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Antibacterial Activity of Optimized Extracellular Biosynthesized Zinc Oxide Nanoparticles using *Corynebacterium* sp. ATCC 6931

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Abstract

Nanoparticle (NP) green synthesis is gaining popularity and has been proposed as a potential substitute for chemical and physical processes. The current study reports the environmentally friendly, low-cost method of optimized zinc oxide nanoparticles (ZnO NPs) utilizing a crude metabolite from *Corynebacterium* sp. ATCC 6931. Aliquot pH 8, 35°C, and an 8:2 (v/v) ratio of bacterial supernatant to zinc nitrate solution were the best conditions for the formation of ZnO NPs. Transmission electron microscopy (TEM), UV-visible spectroscopy (UV-vis), X-ray diffraction (XRD), and Zeta potential analyses were used to characterize the biosynthesized NPs. The synthesized ZnO NPs had sizes ranging from 8 to 17 ± 1.23 nm were mainly spherical in form and had a positive charge of ≈ +18.9 mV. The biosynthesized ZnO NPs in the current study have been applied in antimicrobial applications. Using the agar well diffusion method, different concentrations of biosynthesized ZnO NPs (50 µg/mL, 100 µg/mL, and 150 µg/mL) were used to test the antibacterial activity of the characterized NPs against the Gram-positive and Gram-negative bacteria: *Bacillus cereus*, *Corynebacterium* sp., and *Escherichia coli*. Additionally, the minimum inhibitory concentration (MIC) test was applied, and the results showed that bacterial growth reduces as biosynthesized ZnO NPs concentration increases. Also, compared to Gram-positive bacteria, Gram-negative bacteria appeared to be more sensitive to ZnO NPs.

Keywords: Zinc oxide, nanoparticles, biosynthesis, characterization, optimization, antibacterial activity.

Introduction

Nanoscience and nanotechnology have attracted great interest over the last few years due to their potential impact on many scientific areas such as energy, medicine, pharmaceutical industries, electronics, and space industries. This technology deals with small structures and small-sized materials of dimensions in the range of a few nanometers to less than 100 nanometers. Nanoparticles (NPs) show unique and considerably changed chemical, physical, and biological properties compared to the bulk of the same chemical composition, due to their high surface-to-volume ratio. NPs exhibit size and shape-dependent properties which are of

interest for applications ranging from biosensing and catalysts to optics, antimicrobial activity, computer transistors, electrometers, chemical sensors, and wireless electronic logic and memory schemes. These particles also have many applications in different fields such as medical imaging, nanocomposites, filters, drug delivery, and hyperthermia of tumors (Bhattacharya & Gupta, 2005, Goodsell, 2004, Paull et al., 2003, Salata, 2004).

An important area of research in nanoscience deals with the synthesis of nanometer-size particles of different morphologies, sizes, and monodispersed (Sastry et al., 2003). Physical, chemical, biological, and other hybrid processes are used to synthesize the NPs. For the synthesis of monodispersed NPs, both physical and chemical approaches are particularly effective. These techniques are hazardous in one way or another because the chemicals utilized are hazardous, combustible, and difficult to dispose of in the environment (Kowshik et al., 2002). These techniques cause harmful compounds to become adsorbed on the surface of NPs, which could have negative effects on medicinal applications (Jain et al., 2010). Therefore, developing biocompatible, long-lasting, safe, non-toxic, and environmentally friendly processes for the synthesis of NPs that are found in green approaches, including biological processes (Baka & El-Zahed, 2022). Prokaryotes and eukaryotes like bacteria, fungi, and plants are used most frequently in the biosynthesis processes (Saravanan et al., 2021).

Bacteria are considered a potential bio factory for the synthesis of NPs like silver, gold, zinc, and titanium. Zinc Oxide (ZnO) is most of the bacteria used in NPs biosynthesis example *Aeromonas* sp. SH10 (Rai et al., 2006) *Bacillus subtilis* 168 (Beveridge & Murray, 1980; Southam & Beveridge, 1994), *Rhodobacter sphaeroides* (Rai et al., 2006), *Lactobacillus* strains (Prasad et al., 2007) and *Bacillus cereus* strain RNT6 (Ahmed et al., 2021). Bacteria possess a remarkable ability to reduce heavy metal ions and are one of the best candidates for NPs synthesis. For instance, some bacterial species have developed the ability to resort to specific defense mechanisms to quell stresses like the toxicity of heavy metal ions or metals compared to fungi (He et al., 2011), fish (Lin et al., 2011), algae (Lin et al., 2011) and plants (Lin & Xing, 2008). It was observed that some of them could survive and grow even at high metal ion concentrations (e.g., *Pseudomonas aeruginosa*) (Bridges et al., 1979; Haefeli et al., 1984). The scope of zinc oxide nanoparticles (ZnO NPs) has been a keen area of interest for biologists due to their distinguished antimicrobial and distinct activity which has opened new frontiers to biological sciences (Allahverdiyev et al., 2011; Shinde, 2015).

