillus niger* than for *Penicillium chrysogenum* due to higher amount of total organic acids produced (more than twice on the 14th day of incubation). Moulds are a significant commercial source of organic acids. Citric, gluconic, itaconic, lactic, oxalic, fumaric and malic acids are manufactured via large-scale fungal bioprocesses. *A. niger* can produce up to 200 g/L of citric acid (Magnuson & Lasure, 2004), 13–38 g/l of oxalic acid (Ruijter *et al.*, 1999), 1–16 g/l of malic acid (West, 2011). *Penicillium* sp. are able to produce the oxalic acid (0.3–1.5 g/l) and citric acid (0.9–9.3 g/l) (Cunningham & Kuiack, 1992; Scervino *et al.*, 2011).

The addition of silver nanoparticles (at MIC and ½ MIC) to culture medium decreased the organic acid production from the 3rd day of incubation. Both AgNPs concentrations decreased the acid concentration, however, more significant results were obtained for MIC. The highest decrease was observed after 14 days for *P. chrysogenum* and after 3 days for *A. niger*. The production of organic acids was inhibited more in the case of *P. chrysogenum* than *A. niger*. Oxalic acid production was suppressed the most intensively, while the least suppressed was malic acid. The AgNPs (pH 7) addition to the culture medium increased the pH from 4.73–4.86 to 4.84–4.90 (½ MIC) and to 4.99–5.38 (MIC). The decrease in the pH was slightly lower during the incubation with AgNPs than in the control samples. The cultivation medium can change the amount of organic acids produced by moulds (Gutarowska, 2010). In the case of presented results, the addition of AgNPs decreased the organic acid production.

Moulds are producing a different spectrum of extracellular enzymes on the MEB control medium (Fig. 1). The presence of 10 enzymes was confirmed for *P. chrysogenum* and 8 for *A. niger*.

For *P. chrysogenum,* the highest activity was displayed by α- and β-glucosidase, acid phosphatase and N-acetyl-β-glucosamidase (≥ 40 nmol). For *A. niger,* all 8 enzymes had low activity (5 nmol). The activity of 7 out of 8 enzymes (alkaline and acid phosphatase, esterase (C4), β-galactosidase, α- and β-glucosidase, naphtol-AS-BI-phosphohydrolase) was in agreement with the previous studies for *A. niger* (Coulibaly & Agathos, 2007; Janda *et al.*, 2009). For *P. chrysogenum* 8 out of 10 enzymatic activities (alkaline and acid phosphatase, esterase (C4), esterase lipase (C8), naphtol-AS-BI-phosphohydrolase, β-glucosidase, N-acetyl- β-glucosamidase, leucin arylamidase) were confirmed (Gutarowska *et al.*, 2010; Kołodziejczyk *et al.*, 2014).

The addition of AgNPs caused change in activity of 4 enzymes for *P. chrysogenum* and 8 enzymes for *A. niger*. The higher was the concentration of silver nanoparticles, the greater change was noted. Silver nanoparticles caused a decrease in *P. chrysogenum* culture activity of α-glucosidase and eliminated activity of the esterase lipase. Moreover, new enzymatic activities appeared:

