

New Look on Antifungal Activity of Silver Nanoparticles (AgNPs)

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Abstract

The progress of research on silver nanoparticles (AgNPs) has led to their inclusion in many consumer products (chemicals, cosmetics, clothing, water filters, and medical devices) as a biocide. Despite the widespread use of AgNPs, their biocidal activity is not yet fully understood and is usually associated with various factors (size, composition, surface, red-ox potential, and concentration) and, obviously, specific features of microorganisms. There are merely a few studies concerning the interaction of molds with AgNPs. Therefore, the determination of the minimal AgNPs concentration required for effective growth suppression of five fungal species (*Paecilomyces variotii*, *Penicillium pinophilum*, *Chaetomium globosum*, *Trichoderma virens*, and *Aspergillus brasiliensis*), involved in the deterioration of construction materials, was particularly important. Inhibition of bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*) and yeasts (*Candida albicans* and *Yarrowia lipolytica*) was also assessed as the control of AgNPs effectiveness. AgNPs at the concentrations of 9–10.7 ppm displayed high inhibitory activity against moulds, yeast, and bacteria. The TEM images revealed that 20 nm AgNPs migrated into bacterial, yeast, and fungal cells but aggregated in larger particles (50–100 nm) exclusively inside eukaryotic cells. The aggregation of 20 nm AgNPs and particularly their accumulation in the cell wall, observed for *A. brasiliensis* cells, are described here for the first time.

Key words: silver nanoparticles, antimicrobial activity, *Paecilomyces*, *Chaetomium*, *Trichoderma*, building materials, TEM images, BioscreenC

Introduction

Nowadays nanomaterials within the size of 1–100 nm in at least one dimension with the novel, size-related properties have attracted the attention of many researchers in the area of chemistry, physics, material sciences, medicine, microbiology, and biotechnology (Rai et al. 2009, 2015; Tran et al. 2013). Silver nanoparticles (AgNPs) are one of the most commonly used engineered nanoproducts known for their antimicrobial activity (Kim et al. 2007; Rai et al. 2009; Martinez-Gutierrez et al. 2010). The progress in research led to the inclusion of nanoparticles as biocides in a large number of consumer products: chemicals, cosmetics, clothing, water filters, and medical devices (Kokura et al. 2010; Metak and Ajaal 2013; Zarschler et al. 2016). The application of AgNPs as a contrast agent for mammography

and micro-computed tomography for the ear imaging is based on their electric properties (Anil Kumar et al. 2007; Zou et al. 2015; Zhang et al. 2016b; Lee and Jun 2019). Despite their widespread use, the biocidal activity of AgNPs is not fully understood and the prevalence of either positive or negative effects on humans and the environment are discussed (Bartłomiejczyk et al. 2013; Flores-López et al. 2019).

The nature and the level of AgNPs' molecular cytotoxicity are related to various factors: size, composition, surface area, charge, red-ox potential, and concentration (McShan et al. 2014; Riaz et al. 2017). In general, AgNPs at the concentrations lower than 25–30 ppm do not exhibit toxicity for mammalian cells (Milic et al. 2015; Zhang et al. 2016a). However, some cytostatic and anti-cancer activity is reported at these concentrations (AshaRani et al. 2009). Antibacterial activity of AgNPs

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is rather well documented in the literature, mainly against the following species: *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Yoon et al. 2007; Li et al. 2010; Guzman et al. 2012, Kanawaria et al. 2018). Some information concerning growth inhibition of other bacteria (*Enterococcus* spp.) and yeast (*Candida albicans*) by nanosilver is also available (Kim et al. 2007; Roe et al. 2008; Lara et al. 2015). However, the studies of AgNPs interaction with cell structures of bacteria and yeasts are scarce. Only in few reports the location of AgNPs inside *C. albicans* cells was demonstrated under transmission electron microscopy (TEM) or scanning electron microscopy (SEM) (Kim et al. 2009; Radhakrishnan et al. 2018).

In opposite to bacteria and yeasts, the research on the interactions of AgNPs with filamentous fungi (molds) is still evolving (Kanawaria et al. 2018). The results on AgNPs' effects on fungal growth already reported concern mainly dermatophytes and phytopathogens (Jo et al. 2009; Pulit et al. 2013; Xu et al. 2013), not the molds found in houses or the air. Studies of AgNPs interaction with species involved in biodeterioration of buildings and construction materials are scarce. The degradation of house construction materials could be observed as the action of moulds from *Aspergillus*, *Penicillium*, *Paecilomyces*, *Trichoderma*, and *Chaetomium* genera (Łukaszuk et al. 2011; Kobiałka et al. 2019). Species of those genera are commonly used in the normalized test of resistance to microbial corrosion of construction material components (PN-EN ISO 846, 2002).

The aim of the study was to determine the minimal AgNPs concentrations required for effective growth inhibition of the selected fungal species (possibly occurring as biodeterioration agents) and two unconventional yeast species (*Yarrowia lipolytica* and *C. albicans*). The selected bacterial species: *S. aureus*, *E. coli*, and *P. aeruginosa* that may cause human infections were used to control the effectiveness of the AgNPs tested. The analysis of microbial growth was performed in the microbiological analyzer Bioscreen C (Automated Growth Curve Analysis System, Lab systems, Finland) what allowed for a precise determination (for each species) the inhibitory concentration of AgNPs. TEM was used to localize the AgNPs inside microbial cells and to visualize their aggregation in eukaryotic cells and particular accumulation in the cell wall of *A. brasiliensis*.