Most members of the genus *Corynebacterium* are aerobic Gram-positive bacteria. They are rod-shaped, and during particular stages of life, they take on a more distinctive shape called a club, which gave the genus its name (coryneform or club-shaped). They are widely dispersed in the animal microbiota, which includes the microbiota of humans, and are generally benign, frequently existing in commensal relationships with their hosts (Collins et al., 2004). Some are useful for commerce and industry, such as *C. glutamicum*. Others can cause human disease, most notably *C. diphtheriae*'s causation of diphtheria, which is most notable. They typically are not harmful, but they can occasionally take advantage of unusual access to tissues (through wounds) or weaker host defenses (Poetsch et al., 2011).

The current study aims to examine some optimization parameters and develop a one-step, safe method for the extracellular biosynthesis of antibacterial ZnO NPs using *Corynebacterium* sp. ATCC 6931.

Materials and Methods

Microbial strains

Bacterial strains: *B. cereus* ATCC 14579, *Corynebacterium* sp. ATCC 6931 and *Escherichia coli* ATCC25922 were obtained from the Botany and Microbiology Department, Faculty of Science, Damietta University, Egypt.

Extracellular biosynthesis of ZnO NPs

Corynebacterium sp. were resubcultured from their slants and then incubated at 37°C on nutrient agar plates. The 24-hour bacterial colonies were cultivated aerobically in nutrient broth flasks and incubated for 24 hours at 37°C and 150 rpm. The bacterial culture was centrifuged for 20 minutes at 4000 rpm to obtain the *Corynebacterium* sp. crude metabolite. An autoclaved aqueous solution of 3 mM zinc nitrate ($ZnNO_3$) was added to the

culture supernatant (1:1 v/v). At a temperature of 37°C and 150 rpm, the reaction mixtures were incubated for 72 hours. The reaction mixtures were screened spectrophotometrically using a UV-VIS spectrophotometer (Beckman DU-40) for detection of ZnO NPs formation.

Optimization of ZnO NPs production

Different incubation times (12-72 hours), temperatures (25-50°C), pH values (4-9) and ratios between bacterial crude metabolites and $ZnNO_3$ solution (10-90%, v/v) were tested and optimized to study the best conditions for ZnO NPs biosynthesis.

Characterization of the optimized ZnO NPs

The X-ray diffractometer (model LabX XRD-6000) was used to study the ZnO NPs X-ray diffraction (XRD) pattern. The size and shape of the ZnO NPs were examined using a transmission electron microscope (TEM). Malvern Zetasizer Nano-ZS90 was used to investigate ZnO NPs' zeta potential.

Antibacterial activity of optimized ZnO NPs

The agar well diffusion test was used to investigate the antibacterial activity of ZnO NPs at concentrations of 50, 100, and 150 µg/mL in dimethylformamide (DMF) (CLSI, 2006). The antibacterial activity against Gram-positive and Gram-negative bacteria, *B. cereus*, *Corynebacterium* sp. and *E. coli*, was assessed on Mueller-Hinton agar (MHA) plates. Separate inoculum of each strain (0.5 McFarland, 100 µL) was inoculated into the flasks. ZnO NPs and Penicillin G (as standard drug) colloids (100 µL) were added aseptically to wells (5 mm). For 24 hours, bacterial agar plates were incubated at 37°C. After the incubation times, the mm-sized inhibition zones were measured.

Minimum inhibition concentration (MIC)

Using the broth microdilution technique, the minimum inhibitory concentration of ZnO NPs against the bacterial strains was investigated in accordance with the CLSI protocols (CLSI, 2017). Serial solutions of Penicillin G and ZnO NPs (5-125 µg/mL in water) were investigated. The Mueller-Hinton broth (MHB) was inoculated with approximately 0.5 McFarland standard of bacteria and incubated at 150 rpm, and 37°C. The MIC values were calculated using spectrophotometric measurements of the bacterial growth rates at 600 nm.

Minimal microbicidal concentration (MBC)

According to Stratton et al. (1982), MBC is the dilution that completely prevents the growth of microbial colonies on agar plates. The tested dilutions that completely inhibited the development of the tested microorganisms in the MIC experiment (no apparent microbial growth) were subcultured and injected into solid media in order to determine the least bactericidal concentration. The agar plates were incubated for an entire day at 37°C.

