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Biosynthesis of Silver nanoparticles using *Trichosporon asahii* and study their antibacterial and synergism effects

Alaa M. Madhloom[1]
Mohammed M. Alrufae[2]

[1-] Biology Teacher, Arid Taws Evening School, Kufa Directorate of Education, Iraqi
[2-] Department of Biology, Faculty of Scinece, University of Kufa, Iraq
E. mail : mohammedm.alrufaie@uokufa.edu.iq

Abstract

Nanoparticles (NPs) often have strong antibacterial properties to treat a variety of infections, but their high biotoxicity prevents them from being used directly. The biosynthesis of NPs, as well as their capping/conjugation with natural biopolymers, can improve NPs stability and reduce toxicity. Without using any additional chemical processes, *Trichosporon asahii* was used to directly synthesize silver nanoparticles (AgNPs) by extracellular mechanism. Physical and chemical evaluations such as (solution color change, and UV spectrophotometer), validated the formation of nanoparticles. The AgNPs had similar powerful bactericidal effects against Gram positive (*Staphylococcus aureus*, *Enterococcus Faecalis*, *Bacillus cereus*) and Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*). The goal of this study is to evaluate the efficiency of silver nanoparticles antibacterial activity which produced by *Trichosporon asahii* and use as alternatives to antibiotics.

Keywords: silver nanoparticles, biosynthesis, antibacterial.

Introduction

Silver nanoparticles (AgNP) are one of the most commonly employed nanomaterials in medicinal, antibacterial, and electrical applications (Bartlomiejczyk *et al*., 2013; González-Jiménez and Garcìa, 2020). Despite their long and widespread usage, the mechanism *behind* silver ions' antimicrobial effects remains unknown (Barras *et al*., 2018).

Fungi have a great deal of potential for producing a wide range of chemicals that may be employed in a variety of applications. Microscopic filamentous fungus (ascomycetes and imperfect fungi) and other fungal species are known to generate over 6,400 bioactive compounds (Berdy *et al*., 2005).

Due to their heavy metal tolerance and ability to internalize and bioaccumulate metals, these organisms are commonly utilized as reducing and stabilizing agents. Furthermore, fungi may also be easily grown on a big scale "nanofactories" and create nanoparticles with regulated size and form (Azmath *et al*., 2016; Khan *et al*., 2017).

These nanoparticles are mixed with biomolecules derived from the organism that was used in the synthesis, which can help with stability and may also provide biological activity (Ballottin *et al*., 2016). Biogenic synthesis is simple, clean, long-lasting, and cost-effective, and it improves biocompatibility in nanoparticle applications (Gholami-Shabani *et al*., 2014).

To use fungi to make silver nanoparticles, the fungus must first be cultured on agar before being transferred to a liquid media. The biomass is then transferred to water, where the molecules involved in the synthesis are released. The biomass is

discarded after filtration, and silver nitrate is added to the filtrate (Silva *et al*., 2017; Mekkawy *et al*., 2017).

Given the issues that pathogenic microbes create, researchers are always looking for new ways to combat them. With the advent of nanotechnology, there has been a surge in interest in silver nanoparticles' antimicrobial properties, as well as ways to use them in environmentally friendly ways (Guilger *et al*., 2017; Ottoni *et al*., 2017).

Materials and methods

Biosynthesis of silver nanoparticles

Trichosporon asahii was obtained from a previous study included (Ali and Mohammed, 2021), then grown in potato dextrose broth at 28°C for 48h. Then Suspension separated by filtration. Extracellular synthesis of nanoparticles mechanisms was used by add 0.08 $AgNo_3$ (Bayville, USA) to the filtrate in dark condition and incubate at 28°C,150 rpm for 5 days in shaking incubator (Genex, USA). After incubation period the broth is placed in bottles in a centrifuge (Hettich, Germany) and washed three times 4500 cycles for 30 minutes, then dried in an oven and kept until use.

The mixture was tested for brown color after incubation, which indicated the creation of AgNPs. UV-visible spectrophotometric analysis was used to examine the formation of silver nanoparticles.

UV–visible spectroscopy (Shimadzu, UV-1900i) was used to monitor the creation of silver nanoparticles by recording spectra between 300 and 800 nm. The silver nitrate cell biomass at time zero was used as a control.