Experimental

Materials and Methods

Nanoparticles. AgNPs have been synthesized by the TK Nano according to the procedure described by Koźlecki et al. (2011). The final concentration of

nanoparticles was 107.2 ± 0.8 mg/l. Nanoparticles were characterized by dynamic light scattering (DLS) and TEM. The DLS measurements were performed using a Photocor Complex apparatus (Photocor Instruments), equipped with a 28 mW (657 nm) laser and 288-channel autocorrelator, operating in multi-tau mode. The measurements were carried out in 14.8 mm ID round cells, submerged in analytical grade decalin (Fisher Scientific), as an index-matching liquid; the scattering angle was set at 110° , and the temperature of measurements was fixed at $298 \pm 0.05^\circ\text{K}$. The data analysis was performed with DynaLS ver. 2.8.3 software (Alango Ltd.), using a method similar to the CONTIN algorithm, but more aggressive concerning the noise (Song et al. 2011; Echegoyen and Nerin 2013; Wen et al. 2016).

The imaging with TEM was carried out with a Zeiss EM900 microscope (Carl Zeiss AG). Samples were dipped on a 300-nickel mesh, coated with Formvar (SPI Supplies), and then dried thoroughly. Microphotographs were recorded on photographic film and then scanned using a flatbed scanner with 800×800 DPI resolution (Hewlett-Packard). The images were processed using ImageJ ver. 1.50i software (Pal et al. 2007; Bundschunh et al. 2016; Koziróg et al. 2016).

Microorganisms. Microorganisms used in the study were obtained from the Deutsche Sammlung von Microorganismen und Zellkulturen (DSMZ), and the Microorganisms Collection of the Department of Biotechnology and Food Microbiology (KBiMŻ) at Wrocław University of Environmental and Life Sciences (*Aspergillus brasiliensis* DSMZ1988, *Penicillium pinophilum* DSMZ1944, *Paecilomyces variotii* DSMZ1961, *Trichoderma virens* DSMZ1963, *Chaetomium globosum* DSMZ1962, *Candida albicans* DSMZ1386, *Yarrowia lipolytica* KBiMŻ A101, *Escherichia coli* KBiMŻ, *Pseudomonas aeruginosa* DSMZ939, and *Staphylococcus aureus* subsp. *aureus* DSMZ799).

Petri dishes assay: the plate agar tests on solid medium. Filamentous fungi were cultivated on the surface of a medium composed of 30 g glucose, 2 g NaNO_3 , 0.7 g KH_2PO_4 , 0.3 g K_2HPO_4 , 0.5 g KCl, 0.5 g $\text{MgSO}_4 \times 7\text{H}_2\text{O}$, 0.01 g $\text{MnSO}_4 \times 7\text{H}_2\text{O}$, and 2g agar per 1 l of distilled water. The pH was set to 6.0–6.5 before autoclaving. AgNPs were added directly into Petri dishes before pouring sterilized medium in the following volumes of the stock suspension ($107 \text{ mg/l} = 107 \text{ ppm}$): 0, 0.5, 1 and 5 ml, corresponding to 0 or 2.14, 4.28, and 21.4 mg of AgNPs. On the solidified media, 0.05 ml of the suspension of fungal spores at the concentration of 10^5 CFU/ml was applied to the center of Petri dishes. After incubation for 192 h at 25°C , the diameters of the developed fungal colonies were measured and compared to those observed in medium without AgNPs. The analyses were performed in triplicate for each species of filamentous fungi.

Bioscreen C: the bioassay in liquid media. Biological activity of silver nanoparticles in liquid media was tested on the three groups of microorganisms (bacteria, yeasts, and filamentous fungi). The bacterial cultures were carried out in a liquid broth consisting of 15 g of dry bullion (Biocorp) and 10 g of glucose dissolved in 1 liter of distilled water. Cultures of yeast and fungi were performed in YPG medium composed of 10 g yeast extract, 10 g bacteriological peptone, and 10 g glucose per 1 liter of distilled water. The tests were performed in the automated Bioscreen C system (Automated Growth Curve Analysis System, Lab systems, Finland). The culture volume in the wells of the Bioscreen C was 300 μ l, which comprised 250 μ l of culture medium, 20 μ l of cell suspension (final concentration 10^8 CFU/ml) and 30 μ l of the AgNP stock suspension to the final concentration of 10.7 mg/l. The temperature was maintained at 28°C, and the optical density (OD) of the cell suspensions was measured automatically at 560–600 nm at regular intervals of 30 min during 72–96 hours of cultivation under constant agitation. Each culture variant was performed in 5–10 replications.

The data obtained were analyzed using the spreadsheet software (Microsoft Excel 97) and mean values were calculated from replicates for each cultured microorganism. The overall standard deviation did not exceed 15% (Robak 2007). The mean values were used for plotting growth curves for each strain studied, as the function of the incubation time and OD of the culture. The resultant growth curves were compared to control media.

After the Bioscreen C measurements, the cultures were collected and 1 ml of each sample transferred to 2 ml Eppendorf tubes, centrifuged for 10 min at 2000 \times g, using a Spectrafuge Mini Centrifuge (Labnet International), then 0.7 ml of supernatant was placed on a Costar Spin-X 0.2 μ m filter with polyamide membrane

(Corning Inc.), and centrifuged again at 2000 \times g for 10 min to remove the residual cell debris. The permeate (0.5 ml) was diluted with 1 ml of deionized water and measured using a DLS instrument as described above.

TEM observations of microorganisms and AgNPs. Biological material obtained after removal of the residual medium by centrifugation for 10 min at 2000 \times g was fixed with 2.5% glutaraldehyde and buffered with 0.1 M cacodylate buffer overnight at 4°C. Subsequently, the material was washed three times in 0.1 M cacodylate buffer, followed by post-fixation in 2% osmium tetroxide/1.5% potassium ferricyanide in 0.1 M cacodylate buffer for 1 h at 4°C. Afterward, the material was washed three times with buffer and two times with ultrapure water by centrifugation at 600 \times g for 5 min. Subsequently, the material was incubated with 1% uranyl acetate in ultrapure water overnight at 4°C. Then, the samples were washed three times with ultrapure water and dehydrated using a graded ethanol series (from 30% to 99.9%), followed by infiltration with Epon 812 resin (by replacing the pure ethanol with 1:1 ethanol to resin ratio for 2 h, followed by pure resin). Sample blocks were polymerized for 24 h at 60°C. Ultrathin sections were prepared using an ultramicrotome and glass knife (Leica EM UC7). The preparations were observed using a field-emission scanning electron microscope (FE-SEM, Auriga60, Zeiss) employing a STEM detector, at 20 kV acceleration voltages.