Statistical analysis

The data were statistically assessed using the computer SPSS version 18. All values in the experiments were examined using a one-way analysis of variance (ANOVA) with 0.05 set as the significant level.

Results

Optimization and characterization of ZnO NPs

Corynebacterium sp. had the ability for extracellular biosynthesize ZnO NPs within 12 hours in dark conditions. Different parameters such as incubation periods, pH value, temperature, and mixing ratio between bacterial supernatant and $ZnNO_3$ were optimized to obtain the best conditions for ZnO NPs biosynthesis. The initial sign of the formation of NPs was the emergence of a white precipitate, whose production rate increased as the incubation period lengthened as shown in Figure 1.

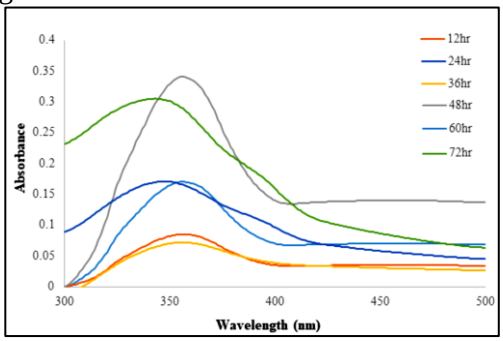

Figure 1. *The UV-VIS spectra of the reaction mixture containing ZnO NPs during different incubation times.*

Figure 2 confirmed that the biosynthesis of ZnO NPs increased with the increase of temperature until 35°C then decreased.

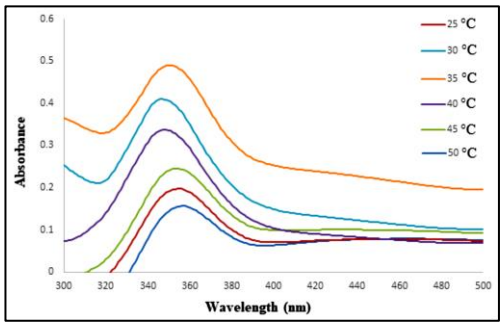

Figure 2. The UV-VIS spectra of the reaction mixture containing ZnO NPs during different temperatures.

The pH value was tested to optimize the ZnO NPs production (Figure 3). pH 8 was the best pH value for the NPs formation.

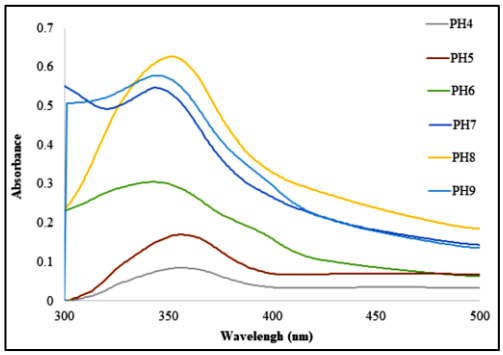

Figure 3. The UV-VIS spectra of the reaction mixture containing ZnO NPs during different pH values.

Different ratios between bacterial supernatant and $ZnNO_3$ solution (v/v%) were tested for the best condition for ZnO NPs production as shown in Figure 4.

The optimized ZnO NPs were characterized by TEM and the results confirmed the formation of spherical-shaped NPs with an average size of ≈ 8 to 17 ± 1.23 nm (Figure 5A). Zeta analysis showed that the biosynthesized ZnO NPs had a positive charge of ≈ +18.9 mV (Figure 5B). The XRD pattern for ZnO NPs (Figure 5C) displayed eight ZnO NPs characteristic peaks at 32°, 37.8°, 46.2°, 48.1°, 57.5°, 63.55°, and 68°, corresponding to respective crystal planes (100), (101), (102), (102), (110), (110), (103), and (112) (Galvez *et al*., 2021).

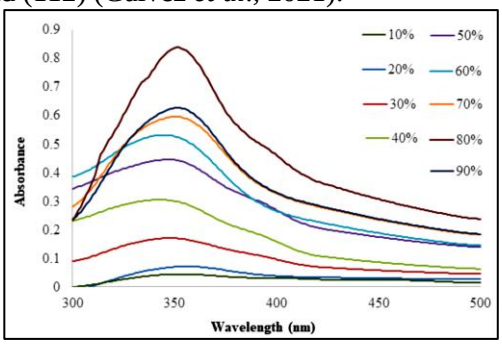

Figure 4. The UV-VIS spectra of the reaction mixture containing ZnO NPs during different volume ratios between bacterial supernatant and $ZnNO_3$ solution (v/v%).