Antibacterial activity of antibiotic and silver nanoparticles

Antibacterial activity of silver nanoparticles made from *Trichosporon asahii* was tested against Gram positive bacteria (*Staphylococcus aureus*, *Enterococcus Faecalis*, *Bacillus cereus*) and Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia***)**.

Each bacteria tested firstly with eight antibiotic agents (Bioanalyse/UK) include; Nitrofurantoin (300 mcg), Imipenem (10 mcg), Cephalexin (30 mcg), Trimethoprim (5 mcg), Gentamicin (10 mcg), Pencillin G (10 U), Tetracycline (10 µg), Ciprofloxacin (5 µg), by disc diffusion method on Mueller–Hinton agar plates and Clinical Laboratory Standards Institute (CLSI) standards for fastidious bacteria (CLSI, 2018) and CLSI criteria for Enterobacteriaceae (CLSI, 2019), were used to record and interpret the results.

The same bacteria were tested with silver nanoparticles with three different concentrations 50µl, 100µl and 200µl by cork borer method on agar plate with added 200µl of each concentration to three well on same plate in order to know the effect of these concentrations on bacterial growth by measuring the inhibition zone.

Synergism of AgNPs with Antibiotics Test

For synergism of AgNPs with antibiotics test, two types of bacteria were selected for this examination, namely (*E. coli* and *B. cereus*) for two types of antibiotics that are resistant to the two types of bacteria mentioned; Tetracycline

(TE10 µg) and Cephalexin (CL30 mcg), by adding of 20µl AgNPs in concentration 100µg/ml to each antibiotics for each bacteria, then incubated, and measured inhibition zone diameter.

Results

Silver nanoparticles formation

Trichosporon asahii generated silver nanoparticles from silver nitrate. Within 2–4 hours, the reaction began, and the color of the solution changed to a yellowish brown, suggesting that *T. asahii* had produced silver nanoparticles. The differences in color between before and after adding $AgNO_3$ and incubation period of *T. asahii* showed in Figure (1)

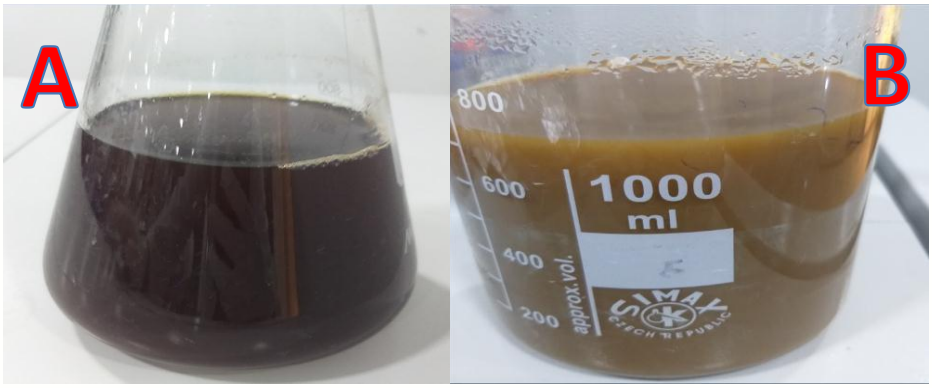

Figure (1): A-The cell free supernatant before adding of $AgNO_3$.
B- The color change after incubation for 24 hours , with 10 mM concentration of $AgNO_3$.

Characteristics of silver nanoparticles

The creation of silver nanoparticles by *T. asahii* was investigated further using UV–visible spectroscopy, which revealed surface plasmon resonance bands. *T. asahii* demonstrated that silver nanoparticles have a peak between 300 and 800 nm and contribute to the absorption bands at 430 nm in the UV–visible spectra. The UV–Vis spectra of aqueous silver nitrate solution (control) did not reveal any at 300–800 nm when monitored separately. Figure (2)

