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Fungal Nanotechnology. Fernandes S, Lima N, Santos C. Universidade do Minho, Campus de Gualtar, Portugal, Braga. sara.fernandes@deb.uminho.pt. [Fungal Nanotechnology]

Abstract:

Due to the outbreak of infectious diseases caused by different pathogenic microorganisms and the development of drug resistance, nanoscale materials have emerged up as novel antimicrobial agents and the well known activity of silver ions and silver-based compounds has promoted research in this field. For this reason, there is an essential need to develop environmentally benign procedures for synthesis of silver nanoparticles for commercialization purposes. In this study, silver nanoparticles were synthesised extracellularly from silver nitrate using the fungi supplied by Micoteca da Universidade do Minho (MUM) fungal culture collection, and the morphology of the nanoparticles was characterised The potential to manipulate key parameters, which control growth and other cellular activities, to achieve an optimised production of nanoparticles were also investigated. In addition, a preliminary study was performed to assess the anti-fungal silver nanoparticles activity against bacteria. Introduction

The ability to uncover the structure and function of biosystems at the nanoscale, stimulates research leading to improvement in biology, biotechnology, medicine and healthcare. The integration of nanomaterials with biology has led to the development of diagnostic devices, analytical tools, physical therapy applications, and drug delivery vehicles (Chan, 2006). The importance of bactericidal nanomaterials study due to the increase in new resistant strains of bacteria and fungi against most potent antibiotics has promoted research in the well known activity of silver ions and silver-based compounds, including silver nanoparticles (Monteiro et al., 2009).

The different size and shape of nanomaterials can be synthesized by conventional chemical methods, but most of them are regarded as highly environmental cost. Generally, the chemical methods are low-cost for high volume; however, their drawbacks include contamination from precursor chemicals, use of toxic solvents, and generation of hazardous by-products (Tien et al., 2008). To accomplish the development of a reliable and ecofriendly process for synthesis of metallic nanoparticles, the use of natural sources like biological systems becomes essential. A vast array of biological resources available in nature including plants and plant products, algae, fungi, yeasts, bacteria, and viruses could all be employed for synthesis of nanoparticles (Mandal et al., 2006).

Importantly, for commercialization purposes, it would be advantageous to have a nonpathogenic biological system that produces the metallic nanoparticles. In relation to other microorganisms fungi present key characteristics such as tolerance and metal bioaccumulation abilities that are advantageous for production of nanoparticles (Mandal et al., 2006). Another advantage of using fungi in nanoparticle synthesis is the ease in their scale-up. Given that fungi are extremely efficient secretors of extracellular enzymes, it is thus possible to easily obtain large-scale production of enzymes (van den Hondel et al., 1992).

Hence, the present study was undertaken to prove the potential in extra-cellular biosynthesis of silver nanoparticles by different fungi supplied by Micoteca da Universidade do Minho (MUM, www.micoteca.deb.uminho.pt) fungal culture collection and investigate the antimicrobial effect in *E. coli*. Materials and Methods

Microorganisms

The fungi *Aspergillus* oryzae MUM 97.19 and Penicillium chrysogenum MUM 03.22 were obtained from Micoteca da Universidade do Minho fungal culture collection and maintained in potato dextrose agar plates at 25 oC. The bactericidal experiments were carried out with gram negative bacteria *Escherichia coli* that was sub-cultured in LB agar medium. Synthesis of silver nanoparticles

To prepare the biomass for biosynthesis studies the fungus was grown aerobically in MYGP liquid medium. The culture flask was incubated in an orbital shaker at 30 oC and agitated at 150 rpm. After 72 h of growth the biomass was harvested followed by extensive washing with sterile double distilled water to remove any medium components from the biomass. Typically 10 g (wet weight) was brought into contact with 100 mL sterile MiliQ water for 72 h at 30 oC in an Erlenmeyer flask and agitated as described earlier. After the incubation the cell filtrate was obtained by passing it through Whatman

filter paper No. 1. For synthesis of silver nanoparticles, 1 mM AgNO3 was added to 100 mL of cell filtrate and agitated at 30 oC in dark conditions. Control flasks without the AgNO3 were incubated at same conditions. Characterization of silver nanoparticles

Samples of 1mL were withdrawn at different time intervals and the localised surface plasmon resonance of silver nanoparticles was characterised by using UV-Vis spectrophotometer (Jasco V-560). The fungal cellular filtrate embedded with the silver nanoparticles was freeze-dried and used for scanning electron microscopy (SEM) analysis (FEI Nova 200). Disk diffusion test

A 10 ml suspension of nanoparticles was filtered through a 0.22 µm membrane. The nanoparticle laden filter paper was dried in an oven for 1 h and small disks of uniform size (6 mm diameter) were punched out and stored in a desiccator at room temperature. The bacterial suspension (100 mL of 104 CFU ml-1) was smeared uniformly on the surface of a LB agar plate before placing the disks on the plate. The plates were incubated at 37 oC for 24 h. Results and Discussion

The fungi *A. oryzae* and *P. chrysogenum* were used for the synthesis of silver nanoparticles. The fungal biomass after 72 hours of incubation was filtered and the filtrate was treated with AgNO3. Fungal culture filtrate exhibited a gradual change in colour towards brown when it was incubated at 30 oC at dark conditions indicating that the reduction of Ag+ ions takes place extracellularly.