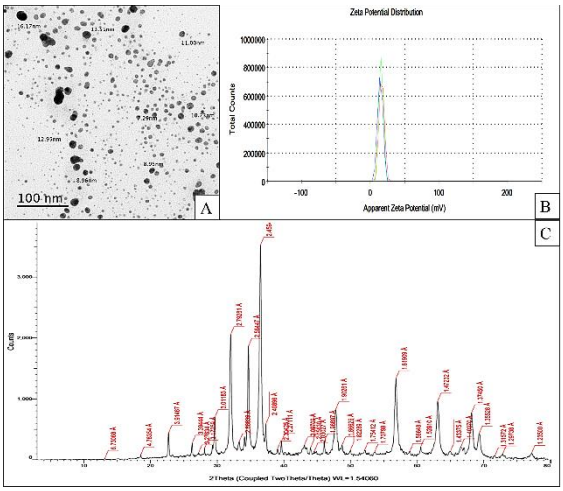

Figure 5. (A) TEM micrographs of ZnO NPs (scale bar = 100 nm). (B) Zeta potential result of ZnO NPs. (C) The XRD pattern of ZnO NPs.

Antibacterial activity of optimized ZnO NPs

The optimized ZnO NPs revealed good antibacterial activity during the antimicrobial tests (Tables 1 and Figure 6). ZnO NPs showed higher antibacterial activity against Gram-negative bacteria than Gram-positive bacteria.

Table 1. Antibacterial activity of ZnO NPs in comparison with Penicillin G (Highly significant = *p<0.05; n = 3).

Antibacterial agents	Concentration, µg/mL	Zone of inhibition (mm, mean ± SD)		
		Gram-positive bacteria		Gram-negative bacterium
		B. cereus	***Corynebacterium* sp.**	***E. coli***
ZnO NPs	50	16 ± 0.04*	12 ± 0.03*	14 ± 0.06*
	100	21 ± 0.06*	14 ± 0.10*	19 ± 0.05*
	150	25 ± 0.13*	18 ± 0.21*	23 ± 0.11*
Penicillin G	50	11 ± 0.14*	8 ± 0.14*	16 ± 0.12*
	100	14 ± 0.11*	11 ± 0.18*	21 ± 0.16*
	150	16 ± 0.02*	14 ± 0.03*	24 ± 0.01*

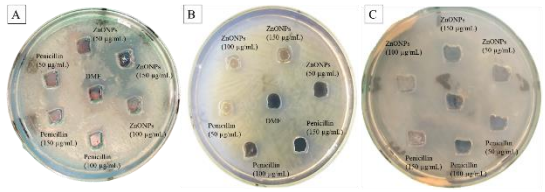

Figure 6. Antibacterial activity of ZnO NPs compared to Penicillin G as standard drug against pathogenic bacterial strains: (A); *B. cereus*, (B); *Corynebacterium* sp. and (C); *E. coli*.

The ZnO NPs MIC findings are shown in Figure 7A. The most effective concentration to inhibit *B. cereus* and *E. coli* growth was 20 µg/mL of ZnO NPs while it was 40 µg/mL for complete inhibition of *Corynebacterium* sp. Figure 7B displays the MBC results which confirmed the potent antibacterial action of the optimized biosynthesized ZnO NPs.

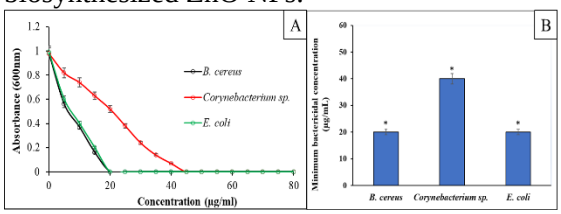

Figure 7. (A) Minimal inhibition concentration of ZnO NPs. (B) Minimal microbicidal concentration of ZnO NPs.

Discussion

A promising method for the synthesis of NPs is the generation of nanoscale materials by microbial cells. Potential biofactories for the production of zinc, gold, silver, and cadmium sulfide nanoparticles are thought to be microbial cells. It is known that bacteria can produce inorganic compounds either intracellularly or extracellularly. Zinc ions are reduced to nanoscale range by extracellular reductase enzymes made by microorganisms. A protein assay of microorganisms demonstrates that the enzyme NADH-dependent reductase is involved in the bioreduction of zinc ions to zinc nanoparticles (Hulkoti & Taranath, 2014).