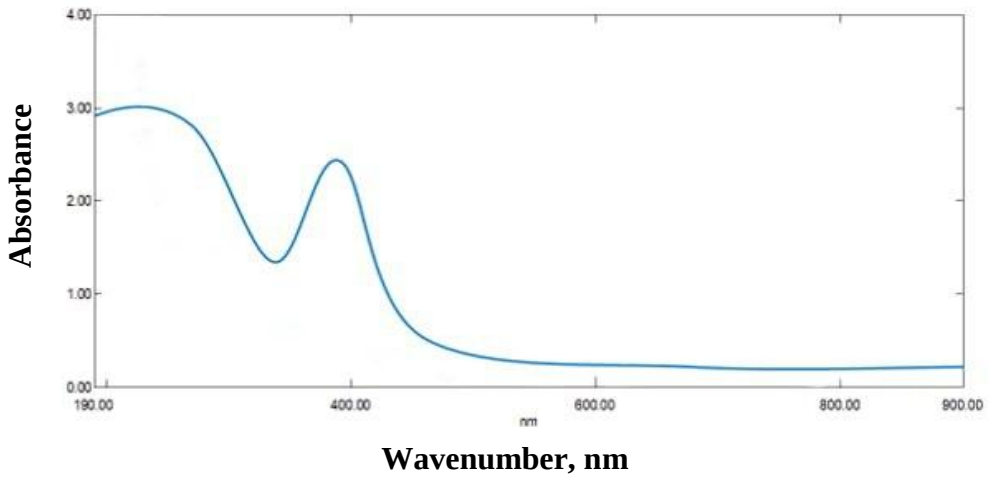

Figure (2): The absorption bands of *T. asahii* at 430 nm in the UV–visible spectra.

Antibacterial activity of antibiotic

The results recorded as resistance, intermediate, and sensitive according to CLSI, intermediate results considered as sensitive in the current time. All bacteria isolates were multi drug resistance, that showed resistance to three or more different antimicrobial classes and all isolates 6/6 (100%) showed complete resistance to Pencillin G, Cephalexin, and Nitrofurantoin.

Also *B. cereus*, *E. coli*, *E. Faecalis*, *P. aeruginosa* showed complete resistance toward Trimethoprim except *K. pneumonia* and *S. aureus* were sensitive to it. However all isolates showed complete susceptibility against Imipenem, and the highest value of the inhibition zone was 35 against *E. Faecalis* bacteria. While all isolates except *E. coli* and *S. aureus* were sensitive to Gentamicin.

B. cereus, *E. coli*, *K. pneumonia* and *P. aeruginosa* were resistance to Tetracycline but *E. Faecalis* and *S. aureus* were sensitive to it. Finally, *E. coli* and *E. Faecalis* were resistance to Ciprofloxacin among other bacteria tested. as showed in the Table (1)

Table (1): Bacteria isolates with their susceptibility results against antibiotic agents

Antibiotic / Bacteria	P	TMP	CL	CN	TE	IMP	F	CIP
B. cereus	0	0	0	20	0	26	13	25
E. coli	0	0	0	10	0	24	16	12
E. Faecalis	0	0	0	16	22	35	0	0
K. pneumonia	7	20	0	16	8	20	13	22
P. aeruginosa	7	0	0	20	0	28	0	17
S. aureus	0	16	0	0	17	18	14	26

P= Pencillin, TMP=Trimethoprim, CL= Cephalexin, CN=Gentamicin, TE= Tetracycline, IMP=Imipenem, F=Nitrofurantoin, CIP= Ciprofloxacin

Antibacterial activity of silver nanoparticles

Gram negative and Gram positive bacteria were effectively suppressed by the silver nanoparticles that were generated. Our findings showed that biosynthesized silver nanoparticles exhibited higher antibacterial activity against all bacteria were tested in all three concentration, Whereas, the highest inhibition zone (23) was at a concentration of 200 µg/ml against *P. aeruginosa*, as well as (13) at a concentration of 50 µg/ml against both *B. cereus* and *E. Faecalis* bacteria. as showed in the Table (2).

Table (2): Antibacterial activity of silver nanoparticles

Bacteria	Inhibition zone of AgNPs concentration		
	200 µg/ml	**100** µg/ml	**50** µg/ml
B. cereus	20	19	13
E. coli	20	19	15
E. Faecalis	18	14	13
K. pneumonia	21	20	16
P. aeruginosa	23	22	14
S. aureus	20	19	14

Synergism of AgNPs with Antibiotics Test

Interestingly, the AgNPs was observed to effective with two types of bacteria *E. coli* and *B. cereus* for two types of antibiotics that were resistant; Tetracycline (TE10 µg) and Cephalexin (CL30 mcg), However, the synergy did not give a good result compared to nanoparticles if used alone. Table (3).

