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**GREEN SYNTHESIS OF SILVER NANOPARTICLES: FOCUS ON
MONOSACCHARIDES AND FUNGI ISOLATED FROM BRAZILIAN SOIL**

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**GREEN SYNTHESIS OF SILVER NANOPARTICLES: FOCUS ON
MONOSACCHARIDES AND FUNGI ISOLATED FROM BRAZILIAN SOIL**

Tese de Doutorado apresentada à Coordenação do Curso de Pós-Graduação em Química da Universidade Federal do Ceará, como parte dos requisitos para obtenção do Título de Doutor em Química. Área de Concentração: Química.

Orientador: Prof. Dr. Pierre Basílio Almeida
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RESUMO

Nanopartículas de prata (AgNPs) são estruturas com tamanho até 100 nm e que exibem propriedades diferentes daquelas encontradas no material de origem. AgNPs possuem diversas aplicações tecnológicas e podem ser sintetizadas por métodos físicos, químicos e biológicos. O uso de micro-organismos, açúcares e plantas para biossíntese de AgNPs é considerado como tecnologia verde, pois não envolve substâncias químicas tóxicas. Entre os açúcares, os monossacarídeos (glicose) são promissores devido a sua disponibilidade, custos baixos e atoxicidade. Já as leveduras possuem uma grande variedade de enzimas e são fáceis de manusear. Entretanto, existem poucos trabalhos explorando a capacidade biossintética de AgNPs por leveduras. Os resíduos da produção de AgNPs, gerados por fungos, podem ser usados com finalidade catalítica, desde que suportado em matrizes poliméricas como o alginato. Os objetivos desse estudo foram usar glicose e leveduras para produzir AgNPs, caracterizar essas estruturas e avaliar as atividades antibacterianas, antifúngicas, citotóxica e catalíticas. Além disso, os resíduos gerados pelos fungos foram usados para a produção de esferas de alginato que foram utilizadas com objetivos catalíticos. A síntese de AgNPs foi realizada com glicose e dodecil sulfato de sódio (SDS) foi usado com estabilizador. As AgNPs de origem química foram associadas com o antibiótico ciprofloxacina e testadas contra *Escherichia coli*. As AgNPs elevaram a atividade da ciprofloxacina em 40%. A ação sinérgica de AgNPs e anfotericina B e nistatina foram avaliadas contra *C. parapsilosis*. AgNPs quando combinadas com anfotericina B e nistatina mostraram potente ação antifúngica e aumentaram a zona de inibição em torno dos discos antifúngicos em 222,6 e 319,3%, respectivamente. A combinação de AgNPs e anfotericina B ou Nistatina pode apresentar uma vantagem tecnológica contra fungos resistentes. Em outra vertente, duas leveduras *Rhodotorula glutinis* e *Rhodotorula mucilaginosa* foram isoladas do solo cearense e avaliadas quanto a sua capacidade de produzir AgNPs. As AgNPs foram caracterizadas pelas técnicas UV-vis, DLS, FTIR (Infra-vermelho com transformada de Fourier), XRD (difração de Raios-X), EDX, MEV (Microscopia Eletrônica de Varredura), MET (Microscopia Eletrônica de Transmissão) e MFA (Microscopia de Força Atômica). AgNPs produzidas pelas leveduras mostraram atividades antifúngicas e catalíticas. AgNPs produzidas por *R. glutinis* e *R. mucilaginosa* mediram 15,45 nm ± 7,94 e 13,70 nm ± 8,21 (média ± DP), respectivamente, quando analisada por MET. As AgNPs mostraram alta atividade catalítica na degradação de 4-nitrofenol e azul de metileno. As 35 cepas de *C. parapsilosis* mostraram grande sensibilidade as AgNPs e essas partículas também aumentaram as propriedades antifúngicas do fluconazol (42,2% *R. glutinis* e 29,7 % *R. mucilaginosa*). A atividade citotóxica das AgNPs foi detectada em concentrações acima daquelas que exerceram atividade biológica, mostrando a segurança dessas partículas. As esferas de alginato produzidas com AgNPs de origem fúngica foram capazes de degradar o 4-nitrofenol e o antibiótico ceftazidima eliminando sua atividade microbiológica. Finalmente, duas leveduras com habilidade de produzir AgNPs foram isoladas do solo brasileiro (Ceará) e apresentaram multifuncionalidades que podem representar uma alternativa tecnológica com potenciais aplicações em diferentes áreas. O uso de monossacarídeos e leveduras na síntese de AgNPs pode representar uma alternativa a síntese convencional e reduzir o impacto ambiental que essas substâncias causam.