Catalase-positive Gram-positive bacilli, often known as "diphtheroids" or "coryneform" bacteria, were historically almost always brushed off as contaminants when they were collected from patients, but they are now more frequently being linked to serious diseases. These taxa were poorly documented and were taxonomically illogical. Rarely were the pathogenic mechanisms examined, particularly for newly discovered taxa. Data on antibiotic susceptibility were limited and their resistance were reported (Bernard, 2012). The current study aimed to biosynthesize ZnO NPs using the crude metabolite of *Corynebacterium* sp. and then use it as strong antibacterial agent against itself and other bacterial strains.

Within 12 hours in the dark, *Corynebacterium* sp. was able to extracellularly biosynthesize ZnO NPs and the production rate increased with increasing in the reaction time. To provide the optimum conditions for ZnO NPs production, several factors including incubation times, pH levels, temperatures, and mixing ratios between bacterial supernatant and $ZnNO_3$ were tested. This production also increased by increasing the temperature until 35°C then decreased. This depletion in the biosynthesis rate at high temperatures might be due to the denaturation of bacterial proteins that are responsible for the biosynthesis process (Mohd-Yusof et al., 2019). The presence of proteins and carbohydrates during the biogenic formation of ZnO NPs was reported during different bacterial syntheses in several studies (El-Nour et al., 2023, Mohd-Yusof et al., 2019; Mohd-Yusof et al., 2020). In addition, the NPs formation increased with the increase of bacterial supernatant. On the other hand, the peak of ZnO NPs was shifted into long wavelengths indicating to formation of less stable and larger NPs (El-Dein et al., 2021; Fayed et al., 2023).

One of the most crucial characteristics of their employment in pharmaceutical and medical applications is NPs' stability. Some ideas contend that the stability of NPs depends critically on their form, size, functional bacterial groups, and surface charge (Bian et al., 2011). The optimized ZnO NPs were spherical shaped, positively charged NPs with small, scaled size. According to Singh et al. (2021) this zeta potential value often exhibits a high level of stability. The XRD pattern for ZnO NPs confirming the formation of ZnO NPs with good stability and crystallin nature (Galvez *et al*., 2021).

During the antimicrobial tests, the optimized ZnO NPs showed good antibacterial activity. Gram-negative bacteria were more resistant to ZnO NPs' antibacterial effects than Gram-positive bacteria. This difference effect might be due to the difference of cell wall structure between both types of bacteria. Gram-positive bacteria contain a thick layer of peptidoglycan which has an acidic nature and strongly binds

with the positively charged ZnO NPs in contrast to Gram-negative bacteria that have thin layers and low amounts of peptidoglycan (El-Zahed *et al*., 2022; Sirelkhatim *et al*., 2015). Moreover, Gram-negative bacteria have an outer lipid layer that may make them resistant to the NPs in contrast to Gram-positive bacteria.

The MIC is the antimicrobial agent concentration below which no measurable bacterial growth is observed. ZnO NPs strong antibacterial action against *B. cereus* followed by *E. coli* while exhibiting moderate activity against the Gram-positive bacterium *Corynebacterium* sp. The antibacterial activity of *B. subtilis* ZBP4 ZnO NPs against several food-pathogenic Gram-positive and Gram-negative bacteria was reported by Hamk et al. in 2022. Additionally, Rajabairavi et al. (2017) validated the antibacterial activity of ZnO NPs against *Enterobacter aerogens* and *Pseudomonas aeruginosa* by biosynthesizing them using *Sphingobacterium thalpophilum*. Mohd-Yusof (2020) reported bacterial suppression of *L. plantarum* ZnO NPs at doses of 2500 µg/ml for *E. coli* and 1250 µg/ml for *S. aureus*, which contrasts with the obtained results.

The exact antibacterial mechanism of ZnO NPs is still unknown, but some reported hypotheses suggested that ZnO NPs may interact with cellular proteins in cell membranes, attacking respiration and cell division, and ultimately killing cells. In addition, the bactericidal effects of ZnO NPs were reported in various studies including formation of reactive oxygen species, cell wall damage, and injuries in membrane permeability (El-Zahed *et al*., 2022; Fayed et al., 2023; Sirelkhatim *et al*., 2015). Further research may help to clarify the antibacterial mechanism of ZnO NPs. But in general, the biosynthesized ZnO NPs could be utilized as pharmaceutical drugs with high antibacterial efficiency.

Conclusion

The crude metabolite of *Corynebacterium* sp., which serves as an efficient reducing and stabilizing agent, is used in the present work to disclose a green, environmentally friendly, and inexpensive method for the biosynthesis of ZnO NPs. The biosynthesized ZnO NPs had a positive charge of +18.9 mV, were spherical, and ranged in size from 8 to 17 ± 1.23 nm. The synthesized ZnO NPs effectively inhibited *B. cereus*, *Corynebacterium* sp., and *E. coli*. Both Gram-positive and Gram- negative bacteria were greatly negatively affected depending on the concentration of biosynthesized ZnO NPs. We can therefore draw the conclusion that the disclosed biologically synthesized ZnO NPs can function as a strong antibacterial agent.

