

The miracle of Ag-NPs biosynthesis using lactobacilli

El.Fadly, E G^{1*}, El.Bazz, A M², Magooz, O F², Kamis, E F², Salem, M S², Abdel Atty, N S¹,
Sakr, H K², Salamah, MA², Nashaat, MA², El-Kemary, M A³

¹Faculty of Agriculture Kafr el sheikh univ, ²Agriculture research centre, ³Institution of nanosciences and technology. Kafr el sheik univ. * corresponding author: Enasali.2006@gmail.com

KEYWORDS

Whey, Lactobacilli, Ag-NPs biosynthesis, antibacterial, antifungal, antinematode, biofouling activity, Cytotoxicity; *in vivo*, *in vitro*

SHORT SUMMARY

In this study, we used five lactobacilli strains to bio-synthesize silver nanoparticles using silver nitrate deferent concentrations, characterizing nanoparticles using Uv/Vis spectrum, ZETA-potential, DLS, FTIR, XRD, SEM-Edx, and TEM images. We used the obtained biomass mixture of the alive bacterial cells and Ag-NPs in different concentrations to study the antibacterial, anti-biofouling, fungal, and nematode activity. Also, we examined the in vitro and in vivo cytotoxicity. We studied the growth rate of the used strains to determine the maximum tolerance concentration. We found a tiny effect on the bacterial cells' growth rates under the stress of silver nitrate concentrations (0,1,2,3,4,5,6mμ/L) compared with the control. TEM images proved that the bacterial cell exo-biosynthesize mechanism is well-known. Still, the surprise was how it protects itself by preventing the nanosilver particles from penetrating throw the cell wall. On the other hand, the same biomass with AgNPs had an aggressive antibacterial, fungal, and nematode activity. Another surprising finding was that lactobacilli strains reduced Bio AgNPs toxicity levels compared with the same concentration of the chemically synthesized. We noted These results three times feeding rates with the same levels of bio and chemical-synthesized AgNPs.

EXTENDED ABSTRACT

Novelty

To our knowledge, we are the first study to use whey, the waste product from the dairy industry, as an enriched biosynthesizing medium. Using whey is a novel eco-friendly synthesizing medium and enhanced lactobacilli proliferation compared to the MRS standard medium.

Methodology

- 1- AgNPs tolerable concentrations in whey medium were evaluated as described in [1]
- 2- Characterization of biogenic silver nanoparticles from whey medium
Transmission electron microscope (TEM) studies were performed on a (JEM-2100, JEOL), operating at 200 kV
- 3- Antimicrobial activity
Antibacterial activity was carried out as the agar well diffusion standard method mentioned in [2], [3]. The antifungal activity test was carried out according to CLSI M38 Reference Method, and the antifungal Susceptibility test was carried out [4].

4- Antinematode activity:

Nematode suspension was treated with whey medium containing the bio AgNPs with a final concentration of 4mmol/L for 48h, then light microscopic photos were captured with the lens 10x.

Tables and Figures

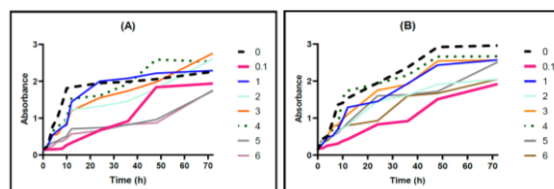


Figure 1 Lactobacilli strain mixture growth rate during bio synthesizing of AgNPs using the concentration of 1- 6mmol/L of AgNO₃ on MRS broth standard medium (A) and whey medium (B)