Results and discussion

Characterization of nanoparticles. The AgNPs were obtained at the concentration of 107.2 mg/l as electrostatically stabilized monodispersed spherical structures (Fig. 1A). The average nanoparticle diameter,

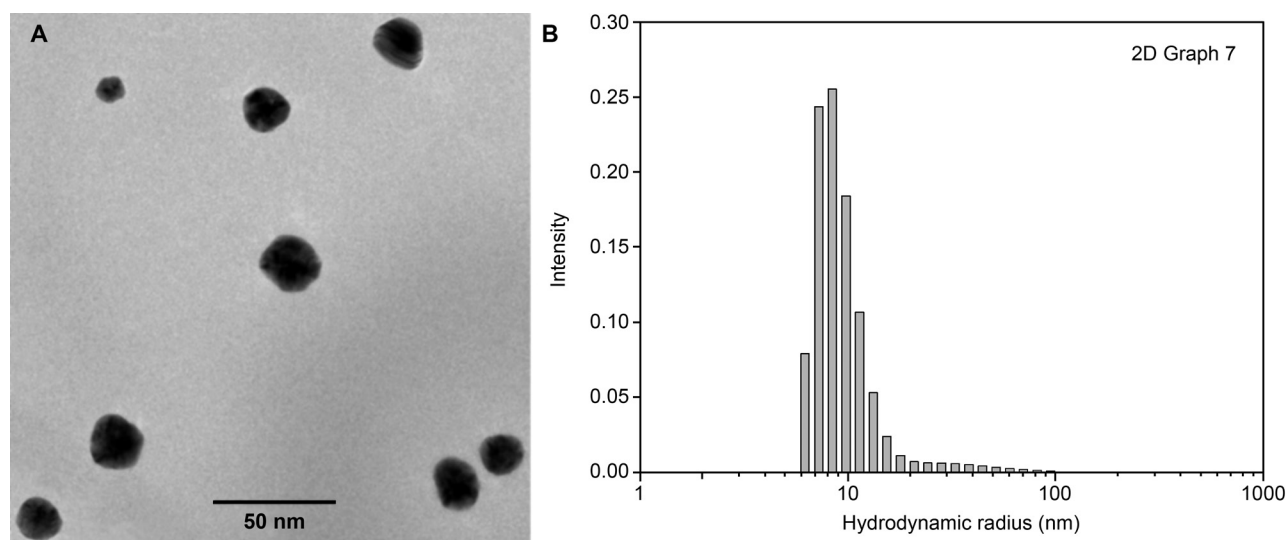


Fig. 1. Nanoparticles' shape (A) and dimensions (B).

Table I
AgNP inhibition of the filamentous fungi growth.

Fungal species	AgNPs in the medium [mg/l]			
	0	2.14	4.28	21.4
Growth [%]				
<i>Aspergillus brasiliensis</i>	100	100	83.1	3.74
<i>Trichoderma virens</i>	100	100	33.9	9.72
<i>Paecilomyces variotii</i>	100	100	0	0
<i>Penicillium pinophilum</i>	100	57.3	44.8	0
<i>Chaetomium globosum</i>	100	63.9	61.2	0

as determined with the dynamic light scattering (DLS) analysis, was 20 nm (Fig. 1B).

Microbiological analysis. The biocidal activity of AgNPs towards five species of filamentous fungi, two yeasts, and two bacterial species was evaluated. Two types of microbial growth analyses were performed: the cultivation on solid agar medium (Petri dishes) and in liquid media (Bioscreen C). The former type of growth is suitable for observation of the size of fungal colonies and the latter allows for the determination of the density of cells and hyphae during micro-batch cultures in Bioscreen C, measured every 30 minutes as an optical density (OD).

Evaluation of filamentous fungal growth on solid agar medium. Inhibition of fungal growth by AgNPs was observed for each of five tested species, however, to a different extent (Table I). The growth of *P. variotii* was completely inhibited by 4.28 mg/l of AgNPs (4.28 ppm). This concentration of nanosilver in the medium had a lower inhibitory effect on the growth of *T. virens* (66%), *P. pinophilum* (55%), *C. globosum* (39%), and *A. brasiliensis* (17%). More pronounced inhibition of growth of the last four species was noted for the highest tested concentration of AgNPs (21.4 mg/l). The growth was completely suppressed for *P. pinophilum* and *C. globosum*, and significantly impaired for *A. brasiliensis* and *T. virens* (by 96% and 90%, respectively). Pulit et al. (2013) reported a 75 – 90% inhibition of the growth of *Aspergillus niger* and *Cladosporium cladosporioides* with a higher concentration of AgNPs (50 mg/l).

This difference in the biocidal effect could be associated with the size of nanoparticles. Pulit et al. (2013) tested AgNPs particles of 60 nm in diameter, in contrast to 20 nm particles analyzed in our study. It has been well documented that smaller nanoparticles (< 10 nm) exhibit more pronounced anti-proliferative activity than larger particles. Nowicka-Krawczyk et al. (2017) demonstrated inhibition of algae by AgNPs of 3 nm in diameter. In turn, Park et al. (2011) compared the effects of AgNPs of 20, 80, and 113 nm in diameter on two mouse cell lines (RAW 264.7 and L929) to conclude more pronounced cytotoxicity for smaller nanoparti-

cles. Higher antimicrobial activity of small nanoparticles was also reported by Duran et al. (2016).