References

Ahmed T, Wu Z, Jiang H, Luo J, Noman M, Shahid M, Manzoor I, Allemailem KS, Alrumaihi F, Li B (2021) Bioinspired green synthesis of zinc oxide nanoparticles from a native Bacillus cereus strain RNT6: characterization and antibacterial activity against rice panicle blight pathogens Burkholderia glumae and B. gladioli. Nanomaterials, 11: 884. https://doi.org/10.3390/nano11040884

Allahverdiyev, A. M., Kon, K. V., Abamor, E. S., Bagirova, M., & Rafailovich, M. (2011). Coping with antibiotic resistance: combining nanoparticles with antibiotics and other antimicrobial agents. Expert Review of Anti-Infective Therapy, 9(11), 1035–1052. https://doi.org/10.1586/eri.11.121

Baka ZA, El-Zahed MM (2022) Antifungal activity of silver/silicon dioxide nanocomposite on the response of faba bean plants (Vicia faba L.) infected by Botrytis cinerea. Bioresources and Bioprocessing, 9: 1-19. https://doi.org/10.1186/s40643-022-00591-7

Beveridge, T. J., & Murray, R. G. (1980). Sites of metal deposition in the cell wall of Bacillus subtilis. Journal of Bacteriology, 141(2), 876–887. https://doi.org/10.1128/jb.141.2.876-887.1980

Bhattacharya, D., & Gupta, R. K. (2005). Nanotechnology and potential of microorganisms. Critical Reviews in Biotechnology, 25(4), 199–204. https://doi.org/10.1080/07388550500361994

Bian S-W, Mudunkotuwa IA, Rupasinghe T, Grassian VH (2011) Aggregation and dissolution of 4 nm ZnO nanoparticles in aqueous environments: influence of pH, ionic strength, size, and adsorption of humic acid. Langmuir, 27: 6059–6068. https://doi.org/10.1021/la200570n

Bridges, K., Kidson, A., Lowbury, E. J., & Wilkins, M. D. (1979). Gentamicin-and silver-resistant pseudomonas in a burns unit. British Medical Journal 1(6161), 446–449. https://doi.org/10.1136/bmj.1.6161.446

Clinical and Laboratory Standards document M2-A9 (2006) Performance standards for antimicrobial

disk susceptibility tests: Approved standard-Ninth Edition. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA.

Clinical and Laboratory Standards document M100-S26 (2017) Performance standards for antimicrobial susceptibility testing: Approved standard-twenty-seven Edition, Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA.

Collins, M. D., Hoyles, L., Foster, G., & Falsen, E. (2004). Corynebacterium caspium sp. nov., from a Caspian seal (Phoca caspica). International Journal of Systematic and Evolutionary Microbiology, 54(3), 925-928. https://doi.org/10.1099/ijs.0.02950-0

El-Dein MMN, Baka ZA, Abou-Dobara MI, El-Sayed AK, El-Zahed M M (2021) Extracellular biosynthesis, optimization, characterization and antimicrobial potential of Escherichia coli D8 silver nanoparticles. Journal of Microbiology, Biotechnology and Food Sciences, 10: 648-656. https://doi.org/10.15414/jmbfs.2021.10.4.648-656

El-Nour, A., Amira, T., Abou-Dobara, M. I., El-Sayed, A. K., & El-Zahed, M. M. (2023). Extracellular biosynthesis and antimicrobial activity of Bacillus subtilis ATCC 6633 zinc oxide nanoparticles. Scientific Journal for Damietta Faculty of Science, 13(2), 39-47. https://doi.org/10.21608/sjdfs.2023.173178.1064

El-Zahed, M. M., Abou-Dobara, M. I. ., El-Sayed, A. K., & Baka, Z. A. M. (2022). Ag/SiO2 nanocomposite mediated by Escherichia coli D8 and their antimicrobial potential. Nova Biotechnologica et chimica, 21(1), e1023. https://doi.org/10.36547/nbc.1023

Fayed, R., Elnemr, A. M., & El-Zahed, M. M. (2023). Synthesis, characterization, antimicrobial and electrochemical studies of biosynthesized zinc oxide nanoparticles using the probiotic Bacillus coagulans (ATCC 7050). Journal of Microbiology, Biotechnology and Food Sciences, e9962. https://doi.org/10.55251/jmbfs.9962

Galvez, A. M., Ramos, K. M., Teja, A. J., & Baculi, R. (2021). Bacterial exopolysaccharide-mediated synthesis of silver nanoparticles and their application on bacterial biofilms. Journal of Microbiology, Biotechnology and Food Sciences, 2021, 970–978. https://doi.org/10.15414/jmbfs.2019.8.4.970-978

Goodsell, D. S. (2004). Bionanotechnology: lessons from nature. John Wiley & Sons.