Palavras-Chaves: Síntese Verde; Nanopartículas de prata; Monossacarídeos; *Rhodotorula glutinis*; *Rhodotorula mucilaginosa*; atividade catalítica; atividade antifúngica.

ABSTRACT

Silver nanoparticles (AgNPs) are structures with sizes up to 100 nm and exhibit properties that are different from those found in the bulk material. AgNPs are structures with several technological applications and can be synthesized by chemical, physical and biological methods. Use of microorganisms, sugars, and plants for the biosynthesis of silver nanoparticles is considered a green technology, as it does not involve any harmful chemicals. Among the sugars, monosaccharides (glucose) are promising because they are readily available, inexpensive, and non-toxic. Yeasts have a wide enzymatic range and are easy to handle. However, there are few reports of yeasts with biosynthetic ability to produce stable AgNPs. The waste generated by fungi in AgNP production can be used for catalytic purposes, provided that it is supported on polymer matrices, such as alginate. The objectives of this study were to use glucose and yeasts to produce AgNPs, characterize these structures, and evaluate their antibacterial, antifungal, cytotoxic and catalytic activities. Additionally the waste generated by fungi was used for the production of alginate beads that were used for catalytic purposes. The synthesis of AgNPs was performed with glucose, and sodium dodecyl sulfate (SDS) was used as a stabilizer. The AgNPs of synthetic origin were associated with the antibiotic ciprofloxacin and tested against *Escherichia coli*. The AgNPs increased the action of ciprofloxacin by 40%. The synergistic action of AgNPs produced with amphotericin B and nystatin against *C. parapsilosis* was evaluated. *Candida spp.* were isolated from candidemia of patients admitted to public hospitals in Ceará. AgNPs, when combined with amphotericin B and nystatin, showed potent antifungal activity and increased the zone around the antifungal disk by 222.6 and 319.3%, respectively. The combination of substances AgNPs and amphotericin B or nystatin can potentiate their effects, therefore showing a large zone of growth inhibition. Two yeasts – *Rhodotorula glutinis* and *Rhodotorula mucilaginosa* – were isolated from Brazilian soil and their ability to produce AgNPs was evaluated. AgNPs were characterized by UV-vis, DLS, FTIR, XRD, EDX, SEM, TEM and AFM. AgNPs produced by yeasts showed catalytic and antifungal activities. The AgNPs produced by *R. glutinis* and *R. mucilaginosa* measured $15.45 \text{ nm} \pm 7.94$ and $13.70 \text{ nm} \pm 8.21$ (average \pm SD), respectively, when analyzed by TEM. The AgNPs showed high catalytic capacity in the degradation of 4-nitrophenol and methylene blue. *C. parapsilosis* showed high sensitivity to AgNPs and also enhanced the antifungal property of fluconazole (42.2% for *R. glutinis* and 29.7 % for *R. mucilaginosa*). Cytotoxic activity of AgNPs was above the concentrations that exerted biological activity, showing the safety of these particles. The alginate spheres produced with these AgNPs of fungal origin were able to degrade 4-nitrophenol and antibiotic ceftazidime, eliminating microbiological action of ceftazidime. Finally, two yeasts isolated from Brazilian soil (Ceará), with the ability to produce AgNPs, were described. These particles (AgNPs) showed multifunctionality and can represent a technological alternative in many different areas with potential applications. The use of monosaccharides and yeasts for AgNP synthesis may be an alternative to orthodox synthesis and reduce the environmental impact that these substances cause.

Keywords: Green synthesis; Silver nanoparticles; Monosaccharides; *Rhodotorula glutinis*; *Rhodotorula mucilaginosa*; catalytic activity; antifungal activity.

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ACRONYMS AND ABBREVIATIONS

AFM= Atomic Force Microscopy
AgNP=Rm= Silver nanoparticles synthesized by *Rhodotorulla mucilaginosa*
AgNP-G= Silver nanoparticles synthesized based on glucose
AgNP-R= Silver nanoparticles synthesized based on ribose
AgNP-Rg= Silver nanoparticles synthesized by *Rhodotorulla glutinis*
AgNPs= Silver nanoparticles
C. parapsilosis= *Candida parapsilosis*
C. tropicalis = *Candida tropicalis*
DLS= Dynamic light scattering
EDX= Energy-dispersive X-ray spectroscopy
FEG= Field-emission gun
FT-IR= Fourier Transform Infrared Spectroscopy
PDI- Polydispersity index
Rg= *Rhodotorulla glutinis*
Rm= *Rhodotorulla mucilaginosa*
SDS= Sodium dodecyl sulfate
SEM= Scanning Electron Microscopy
TEM= Transmission Electronic Microscopy
XRPD= X-ray powder diffraction

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