Evaluation of microbial growth in Bioscreen C. As reported by Żarowska et al. (2015), the lowest AgNP concentration to cease the growth of bacteria (*S. aureus*, *E. coli*, and *Pseudomonas fluorescens*) and yeast species (*C. albicans*, *Y. lipolytica*, and *Saccharomyces cerevisiae*) was found to be nearly 9 mg/l. Here, for Bioscreen C culture evaluation of bacterial, yeast, and fungal growth, the AgNPs in the concentration of 10.7 mg/l were applied and their antimicrobial activity was confirmed. As expected, the growth of bacteria *S. aureus*, *P. aeruginosa*, *E. coli*, and yeast *Y. lipolytica* was completely inhibited (Fig. 2A, 2B).

This followed the results reported in the literature where the AgNPs biocidal effects were observed. Sondi and Salopek-Sondi (2004) reported 70% inhibition of *E. coli* growth by AgNPs in the concentration of 10 mg/l and total inhibition at 50–60 mg/l. Dar et al. (2013) found that AgNPs of an average size of 30–70 nm displayed inhibition of bacterial growth at a concentration ten times lower (5 mg/l). Concerning filamentous fungi, AgNPs at the concentration of 10.7 mg/l seriously influenced and retarded the development of the following species: *P. variotii*, *C. globosum*, *A. brasiliensis*, *P. pinophilum*, and *T. virens* (Table II). As compared to the culture of mixed spores (of five species), the lag phase duration increased from 1.5 to nearly 10 times, and the final cell density (OD) decreased more than 36-fold showing serious growth inhibition. The intensity of *C. albicans* growth fluctuated during the time of analysis, in a period of approximately 10 hours (Fig. 2B, violet line). This fluctuation is difficult to explain but appears to be connected with the presence of AgNPs, which probably influences cell metabolism and the duplication time. According to Radhakrishnan et al. (2018) *C. albicans* cells treated with AgNPs exhibited

Table II

Lag phase duration and final OD after 96 hours of filamentous fungal and yeast growth in BioscreenC without or with AgNPs.

Filamentous fungi and yeast species	Lag phase [h]	OD		
		at 96 h	compared to	
			SM [%]	initial [× folds]
SM: spores mix (control)	10	1.592	100	36
SM: spores mix +AgNPs	20	1.308	82	5.8
<i>A. brasiliensis</i> +AgNPs	40	1.600	100	8.1
<i>P. pinophilum</i> +AgNPs	30	1.517	95	9.2
<i>P. variotii</i> +AgNPs	~68	0.633	40	3.7
<i>T. virens</i> +AgNPs	18	1.306	82	6.5
<i>C. globosum</i> +AgNPs	48	1.063	67	5.2
<i>C. albicans</i> +AgNPs	15	0.953	–	3.8
<i>Y. lipolytica</i> +AgNPs	>96	0.194	–	0