* Corresponding author

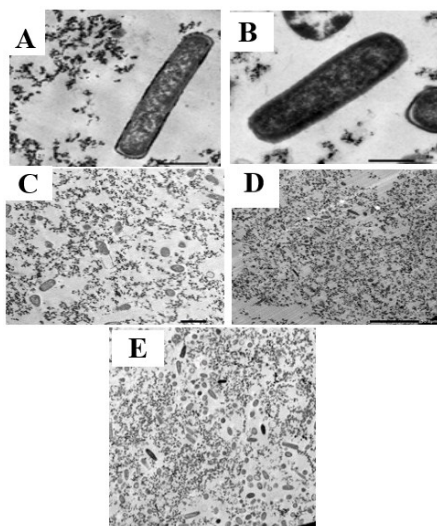


Figure 2 TEM of Lactobacilli strains after bio-synthesizing AgNPs using the concentration of 6mmol/L of AgNO₃ on the whey medium. (A) *Lb delbrueckii* ss *bulgaricus* DSM20080 (B) *Lb acidophilus* DSM 20079 (C) *Lb casei* ss *casei* DSM 20011 (D) *Lb plantarum* ss *plantarum* DSM 20174 (E) *Lb rhamenusus* ATCC 7469

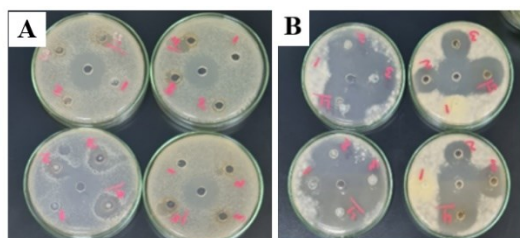


Figure 3 Antibacterial and Antifungal activity after bio AgNPs synthesized on whey medium (Left) and MRS (Right) in different concentrations (0,2,4, and 6mmol/L against (A) *E coli* ATCC 8739 (B) *Aspergillus brasillensis* 16404, respectively

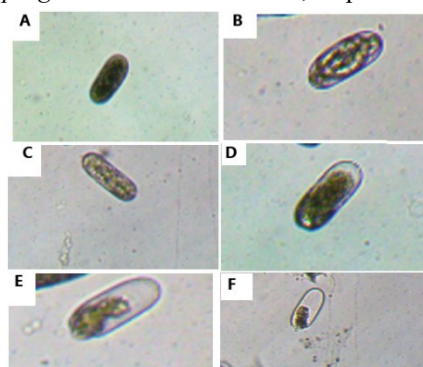


Figure 4(A) Stages of the effect of AgNPs on Egg-hatching of *Meloidogyne javanica* eggs, under laboratory conditions.

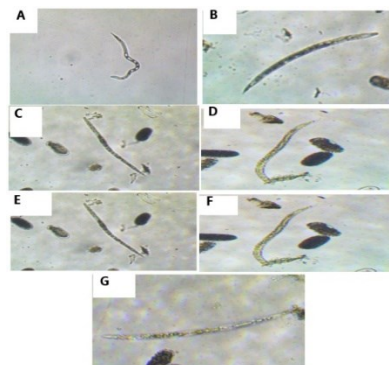


Figure 4(B) Stages of the effect of AgNPs on second-stage juveniles (j2) of *Meloidogyne javanica* under laboratory conditions.

Acknowledgments

The teamwork members express their deep appreciation to Prof Maged El-Kemary, the former president of Kafrelshiekh univ for his support, in every possible way during every step of this work. This project is fully funded by the Academy of Scientific research and Technology, Ministry of Higher Education and Scientific research, Cairo, Egypt.

References

- [1] H. Korbekandi, S. Iravani, and S. Abbasi, "Optimization of biological synthesis of silver nanoparticles using Lactobacillus casei subsp. casei," *Journal of Chemical Technology & Biotechnology*, vol. 87, no. 7, pp. 932–937, Jul. 2012, doi: 10.1002/JCTB.3702.
- [2] R. P. Viorica, P. Pawel, and B. Boguslaw, "Use of Lactobacillus paracasei isolated from whey for silver nanocomposite synthesis: Antiradical and antimicrobial properties against selected pathogens," *J Dairy Sci*, vol. 104, no. 3, pp. 2480–2498, Mar. 2021, doi: 10.3168/JDS.2020-19049.
- [3] FREDERICK KAVANAGH, *ANALYTICAL MICROBIOLOGY*, Vol 2. ACADEMI C PRESS, INC.(LONDON) LTD., 1972.
- [4] "M38 Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi", Accessed: Oct. 12, 2022. [Online]. Available: www.clsi.org.