ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES PRODUCED BY GREEN SYNTHESIS FROM ENDOPHYTIC FUNGI PENICILLIUM SPP

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ABSTRACT: The increase in bacterial resistance to antimicrobials has become a major problem for public health in recent decades. Endophytic fungi, that lives in mutualistic association with plants, are a rich source of bioprospecting for such bioactive compounds. The objective of the present project was to synthesize silver nanoparticles through green synthesis, an ecologically sustainable approach that uses biological organisms as biofactories to the production of metallic nanoparticles. The utilization of metallic nanoparticles can be applied in the study of the control of resistance to traditional antimicrobials using inhibition as prevention of microbial diseases. The endophytic filamentous fungi *Penicillium* spp., isolated from *Polygala paniculata* a plant collected in Brazil, were used in the biosynthesis of silver nanoparticles. The synthesis of AgNPs were conducted utilizing the *Penicillium* spp. fermentation broth with 1 mL de $AgNO_3$ (1mM). To conduct the characterization of AgNPs the 1,5 mL of reactional solution was submmited to the UV-Vis (200 and 800 nm) in 72, 144 and 216 h incubation, and fermentation broth without $AgNO_3$ as control. Subsequently, the nanoparticles were submitted to evaluative assays to test their antimicrobial, effects. The nanoparticles presented inhibition of *Escherichia coli* and *Staphylococcus aureus*, with better results for the Gram negative bacterium. The results show that the green synthesis is a good method to produce metallic nanocomposites utilizing endophytic *Penicillium* spp.

KEYWORDS: *Penicillium* spp.*, endophytes, green synthesis, metallic nanoparticles, $AgNO_3$, microbial inhibition*

EXPERIMENTAL

The use of polimers as metallic nanoparticles is an important and innovative tool, since the generated data can achieve better results when the subject is the study of the microbial resistance to traditional antimicrobials.This protocol can impact directly the resistance as a major public health concern, giving new highlight into the microbial diseases.

To proceed with the development of the endophyte, *Penicillium* spp. (isolated from *Polygala paniculata* Cruz et al., 2021) was submmited to the fermentation broth that was cultivated in BDA, in the absence of light at 28°C, during 10 days incubation (Fig. 1).

The synthesis of AgNPs, was done utilizing 100 mL of fermentation broth with 1 mL of $AgNO_3$, to final concentration of 1 mM. The silver ions were reducted as visualized by color reactional change. The characterization of AgNPs was made utilizing UV-Vis in CDMF, Chemistry Department. The reactional solution was submmited to double beam spectrophotometry, operating in 200 and 800 nm in 72, 144 and 216 h of incubation time. Fermentation broth without AgNO3 was used as the negative control. The evaluation of AgNPs antimicrobial activity were made using the diffusion method in solid media. To do the characterization of AgNPs (CDMF/UFSCar) 1,5 mL of reactional solution was submmited to the UV-Vis (200 and 800 nm) in 72, 144 and 216 h incubation, and fermentation broth without $AgNO_3$ as control (Verassami et al., 2011).

To conduct the bioguided tests, we used the batcterial pathogens *Escherichia coli* and *Staphylococcus aureus* as Gram positive and negative, respectively. The bacterial padronization was made using spectophotometer at 620 nm and an OD between 0,08 e 0,10.

RESULTS AND DISCUSSION

Figure 1 show the studied organism, *Penicillium* spp. In this picture, it can be seen the morphologic colony characteristic of the fungi. Table 1 shows the antimicrobial effect of AgNPs against *E. coli* and *S. aureus,* that were similar, but higher to *S. aureus* with the identified inhibition zones.

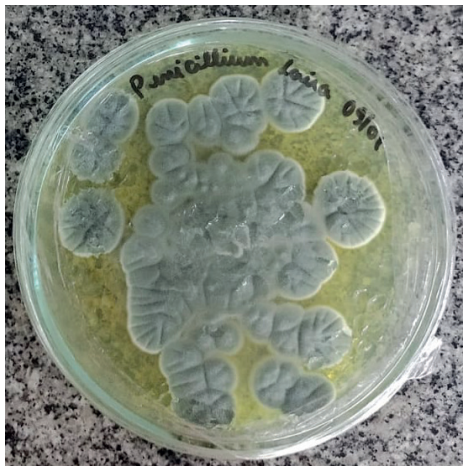


Figure 1. *Penicillium* spp. typical microbial morphology, growing in BDA plate dish.

Figures 2 and 3 shows the UV-vis spectre for the tested samples. AgNPs have unique optical properties due to superficial plasmon ressonance.

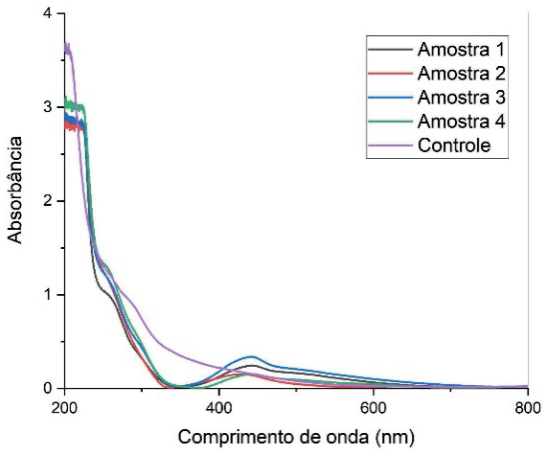


Figura 2. UV-vis spectre with pic near 450 nm in the reactiona solutions, indicating the presence of silver nanoparticles. Control solution showed one pic near 200 nm indicating the presence of reductor molecules in the broth fermentation.

