BRIEF COMMUNICATION

ANTIFUNGAL ACTIVITY OF SILVER NANOPARTICLES OBTAINED BY GREEN SYNTHESIS

Eduardo José J. MALLMANN(1), Francisco Afrânio CUNHA(1,2), Bruno N.M.F. CASTRO(2), Auberson Martins MACIEL(1,2), Everardo Albuquerque MENEZES(2) & Pierre Basílio Almeida FECHINE(1)

SUMMARY

Silver nanoparticles (AgNPs) are metal structures at the nanoscale. AgNPs have exhibited antimicrobial activities against fungi and bacteria; however synthesis of AgNPs can generate toxic waste during the reaction process. Accordingly, new routes using non-toxic compounds have been researched. The proposal of the present study was to synthesize AgNPs using ribose as a reducing agent and sodium dodecyl sulfate (SDS) as a stabilizer. The antifungal activity of these particles against C. albicans and C. tropicalis was also evaluated. Stable nanoparticles 12.5 ± 4.9 nm (mean ± SD) in size were obtained, which showed high activity against Candida spp. and could represent an alternative for fungal infection treatment.

KEYWORDS: Silver nanoparticles; Antifungal activity; Candida spp.
Table 1

| Strains (n) | AgNPs | Amphotericin B |
|-------------|-------|---------------|
|             | Range (mm) | Halo (mm) | Range (mm) | Halo (mm) | P |
| C. albicans (14) | 17-30 | 23 ± 4 | 15-25 | 20 ± 3 | 0.02 |
| C. tropicalis (16) | 12-30 | 21 ± 4 | 15-25 | 20 ± 3 | |

The size of the AgNPs was analyzed on Zetasizer, NanoZS Malvern® by dynamic light scattering (DLS).

In this study, 30 strains of Candida spp. were selected (14 C. albicans and 16 C. tropicalis) and isolated from blood samples of patients hospitalized in the state of Ceará, Brazil. C. albicans was purified and identified in a chromogenic medium, with production of chlamydospores in rice extract agar containing Tween-80, and germ tube formation. This identification was carried out by molecular biology with the primer hwp1 (cr-f-5'-GCT ACC ACT TCA GAA TCA TCA TC-3'; cr-r-5' GCA CCT TCA GTC GTA GAG ACG-3') and the PCR conditions were 95 °C for five min, followed by 30 cycles of 94 °C for 45 s, 58 °C for 40 s, and 72 °C for 55 s; extension was performed at 72 °C for 10 min. The DNA fragment size that was produced had 945 bp. The molecular identification of C. tropicalis was performed using the trf4 gene. The following primers were used (trf4 5'-ATT GCC TGA AAC AGA GGT-3'; trf-4 5'-CAA CCC TGC TAA GTC ATT AC-3') and the PCR conditions were 95 °C for five min, followed by 30 cycles of 94 °C for one min, 50 °C for one min, and 72 °C for 90 s; extension was performed at 72 °C for 10 min. The DNA fragment size that was produced had 324 bp.

The sensitivity of Candida spp. was evaluated by the well diffusion method on a Mueller-Hinton medium supplemented with 2% glucose and 0.05% methylene blue. In mediums containing Candida spp., wells were made and filled with 80 µg of AgNPs. Discs of amphotericin B 10 µg were used as control. The plates were incubated at 35 °C for 24h, and after this period fungal growth inhibition halos were measured (mm). Each test was conducted three times, according to the protocol of CLSI M44-A2(18,19).

Production of AgNPs using ribose as a reducing agent and SDS as a capping agent was simple and easy to perform. The entire process was completed in 30 min. The chemical reaction proposed can be represented by the following equation:

\[ \text{C}_6\text{H}_{12}\text{O}_6 + 2\text{Ag}^+ \rightarrow \text{C}_6\text{H}_{12}\text{O}_6^+ + 2\text{H}^+ + 2\text{Ag}\ (\text{AgNPs}) \]

These AgNPs showed strong spectrophotometric absorbance, around 420 nm, as shown in Figure 1b. This is typical behavior of these structures. Use of SDS as the capping agent provided prolonged stability for up to four months when stored at room temperature and exposed to ambient light. Some sugars have reducing properties and are used in the production of nanoparticles. The process does not harm the environment because it does not produce toxic waste and requires no accelerator(13).

AgNPs produced in this study had a size of 12.5 ± 4.9 nm (mean ± SD), with a narrow particle size distribution, as shown in Figure 1c.

This feature gives a high surface area, better for antimicrobial activity and good order. In previous studies using glucose as the reducing agent, the size of AgNPs was around 15 nm(19).

The AgNPs exhibited high antimicrobial activity, and this property can be very useful, especially against microorganisms resistant to conventional antimicrobials(17). C. albicans and C. tropicalis showed high sensitivity to AgNPs (Fig 1d). The activity of 80 µg of AgNPs can be compared with the activity of amphotericin B, a powerful antifungal (Table 1). Studies highlight this same result with activity of AgNPs against Candida spp(16). The statistical analysis of the results, carried out by Student’s t-test, showed that C. albicans was more sensitive than C. tropicalis (p = 0.02).

In conclusion, AgNPs were easily prepared by green synthesis using ribose as a reducing agent and SDS as a stabilizer. Additionally, they showed high activity against C. albicans and C. tropicalis, a similar activity observed by the antifungal amphotericin B, and may represent an alternative for treating fungal infections.