It is well known that silver nanoparticles exhibit a brown colour in water due to excitation of surface Plasmon vibrations in the metal nanoparticles (Mulvaney, 1996) and a characteristic surface Plasmon absorption band at 420 nm was observed at 24 h that attained the maximum intensity after 96 h. After 96 h of incubation, no change in intensity at 420 nm was observed indicating complete reduction of silver ions.

SEM micrograph of silver nanoparticles obtained after 24 h of incubation showed variable shapes, most of them present in spherical in nature. The size of the particle ranged from 19 to 51 nm. Majority of the silver nanoparticles were aggregates.

Nanoparticle synthesis in terms of colour intensity of culture filtrate was examined at different temperatures (30, 40, 50, 60, 60 and 80 oC), pH (3-8), and sodium chloride concentration (0.1-0.6%). The absorbance spectra exhibited a peak at 420 nm. At this wavelength, the highest optical density was found pH 7.0, temperature of 80 oC, and 0.3% sodium chloride after 24 h of incubation of A. oryzae cellular filtrate with 1 mM of AgNO3 while temperatures between 60 and 80 oC, low percentage of NaCl and pH range between 4 and 8 are ideal conditions for AgNPs synthesis by P. chrysogenum.

Recent studies have confirmed that metal nanomaterials have good antimicrobial activity and among inorganic agents, silver has been employed most widely since ancient times. Silver is known to exhibit a strong toxicity to a wide range of microorganisms (Liau et al., 1997) and for this reason silver-based compounds have been used extensively in many bactericidal applications (Gupta and Silver, 1998; Nomiya et al., 2004). Silver compounds have also been used in the medical field to treat burns and a variety of infections (Feng et al., 2000). The bactericidal effect of silver nanoparticles was shown to be size dependent as smaller particles with a larger

surface-to-volume ratio provide a more efficient means of antibacterial activity against E. coli and *Staphylococcus aureus* (Baker et al., 2005; Panácek et al., 2006).

Our preliminary studies also shows that AgNPs synthesised by *A. oryzae* and *P. chrysogenum* have great promise as antimicrobial agent against *E. coli* based on the inhibition zone measured in the disk diffusion tests conducted in agar plates.

The shape of silver nanoparticles may also interfere with their antimicrobial effect (Pal et al., 2007). For this reason, the development of a protocol to synthesize silver nanoparticles with controlled size and shape is of great importance in the field of material miniaturization because the nanomaterials exhibits optical, magnetic, catalytic, optoelectronic and thermal properties which depends on its sizes (Alivisatos, 1996) and shapes (Jin et al., 2001). Moreover, exhaustive experimental trials and clinical studies are required for a better understanding of the antimicrobial efficiency of silver nanoparticles.

Irrespective of the biological system used, an understanding of the biochemical and molecular mechanism of nanoparticle synthesis is essential for maximal exploitation. This will achieve better control of size and polydispersity of the nanoparticle formation by the cell filtrate. Elucidation of biochemical pathways leading to metal ion reduction in the different classes of microbes is necessary and will lead to the development of a rational microbial nanoparticle synthesis procedure. However we postulate that a reductase enzyme and other meadiators secreted by the fungi used in ours study is responsible for the reduction of Ag+ ions and the subsequent formation of silver nanoparticles. Given this, our aim is the identification and characterisation of the enzymes and putative mediators involved in the biological synthesis of nanoparticles as well as an improvement of performance of these enzyme-mediator complexes. References

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Resultado de parceria entre o IBGE e o Ministério do Meio Ambiente, os biomas brasileiros foram divididos em Amazônia, Cerrado, Caatinga, Mata Atlântica, Pantanal e Pampa (IBGE 2004). Os nomes adotados foram os mais usuais e populares, em geral associados ao tipo de vegetação predominante ou ao relevo. Ainda conceituam bioma como um conjunto de vida (vegetal e animal) constituido pelo agrupamento de tipos de vegetação contíguos e identificáveis em escala regional, com condições geoclimáticas similares e história compartilhada de mudanças, o que resulta em uma diversidade biológica própria. O bioma Amazônia ocupa área de 49,29% e somado ao bioma Pantanal (1,76%) ocupam mais da metade do território brasileiro enquanto o bioma Mata Atlântica ocupa 13,04% do território nacional. Este último constitue o total da cobertura dos Estados do Espitito Santo, Rio de Janeiro e Santa Catarina, 98% do Paraná e cerca de 80% do Estado de São Paulo. Os demais estados situados mais a leste do País tem apenas influencia parcial com pouco mais de 50% no caso de Alagoas, declinando sensivelmente a partir do estado do Rio Grande do Sul aos estados de Minas Gerais, Sergipe, Bahia, Pernambuco, Rio Grande do Sul, Paraíba, Piauí, Rio Grande do Norte, Ceará e Goiás.