Table (3): Synergism of AgNPs with Antibiotic

Bacteria / Synergism	*B. cereus*	*E. coli*
AgNPs 100µg/ml + TE10 µg	10	9
AgNPs100 µg/ml + CL30 mcg	9	9

Discussion

All natural silver has been regarded as a nontoxic, harmless inorganic antibacterial agent. Many bacteria, yeasts, fungus, and algae have been found to produce silver nanoparticles outside of their cells (Rauwel *et al*., 2015). Several previous studies recorded fungi are used to demonstrate the extracellular synthesis of silver nanoparticles (Rahimi *et al*., 2016; Jalal *et al*., 2018; Lotfy *et al*., 2021). Because of the distinctiveness of their physiochemical features, AgNPs have been used in a variety of industrial and medical applications (Abdelghany *et al*., 2017).

Antibiotic resistance is one of the most pressing issues confronting the health-care system today, posing a major threat to public health. If no new antibiotics are created or found by 2050, it is estimated that there will be no effective antibiotic available. MDR gram-negative bacteria, such as *K. pneumonia*, *P. aeruginosa*, and *E. coli*. Extended spectrum beta lactamase (ESBL) producing Enterobacteria, and carbapenem-resistant Enterobacteria (CRE), and vancomycin-resistant Enterococcus (VRE) are thought to be the predominant cause of nosocomial infections (Teerawattanapong *et al*., 2017).

The most common bacterial infections have lately been identified as methicillin-resistant (MRSA) *S. aureus* (Hiramatsu *et al*., 2001). Moreover, there are MDR bacteria isolated from animal-derived foods, water, and animals include *B. cereus* and *E. Faecalis* (Almaaytah *et al*., 2018). This necessitates the quest for alternate antibiotic-resistant disease management strategies such as; Phage therapies, Antibodies, and Nanotherapeutics (Vivas *et al*., 2019).

Silver nanoparticles have the ability to kill bacteria on their own. After anchoring to the cell surface, silver nanoparticles can aggregate in pits that form on the cell wall. Silver nanoparticles that have collected in the cell membrane can cause denaturation. Because of their nanoscale size, silver nanoparticles have the capacity to penetrate bacterial cell walls and modify the structure of the cell membrane (Liao *et al*., 2019).

Denaturation of the cytoplasmic membrane can cause organelles to rupture and potentially cell lysis. Silver nanoparticles may also play a role in bacterial signal transduction. Phosphorylation of protein substrates affects bacterial signal transduction, and nanoparticles can dephosphorylate tyrosine residues on peptide substrates. Cell apoptosis and cell expansion can be halted if signal transduction is disrupted (Li *et al*., 2019).

Several other studies included the effective anti-bacterial activity as bacteria *B. cereus* (Kumar *et al*., 2020), and conclusive results about the mechanism of action of AgNPs against against *S. aureus* and *P. aeruginosa* (Yuan *et al*., 2017), from Candida albicans mycosynthesis of silver nanoparticles and antibacterial efficacy against Escherichia coli and Staphylococcus aureus (Rahimi *et al*., 2016), inhibitory effect of *E. Faecalis* (Wu *et al*., 2014). The manufacture of silver nanoparticles (AgNPs) from Massilia sp. culture supernatant, as well as the antibacterial use of generated AgNPs against multidrug resistant pathogenic *Klebsiella pneumoniae* and *Salmonella Enteritidis* (Huq and Akter, 2021).

The AgNPs was observed to effective with two types of bacteria *E. coli* and *B. cereus* for two types of antibiotics that were resistant; Tetracycline (TE10 µg) and Cephalexin (CL30 mcg). The study's findings clearly show that biosynthesized AgNPs have promising biomedical applications. AgNPs with tetracycline, exhibited efficiency against the multidrug-resistant bacteria Salmonella typhimurium (Deng *et al*., 2016). In separate circumstances, AgNPs were found to have synergistic effects with gentamicin, kanamycine, cephalosporin, and penicillin. *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Ebrahimi *et al*., 2018).

Conclusion

The results of this work show that silver nanoparticles can be made in a simple, safe, cost-effective, and environmentally friendly manner utilizing yeast *Trichosporon asahii*. The biosynthesized nanoparticles had antibacterial action against both Gram-negative and Gram-positive bacteria. As a result, the use of biosynthesized silver nanoparticles could lead to the development of new pharmacological and industrial products.

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