RESUMO

Atividade antifúngica de nanopartículas de prata obtidas por síntese verde

Nanopartículas de Prata (AgNPs) são estruturas metálicas em escala nanométrica. AgNPs apresentam atividades antimicrobianas contra fungos e bactérias; no entanto, a síntese de AgNPs pode gerar resíduos tóxicos e devido a isso novas rotas utilizando compostos atóxicos têm sido buscadas. O objetivo desse estudo foi sintetizar AgNPs utilizando a ribose como agente redutor e dodecil sulfato de sódio (SDS) como estabilizador e avaliar a atividade antifúngica dessas partículas contra C. albicans e C. tropicalis. Foram sintetizadas nanopartículas estáveis com 12.5 ± 0.2 nm (média ± DP) que apresentaram elevada atividade contra Candida spp. e podem representar boa alternativa no tratamento de infecções fúngicas.

ACKNOWLEDGMENTS

The financial support received from CNPq (Brazilian agency), Process 473417/2007.

REFERENCES

1. Barie PS. Multidrug-resistant organisms and antibiotic management. Surg Clin North Am. 2012;92:345-91.
2. Bhaduri GA, Little R, Khomane RB, Lokhande SU, Kalakarni BD, Mendis BG, et al. Green synthesis of silver nanoparticles using sunlight. J Photochem Photobiol A: Chemistry. 2013;258:1-9.

3. Clinical and Laboratory Standards Institute. Method for antifungal disk diffusion susceptibility testing of yeasts: approved standard M44-A2. Wayne: Clinical and Laboratory Standards Institute; 2008.

4. Cornstein W, Mora A, Orellana N, Capparelli FJ, Castillo M. Candida: epidemiologia y factores de riesgo para especies no albicans. Enferm Infeccc Microbiol Clin. 2013;31:380-4.

5. Gao X, Wei L, Yan H, Xu B. Green synthesis and characteristic of core-shell structure silver/starch nanoparticles. Mater Lett. 2011;65:2963-5.

6. Iravani S. Green synthesis of metal nanoparticles using plants. Green Chem. 2011;13:2638-50.

7. Kang Y, Iida S, Yamamoto S, Kogure T, Tanaka R, Mikami Y. Trf4 is a useful gene for discrimination of Candida tropicalis from other medically important Candida species. Nikon Ishinkin Gakkai Zasshi. 2008;49:39-43.

8. Kashyap PL, Kumar S, Srivastava AK, Sharma AK. Myconanotechnology in agriculture: a perspective. World J Microbiol Biotechnol. 2013;29:191-207.

9. Kumar P, Selvi SS, Govindaraju M. Seaweed-mediated biosynthesis of silver nanoparticles using Gracilaria corticata for its antifungal activity against Candida spp. Appl Nanosci. 2013;3:495-500.

10. Lanje AS, Sharma SJ, Pode RB. Synthesis of silver nanoparticles: a safer alternative to conventional antimicrobial and antibacterial agents. J Chem Pharm Res. 2010;2:478-83.

11. Lockhart SR, Igbal N, Cleveland AA, Farley MM, Harrison LH, Bolden CB, et al. Species identification and antifungal susceptibility testing of Candida bloodstream isolates from population-based surveillance studies in two U.S. cities from 2008 to 2011. J Clin Microbiol. 2012;50:3435-42.

12. Menezes EA, Cunha MCSO, Cunha FA. Identificação preliminar de algumas espécies do gênero Candida spp. em meio crômogeno: resultados de dois anos de um estudo multicêntrico realizado no Ceará. Rev Patol Trop. 2011;40:297-303.

13. Oluwafemi OS, Lucwaba Y, Gura A, Masabeya M, Ncapayi V, Olujimi OO, et al. A facile completely ‘green’ size tunable synthesis of maltose-reduced silver nanoparticles without the use of any accelerator. Colloids Surf B Biointerfaces. 2013;102:718-23.

14. Panácek A, Kolár M, Vicerová V, Prucek R, Soukopová J, Krystof V, et al. Antifungal activity of silver nanoparticles against Candida spp. Biomaterials. 2009;30:6333-40.

15. Quester K, Avalos-Borja M, Castro-Longoria E. Biosynthesis and microscopic study of metallic nanoparticles. Micron. 2013;54-55:1-27.

16. Romeo O, Criseo G. First molecular method for discriminating between Candida africana, Candida albicans and Candida dubliniensis by using hwp1 gene. Diagn Microbiol Infect Dis. 2008;62:230-3.

17. Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: green synthesis and their antimicrobial activities. Adv Colloid Interface Sci. 2009;145:83-96.

18. Shenshena MA, El-Safty SA, Elshehy EA. Synthesis, morphological control, and properties of silver nanoparticles in potential applications. Part Part Syst Charact. 2014;31:293-316.

19. Sintubin L, Verstraete W, Boon N. Biologically produced nanosilver: current state and future perspectives. Biotechnol Bioeng. 2012;109:2422-36.

20. Vasconcelos Júnior AA, Menezes EA, Cunha FA, Cunha MCSO, Bráz BHL, Capelo LG, et al. Comparação entre microdiluição e disco difusão para o teste de susceptibilidade aos antifúngicos contra Candida spp. Semina Ciênc Biol Saúde. 2012;33:135-42.

Received: 30 April 2014
Accepted 14 July 2014