Figure 3 shows the UV-vis spectre with results from sample 3 in different incubation times. It can be seen that was a considerable pic after 72 hours of incubation. However, after 144 hours, the pic presented a little growth indicating that the synthesis was approching the end time.

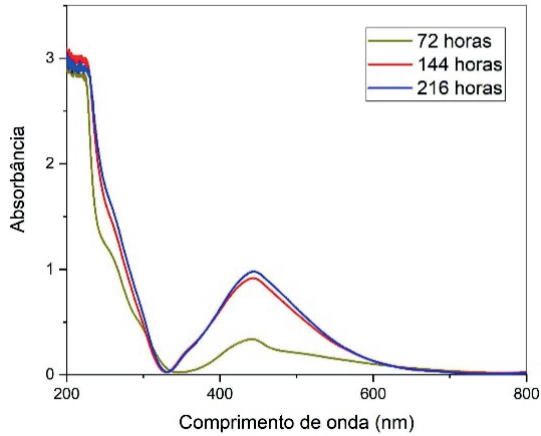


Figure 3. UV-vis spectre results from sample 2 in different incubation times.

Based on literature (Halkai et al., 2017), it is expected that the NPs synthetised from the *Penicillium* spp. fermentation broth presents morphology with 16-40 nm and spherical aspect.

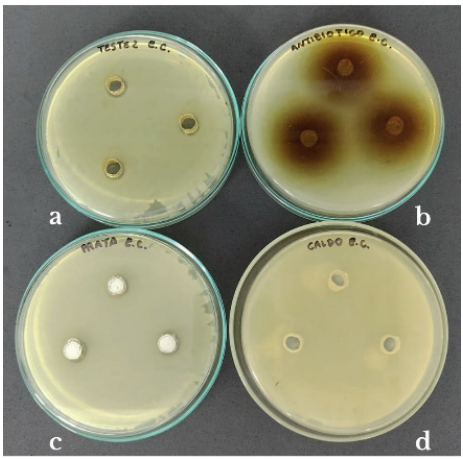
The results presented in Table 1 shows the presence of halos numbers and data is similar to work conducted by Jaidev and Narasimha (2010).

Samples	***E. coli***	***S. aureus***
AgNPs	1	2-4
AgNO3	1	1
Fermentation broth (- control)	-	-

Table 1. Nanoparticles inhibition zones (mm) sinthetized by *Penicillium* spp. broth fermentation against *E. coli* and *S. aureus.*

In Figure 4 it is showed the inhibition halos against *Escherichia coli*. DA MOTA et al., (2018) worked with Gram negative bacteria and these authors results are similar to this presented here.

The Figure 4 shows the bioguided test with inhibition halos. Plates with *E. coli* in the a) AgNPs reactional solution, b) tetraciclin, c) AgNO3 and d) fermentation broth.



Source: Authors

In the figure 5, the results show the evaluation of AgNPs with charcteristic results. These data present some difference from Bogas et al., 2022.

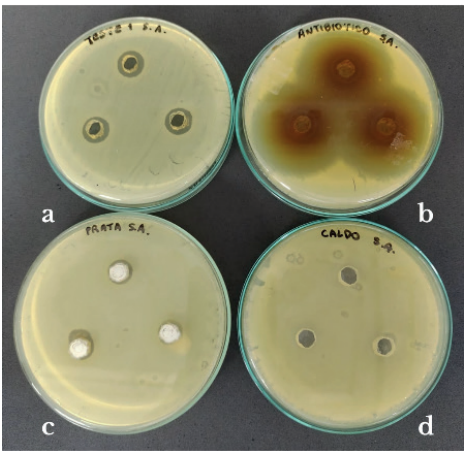


Figure 5. *S. aureus* plates with (a) AgNPs reactional solution (b) tetraciclin, (c) AgNO3 solution and (d) fermentation broth.

Source: Authors

There was a small inhibition halo formation in the fermentation broth plate (a) and no halo in the tetraciclin (b). In the others tests the inhibition halo was present.

As can be seen in Figures 2 and 3, the plates with the AgNPs showed inhibition halos for both pathogens, with better results for S. aureus. On the other hand, the plates with AgNO3 (1 mM) had a small inhibition halo and the plates with fermentation broth presented no halo, as expected.

CONCLUSION

The extracellular syntesis of AgNPs from *Penicillium* fermentation broth was done succesfully.

The antimicrobial activity of nanoparticles presented activity against *E. coli* and S*. aureus.* The green synthesis can provide good results and an ecologically sustainable approach that uses biological organisms as biofactories in the production of metallic nanoparticles.

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