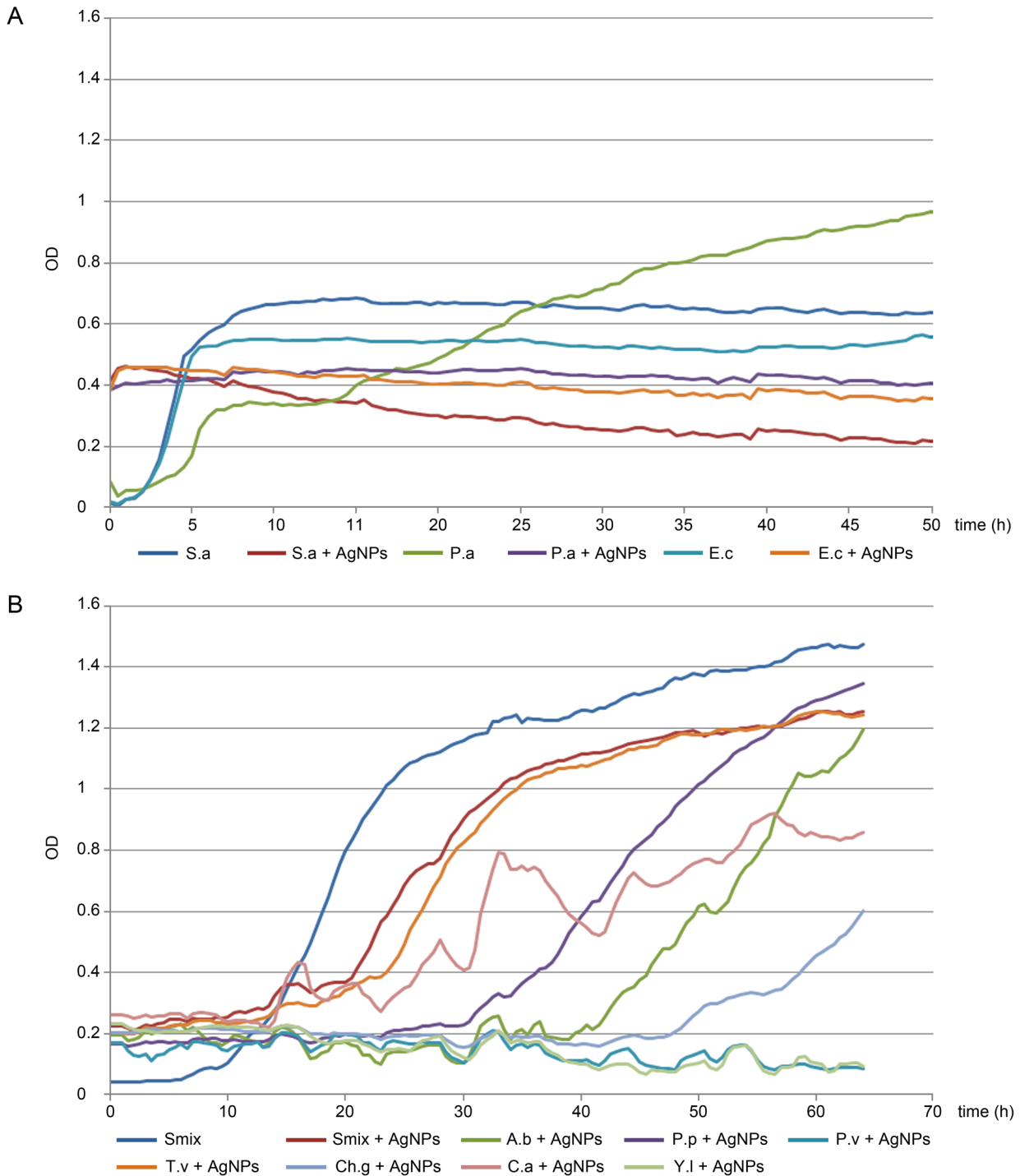


Fig. 2. Microorganism growth in BioscreenC with and without AgNPs: (A) Bacteria: S.a, *Staphylococcus aureus*; P.a, *Pseudomonas aeruginosa*; E.c, *Escherichia coli*; (B) yeasts Y.I, *Yarrowia lipolytica*, and fungi: Smix, the mixture of spores of five species of filamentous fungi; A.b, *Aspergillus brasiliensis*; P.p, *Penicillium pinophilum*; P.v, *Paecilomyces variotii*; T.v, *Trichoderma virens*, and Ch.g, *Chaetomium globosum*.

altered surface morphology and cellular ultrastructure, membrane fluidity, as well as ergosterol and fatty acids content. It was noted that the AgNP-mediated inhibition by was not only induced by ROS (reactive oxygen species) formation. The action of AgNPs on *C. albicans* was also size-dependent. According to Kim et al. (2009), AgNPs of 5 nm diameter and at the concentration of

2 mg/l effectively killed *C. albicans* yeasts. A comparable concentration (2.5 mg/l) of AgNPs was sufficient for the inhibition of *Aureobasidium pullulans*, and ten times higher concentration was needed for the *A. niger* growth inhibition (Žarowska et al. 2015). Therefore, the activity of AgNPs against yeasts and filamentous fungi requires further studies.

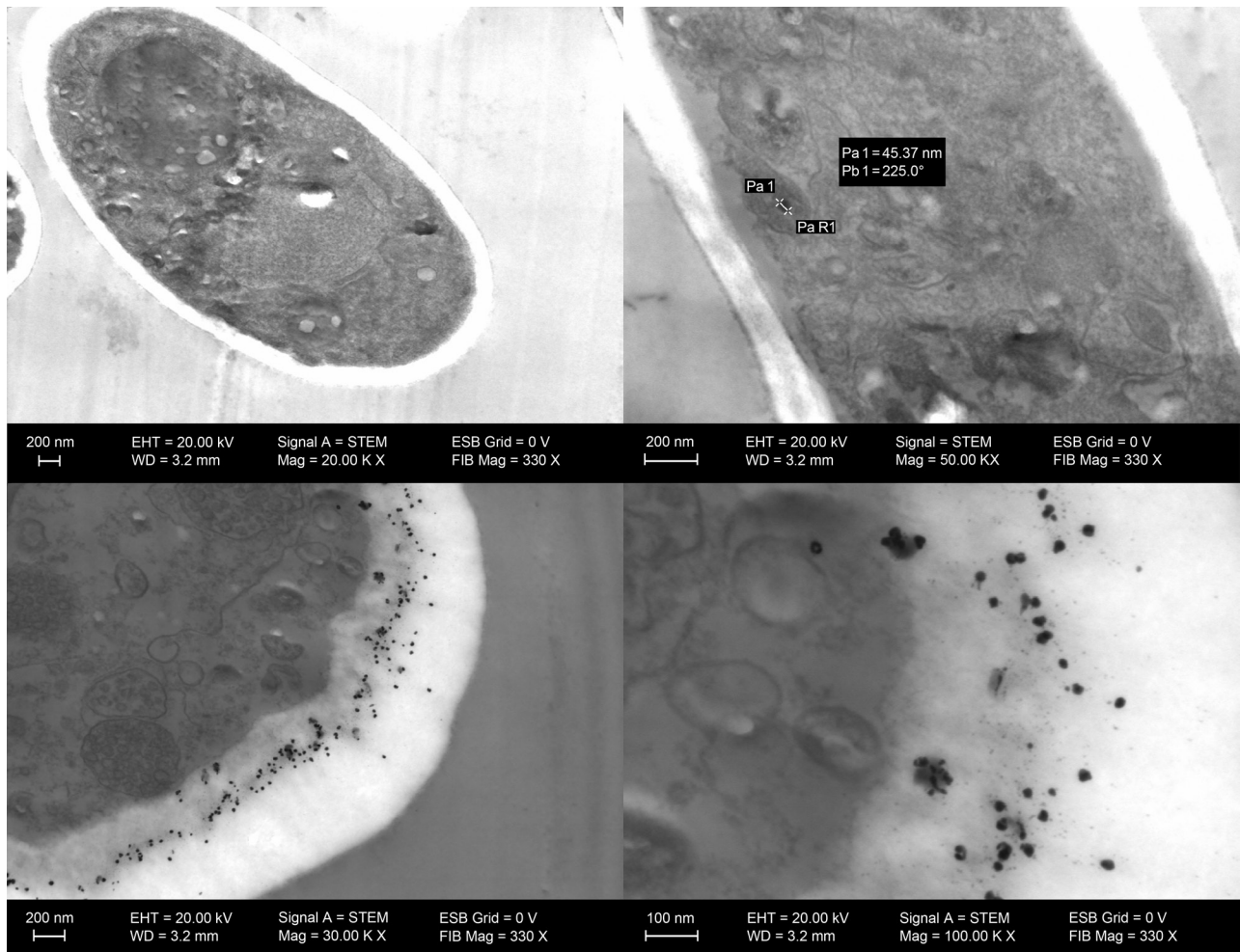


Fig. 3. TEM images of *Aspergillus brasiliensis* after the growth in the BioscreenC: (a) without AgNPs; (b) with AgNPs visible inside the cell (black points); (c, d) accumulated within the cell wall.