Haefeli, C., Franklin, C., & Hardy, K. (1984). Plasmid-determined silver resistance in Pseudomonas stutzeri isolated from a silver mine. Journal of Bacteriology, 158(1), 389–392. https://doi.org/10.1128/jb.158.1.389-392.1984

Hamk, M., Akçay, F. A., & Avcı, A. (2022). Green synthesis of zinc oxide nanoparticles using Bacillus subtilis ZBP4 and their antibacterial potential against foodborne pathogens. Preparative Biochemistry & Biotechnology, 1-10. https://doi.org/10.1080/10826068.2022.2076243

He, L., Liu, Y., Mustapha, A., & Lin, M. (2011). Antifungal activity of zinc oxide nanoparticles against Botrytis cinerea and Penicillium expansum. Microbiological Research, 166(3), 207–215. https://doi.org/10.1016/j.micres.2010.03.003

Jain, D., Kachhwaha, S., Jain, R., Srivastava, G., & Kothari, S. L. (2010). Novel microbial route to synthesize silver nanoparticles using spore crystal mixture of Bacillus thuringiensis. Indian Journal of Experimental Biology 48(11), 1152-1156. PMID: 21117457

Kowshik, M., Ashtaputre, S., Kharrazi, S., Vogel, W., Urban, J., Kulkarni, S. K., & Paknikar, K. M. (2002). Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3. Nanotechnology, 14(1), 95. https://doi.org/10.1088/0957-4484/14/1/321

Lin, D., & Xing, B. (2008). Root uptake and phytotoxicity of ZnO nanoparticles. Environmental Science & Technology, 42(15), 5580–5585. https://doi.org/10.1021/es800422x

Lin, S., Zhao, Y., Xia, T., Meng, H., Ji, Z., Liu, R., George, S., Xiong, S., Wang, X., & Zhang, H. (2011). High content screening in zebrafish speeds up hazard ranking of transition metal oxide nanoparticles. ACS Nano, 5(9), 7284–7295. https://doi.org/10.1021/nn202116p

Mohd-Yusof, H., Mohamad, R., Zaidan, U. H., & Abdul Rahman, N. A. (2019). Microbial synthesis of zinc oxide nanoparticles and their potential application as an antimicrobial agent and a feed supplement in animal industry: a review. Journal of Animal Science and Biotechnology, 10, 1-22. https://doi.org/10.1186/s40104-019-0368-z

Mohd-Yusof, H., Rahman, A., Mohamad, R., Zaidan, U. H., & Samsudin, A. A. (2020). Biosynthesis of zinc oxide nanoparticles by cell-biomass and supernatant of Lactobacillus plantarum TA4 and its antibacterial and biocompatibility properties. Scientific Reports, 10(1), 1-13. https://doi.org/10.1038/s41598-020-76402-w

Paull, R., Wolfe, J., Hébert, P., & Sinkula, M. (2003). Investing in nanotechnology. Nature Biotechnology, 21(10), 1144–1147. https://doi.org/10.1038/nbt1003-1144

Poetsch, A., Haußmann, U., & Burkovski, A. (2011).

Proteomics of corynebacteria: From biotechnology workhorses to pathogens. Proteomics, 11(15), 3244-3255. https://doi.org/10.1002/pmic.201000786

Prasad, K., Jha, A. K., & Kulkarni, A. R. (2007). Lactobacillus assisted synthesis of titanium nanoparticles. Nanoscale Research Letters, 2(5), 248–250. https://doi.org/10.1007/s11671-007-9060-x

Rai, A., Singh, A., Ahmad, A., & Sastry, M. (2006). Role of halide ions and temperature on the morphology of biologically synthesized gold nanotriangles. Langmuir, 22(2), 736–741. https://doi.org/10.1021/la052055q

Rajabairavi, N., Raju, C. S., Karthikeyan, C., Varutharaju, K., Nethaji, S., Hameed, A. S. H., & Shajahan, A. (2017). Biosynthesis of novel zinc oxide nanoparticles (ZnO NPs) using endophytic bacteria Sphingobacterium thalpophilum. In: Ebenezar, J. (eds) Recent Trends in Materials Science and Applications. Springer Proceedings in Physics, vol 189. Springer, Cham. https://doi.org/10.1007/978-3-319-44890-9_23