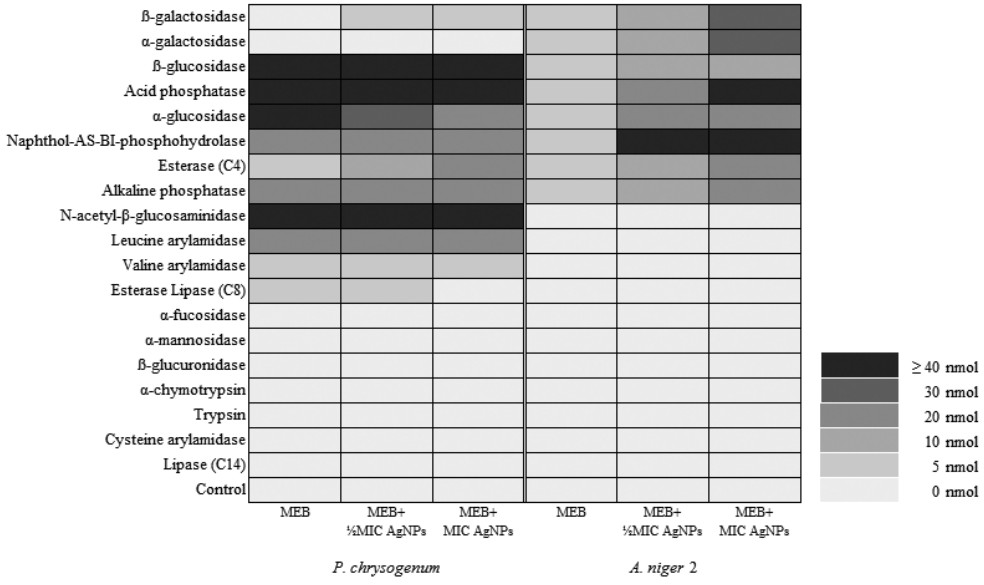

Figure 1. Two-way joining graph of the concentration of extracellular enzymes (nmol) produced by moulds

Table 4. Influence of AgNPs on organic acids produced by moulds

Incubation time (day)	Sample	pH	Organic acid (g/100 ml)			
			Oxalic	Citric	Malic	Succinic
P. chrysogenum						
0	MEB	4.73±0.00	nt	nt	nt	nt
3		4.78±0.02	2.764	0.712	0.036	0.013
7		3.69±0.00	0.913	0.075	0.006	0.010
14		3.94±0.12	0.244	0.028	0.006	0.003
0	MEB +½MIC AgNPs	4.90±0.15	nt	nt	nt	nt
3		4.89±0.07	2.344	0.604	0.027	0.007
7		3.77±0.11	0.575	0.047	0.016	0.006
14		4.22±0.06	0.141	0.014	0.007	0.002
0	MEB +MIC AgNPs	5.38±0.05	nt	nt	nt	nt
3		5.13±0.04	1.285	0.430	0.024	0.005
7		3.98±0.16	0.231	0.027	0.008	0.006
14		4.98±0.01	0.060	0.005	0.005	0.000
A. niger 2						
0	MEB	4.86±0.18	nt	nt	nt	nt
3		4.73±0.06	2.846	0.708	0.053	0.010
10		2.18±0.04	0.556	0.253	0.006	0.003
14		2.25±0.00	0.322	0.232	0.004	0.000
0	MEB +½MIC AgNPs	4.84±0.11	nt	nt	nt	nt
3		4.73±0.01	2.722	0.603	0.044	0.007
10		2.23±0.04	0.503	0.283	0.018	0.003
14		2.30±0.06	0.288	0.293	0.005	0.000
0	MEB +MIC AgNPs	4.99±0.25	nt	nt	nt	nt
3		5.01±0.11	1.879	0.558	0.039	0.008
10		2.24±0.08	0.388	0.251	0.014	0.003
14		2.48±0.08	0.113	0.233	0.012	0.000

mean value±standard deviation; nt – not tested

β-galactosidase and esterase (C4), also at a lower AgNPs concentration. In the case of *A. niger,* all 8 enzymes increased their activity, the highest (from 5 nmol to more than 40 nmol) increase was noted for acid phosphatase and naphtol-AS-BI-phosphohydrolase. Generally, silver nanoparticles increased the total enzymatic activity of the moulds tested. The modification of the culture media can change the produced enzymes by increasing or decreasing their activity but also by activation of new features (Gutarowska *et al.*, 2010), which was confirmed in the presented study with AgNPs addition.

The conducted study showed that silver nanoparticles can change the metabolism and toxicity of moulds. The higher concentration is used, the more significant changes are observed. AgNPs decrease the mycotoxin production of *Aspergillus* sp. (81-96%) and reduce mould cytotoxicity (50-75%). AgNPs influences the organic acid production of *A. niger* and *P. chrysogenum* by decreasing their concentration (especially oxalic and citric acid). Also, the change in the extracellular enzyme profile of *A. niger* and *P. chrysogenum* was observed, however, the total enzymatic activity was increased.

In further studies, changes in the ultrastructure of moulds due to silver nanoparticle action should be examined, as well as mould proteins to which AgNPs are able to bind should be determined.

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