Also, difficult to explain is the recovery of growth after 20–48 hours of the lag phase (Table II) by four species of filamentous fungi, except *P. variotii*. The recovery of cell growth could result from at least two factors. One of them could be the microorganism's ability to synthesize and extracellularly secrete nanoparticles. This phenomenon was supported by the results of Dar et al. (2013) who proved that AgNPs could be synthesized by *Cryphonectria* spp. from silver nitrate and extracellularly secreted. Also, other mold species can synthesize and secrete AgNPs (Tran et al. 2013, Akter et al. 2018). Even autoclaved (inactivated) biomass of *Aspergillus aculeatus* was able to synthesize silver nanoparticles (Salvadori et al. 2014).

The second factor could be connected to the known fungal resistance to metals, including silver (Abou-Shanab et al. 2007). The resistance to metal as well as the nanoparticle biosynthesis could result from the activity of the same enzyme, nitrate reductase (EC 1.7.99.4). Shahverdi et al. (2007) identified nitroreductase as being responsible for the reduction of AgNO_3 to AgNPs by *Klebsiella pneumoniae*, and Anil Kumar et al.

(2007) described the green synthesis of AgNPs by this enzyme isolated from *Fusarium oxysporum*.

TEM analysis. The observations under TEM revealed the sites of AgNPs interaction with cells and filaments after nearly four days of the exposition. Nanoparticles accumulated in the cell wall without any aggregation, which was especially visible for *A. brasiliensis* (Fig. 3), and with aggregation in the cells of *P. variotii* and *P. pinophilum*. The TEM analysis was performed on microbial biomass collected after 92 h of culture with AgNPs added to the medium. Hence, at the time of microscopic observations, the fungal cells had already been adapted to the biocide (as shown in Fig. 2), and the observed accumulation of AgNPs was probably a result of its expulsion from the cells as in *A. brasiliensis*. This particular AgNPs deposition site could be due to their association with proteins present on the outer side of the cell membrane or with membrane lipids. The protein corona formation on AgNPs has been described elsewhere (Rahman et al. 2013; del Pino et al. 2014; Bargheer et al. 2015); however, the AgNPs deposition between the cell wall and cytoplasmic membrane could also involve

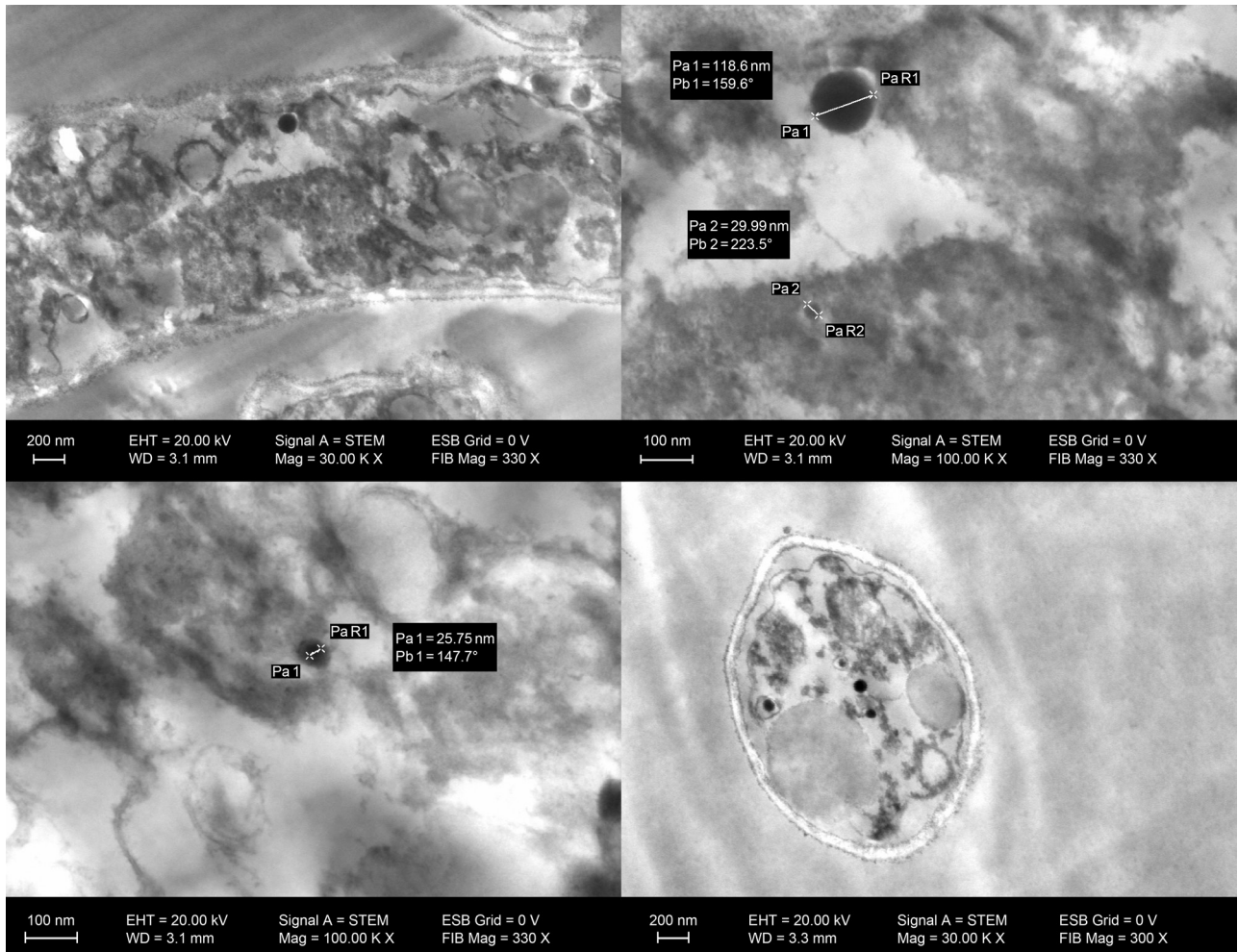


Fig. 4. TEM images of filamentous fungi after the growth with AgNPs in the Bioscreen C: (a, b) *Paecilomyces variotii*; (c, d) *Penicillium pinophilum*; AgNPs, the black structures observed inside the cell and hypha.