Salata, O. V. (2004). Applications of nanoparticles in biology and medicine. Journal of Nanobiotechnology, 2(1), 1–6. https://doi.org/10.1186/1477-3155-2-3

Saravanan, A., Kumar, P. S., Karishma, S., Vo, D. V. N., Jeevanantham, S., Yaashikaa, P. R., & George, C. S. (2021). A review on biosynthesis of metal nanoparticles and its environmental applications. Chemosphere, 264, 128580. https://doi.org/10.1016/j.chemosphere.2020.128580

Sastry, M., Ahmad, A., Khan, M. I., & Kumar, R. (2003). Biosynthesis of metal nanoparticles using fungi and actinomycete. Current Science, 162–170. https://doi.org/10.1186/1477-3155-2-3

Shinde, S. S. (2015). Antimicrobial activity of ZnO nanoparticles against pathogenic bacteria and fungi. Science Medical Central, 3, 1033.

Singh, A., Madhavi, B. L. R., & Sagar, M. N. N. (2021). An overview of green synthesis mediated metal nanoparticles preparation and its scale up opportunities. Journal of Drug Delivery and Therapeutics, 11(6), 304–314. https://doi.org/10.22270/jddt.v11i6.5082

Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N. H. M., Ann, L. C., Bakhori, S. K. M., ... & Mohamad, D. (2015). Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. Nano-micro Letters, 7(3), 219-242. https://doi.org/10.1007/s40820-015-0040-x

Southam, G., & Beveridge, T. J. (1994). The in vitro formation of placer gold by bacteria. Geochimica et Cosmochimica Acta, 58(20), 4527–4530. https://doi.org/10.1016/0016-7037(94)90355-7

Stratton, C. W., Weinstein, M. P., & Reller, L. B. (1982). Correlation of serum bactericidal activity with antimicrobial agent level and minimal bactericidal concentration. Journal of Infectious Diseases, 145(2), 160-168. https://doi.org/10.1093/infdis/145.2.160 .

الملخص العربي

عنوان البحث: النشاط المضاد للبكتيريا للجسيمات النانومترية المحسنة لأكسيد الزنك والمُصنّعة حيويًا خارج الخلية باستخدام بكتيريا كورينباكتريوم

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احتل التصنيع الأخضر للجسيمات النانومترية شعبية كما تم اقتراحه كبديل لعمليات التصنيع الكيميائية والفيزيائية. تشير الدراسة الحالية إلى طريقة صديقة للبيئة ومنخفضة التكلفة لجسيمات أكسيد الزنك النانوية المحسنة باستخدام المستخلص البكتيري الخام لبكتيريا كورينباكتريوم. يعد الرقم الهيدروجيني ٨، ٣٥ درجة مئوية، ونسبة ٨: ٢ من حجم مستخلص البكتيريا إلى حجم محلول نترات الزنك هي الظروف المثلى لصنيع جسيمات الزنك النانومترية. تم استخدام المجهر الإلكتروني النافذ، التحليل الطيفي للأشعة فوق البنفسجية والمرئية، حيود الأشعة السينية وتحليل زيتا لتوصيف جسيمات النانو المُصنّعة حيويًا. تتراوح أحجام جسيمات الزنك النانومترية المُصنَّعة من ٨ إلى ١٧ ± ١,٢٣ نانومتر، وهي جسيمات كروية الشكل ذات شحنة موجبة ≈ +١٨,٩ مللي فولت. تم تطبيق جسيمات الزنك النانومترية المُصنّعة حيويًا في الدراسة الحالية في التطبيقات المضادة للميكروبات من خلال اختبار ثلاث تركيزات مختلفة (٥٠، ١٠٠ و١٥٠ ميكروجرام/مل) ضد بكتيريا موجبة وسالبة لصبغة جرام مثل بكتيريا باسيلس سيريس، بكتيريا كورينباكتريوم، وبكتيريا إشريكية كولاي. بالإضافة إلى ذلك، تم تطبيق اختبار التركيز الأدنى المثبط لنمو البكتيريا، وأظهرت النتائج أن نمو البكتيريا ينخفض مع زيادة تركيز جسيمات الزنك النانومترية المصنعة حيويا. أيضًا، بالمقارنة مع البكتيريا الموجبة لصبغة جرام، كانت البكتيريا السالبة لصبغة جرام أكثر استجابة وتأثرا بجسيمات الزنك النانومترية.