the binding of nanoparticles to fatty acids present as phospholipids. Recently, Radhakrishnan et al. (2018) reported an altered profile of fatty acids in the *C. albicans* cells exposed to AgNPs. However, in our study, no such abundant AgNPs deposition was denoted in any species studied, and possibly a particular cell wall compound of *A. brasiliensis* could be involved. In *A. fumigatus* the major component of the cell wall (30%) is the branched β -1,3 and β -1,6 glucans linked to chitin (Latge et al. 2005). The β -1,3 and β -1,6 glucans are not present in yeast and bacteria. So, these glucans could be involved in AgNPs localization in cell wall of *A. brasiliensis* also. It is noteworthy that for *P. variotii* and *P. pinophilum* no such type of nanoparticle accumulation was observed.

Here, besides their accumulation in cell walls, AgNPs were also found inside fungal and bacterial cells, especially in the cytoplasm, and outside of destroyed cells of *P. aeruginosa* (Fig. 4 and 5). Morones et al. (2005) have already presented similar images of AgNPs located outside *P. aeruginosa* cells.

According to many reports, the activity of AgNPs against the cell may involve generation of reactive oxy-

gen species (ROS), damage of macromolecules (DNA, proteins), and perforation of membranes due to destabilized conformation of their components, especially proteins, lipids, and glycans (Bartłomiejczyk et al. 2013; Duran et al. 2016). It appears that the observed damage was exerted by the release of Ag^+ from nanostructures (Duran et al. 2016). The mechanism of interaction of silver with the cell is best described for Gram-negative bacteria, notably *E. coli* (Kędziora et al. 2016). AgNPs uptake by the cell involves special membrane transporters, proteins of P-type ATPases (Li et al. 1997). Those P-type ATP-ases are responsible for the import of inorganic cations to the cytoplasm and the export of these ions outside the cytoplasm (Chong et al. 2012). According to Galván Márquez et al. (2018) the decreased transcription, reduced endocytosis, and dysfunctional electron transport system were observed in *S. cerevisiae* cells exposed to AgNPs.

The TEM analysis (based on the careful inspection of 300 images) allowed for the measurement of the size of the nanoparticles after interaction with the growing microorganisms. In 79% of the observed and measured

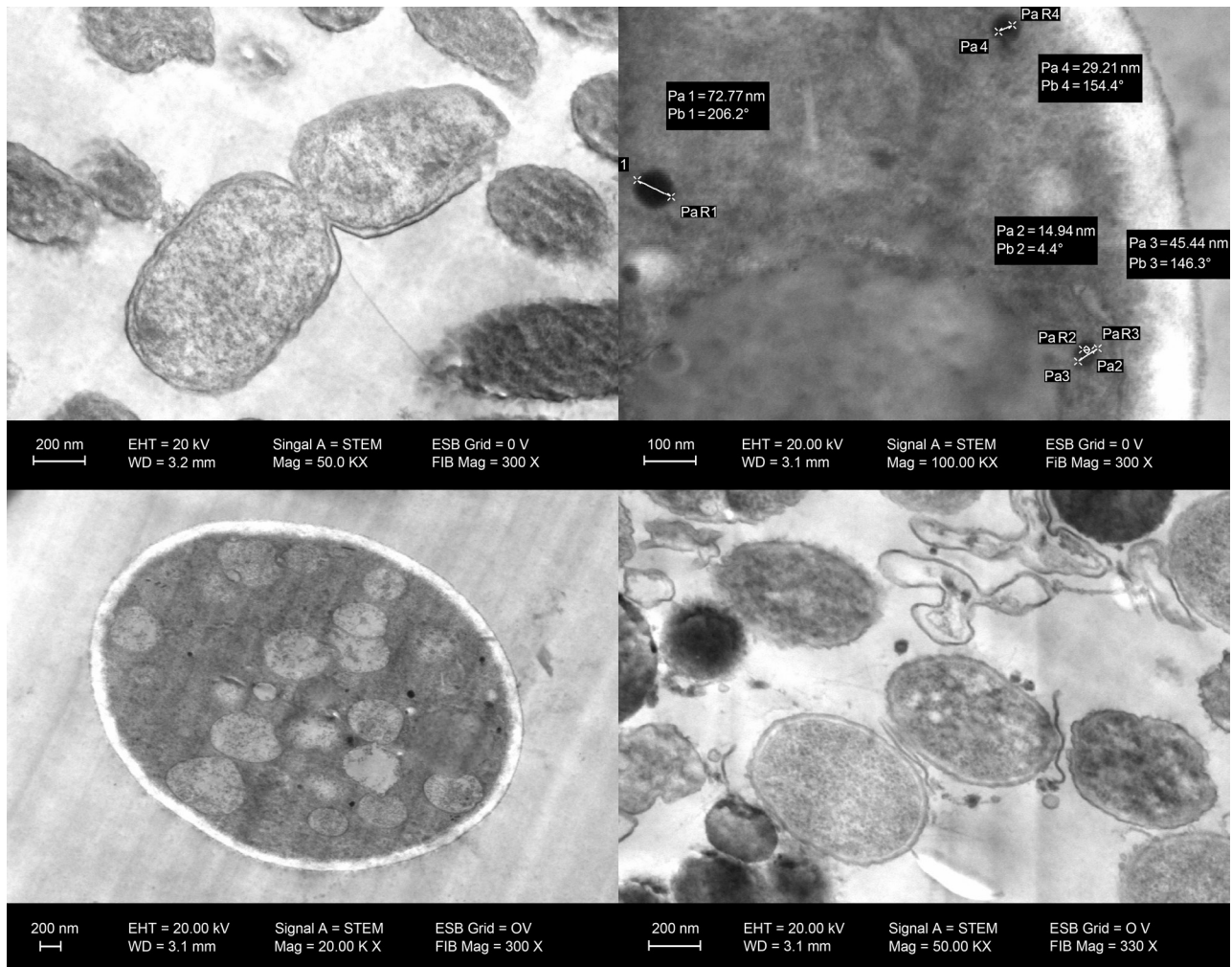


Fig. 5. TEM images of the bacterium and yeasts after the growth in the BioscreenC: (a) *Pseudomonas aeruginosa* without AgNPs; (b) *P. aeruginosa* with AgNPs; (c, d) *Candida albicans* with AgNPs, the black structures visible inside and outside of cells.

AgNPs particles, the diameter was close to 20 nm, so was the size of nanoparticles used in the study. In nearly 14%, the size was 2–2.5 times greater and in 7% diameter was 4–5 times larger. The distribution of AgNPs varied with particle diameter. Large AgNPs prevailed inside the cells while small ones dominated outside (Table III, Fig. 5).

Table III

The size and distribution (inside, outside, and within the cell wall) of AgNPs after their interaction with microorganisms.

AgNPs Size [nm]	[%]	Cell distribution according to the size [%]			
		Inside	Outside	In the cell wall	Total
21.45 ± 5.9	79.1	37.4	51.6	11	100
54.49 ± 9.1	13.9	93.8	6.25	0	100
94.62 ± 18.16	7.0	85.7	14.3	0	100
Total	100	48.7	42.6	8.7	100

The higher size of AgNPs inside the cells was probably due to cytoplasm properties to engender aggregation of AgNPs or protein corona formation. Such agglomeration was described by Rahman et al. (2013) and Bargheer et al. (2015) as the result of surface properties of proteins, as well as nanoparticles. The relatively high initial OD value for the samples with AgNPs observed in Bioscreen C microcultures (Fig. 2A and B; time 0 h) may result from the AgNP protein corona formation with the medium peptides, finally making the medium less transparent. However, the size of AgNPs found after *S. aureus* growth in Bioscreen C (with different nanoparticle doses) was not changed.

Many authors have studied the antimicrobial activity of AgNPs and found some peculiarities. Kaiser et al. (2017) described the influence of medium composition on nanosilver cytotoxicity, especially after the addition of serum and chloride. Also, food components influence the level of Ag⁺ release from the packaging material (Song et al. 2011), where Ag⁺ migration from food containers manufactured with silver and nanosil-

ver was revealed (Echegoyen and Nerin 2013). Silver ions are more toxic than AgNO₃ and nanoparticles. The minimal toxic dose of silver ions for humans is 0.014 mg/kg/day and only 1–2% accumulate in the body. Wen et al. (2016) reported that AgNPs at concentrations below 0.1 mg/l had low but observable cytotoxicity toward human buccal epithelial cells. The same concentration was the secondary maximum contaminant level of silver in drinking water (EPA 2017).

The antimicrobial activity of AgNPs also depends on the shape of nanoparticles. Pal et al. (2007) noted a stronger biocidal effect for truncated triangular silver nanoplates. These authors affirmed that for the safe use of AgNPs some questions had to be resolved (Bundschuh et al. 2016; Zarschler et al. 2016). Bundschuh et al. (2016) pointed out that long term consequences of the uptake by organisms of the engineered nanoparticles that are transferred throughout the food chain and may affect the microbial, plant, invertebrate, and fish communities, as well as the environment, is only partially understood and requires further systematic investigation. The study performed on nanoparticles used as antimicrobial agents against the molds causing deterioration of buildings and construction materials could also improve our living conditions. The environment protection becomes a very important issue for the human future and the use of AgNPs as antimicrobial agents could be in favor to overcome pollution by chemicals. Biocides used as antimicrobial agents are applied at rather high concentrations. Koziróg et al. (2016) tested seven compounds for their biocidal activity against microorganisms isolated from a wooden surface at the Auschwitz II-Birkenau Former German Nazi Concentration and Extermination Camp. Three of them were active, exhibiting biocidal activity at the concentration of 0.02–2% (200–20 000 mg/l). These active compounds were the ingredients of eight commercial biocides tested by the same researchers on wood pieces contaminated with microorganisms by triple spraying of the surface. According to the authors some of those biocides inhibited growth at the concentration of 6% (v/v), and others only at 30% (v/v). We found that the concentration of 9–10.7 mg/l of AgNPs (sized 20 nm) is sufficient to exhibit high anti-mold, anti-yeast, and anti-bacterial activity in the laboratory tests. The supplementation of materials used in the house construction with AgNPs at the low concentration (10.7 mg/l = 0.00107%) could be a better solution for the environment and human life.

Conclusions

The AgNPs tested inhibited the growth of *A. brasiliensis*, *C. globosum*, *P. pinophilum*, *P. variotii*, and *T. virens*. Therefore, AgNPs can be used to prevent

molds invasion on construction materials. The sensitivity to AgNPs depends on the molds species. Total inhibition of *P. variotii* growth was observed at a very small concentration of AgNPs (4.28 mg/l).

The TEM images revealed that AgNPs entered into the bacterial, yeast, and fungal cells and aggregated in larger particles exclusively inside cells of eukaryotic microorganisms. Such types of nanosilver aggregation have not yet been reported in literature and accumulation of AgNPs in the cell wall of *A. brasiliensis* cells was observed for the first time. Peculiarities of fungal interaction with AgNPs presented in this paper could be an interesting area of future research.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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