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# Biosynthesis of Silver Nanoparticles by Food-Origin *E. coli* and *Candida Species* and Testing Its Antimicrobial Activity against Pathogenic Bacteria and Fungi

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***Abstract:*** *Due to the growing demand to improve an environmentally friendly, nonhazardous and cost-effective technology as a biocide to control the drug-resistant microorganism, thus in the present study; food-origin Escherichia coli, Candida zeylanoides and C. krusei were used for the biosynthesis of silver nanoparticles.*
*The biosynthesis of silver nanoparticles was monitored upon the colour change of the reaction mixture by ultraviolet-visible spectroscopy. Furthermore, the product was explored by Atomic Force Microscopy; the results revealed the formation of silver nanoparticles in the reaction mixture of the used microorganisms. The biosynthesized silver nanoparticles from E. coli, C. zeylanoides and C. krusei showed a maximum absorption at 423 nm, 415 nm and 426 nm respectively at range between 300 to 800nm wavelength and the size was 64.93 nm, 102.86 nm and 95.37 nm respectively. The biosynthesized silver nanoparticles were tested for their antimicrobial activity against various pathogens Pseudomonas flourescence, Klebsiella pneumonia, E.coli, Candida guilliermondii, and C. albicans; the results showed that the silver nanoparticles that biosynthesized in the current research exhibited an effective antimicrobial activity.*
***Key words****: Biosynthesis, Silver nanoparticles, C. zeylanoides, C. krusei, E. coli, Atomic Force Microscopy*

## I. Introduction

Nanoparticles (Nps) may be defined as particles of 1-100 nm diameters with sole surface, optical and microscopic properties, there are different methods to synthesize Nps include: physical, chemical and biological procedures; biological methods have become progressively conspicuous because they are cheap and mild conditions are used in a diversity of hosts; additionally; stable Nps with controlled dimensions can be produced using bacteria, fungi and plants [1]. Pathogenic bacteria developed a variety of mechanisms to resist antibiotics; this subject is of a great concern from a medical point of view, so the researchers always aimed to improve new choices to control pathogens [2].

It was reported that fungi, yeasts, actinomycetes, bacteria and viruses were used for the biosynthyesis of different metal nanoparticles: tellurium, zirconia, platinum, silica, selenium, silver, gold, magnetite titanium, palladium and uraninite [3,4]. This biosynthesis occurred via clutching the target ions by the microorganism from their surroundings, and thereafter turning these metal ions to nanoparticles [5].

Metal nanoparticles are an active method to sequester many antibiotic-resistant microorganisms. Nanoparticles are relevant in different areas such as electronics, biosensors, diagnostic agents, coatings, imaging, environmental remediation, cosmetics, medicine, drug and gene delivery. Because of the unique properties of their conductivity, chemical stability, optical behavior, and catalytic antibacterial activity; silver nanoparticles was the most studied metal [2,6].Chemically synthesized nanoparticles are toxic naturally, therefore there is an emergent necessity to improve cost effective, accessibly, environment friendly and reproducible approaches of nanoparticle synthesis. Furthermore, nanoparticles have an amplified surface area and consequently increasing the interaction area with the pathogens.

Due to their smaller size, nanoparticles have the ability to enter the microbial surfaces more effective than the micron particles, and thus having stronger effects on microbial targets [7,8].
This study aimed to:

1- Biosynthesize silver nanoparticles using food origin isolates of bacteria and yeasts
2- Investigation of the antimicrobial properties of the biosynthesized AgNps against multidrug resistant clinical isolates of bacteria and yeasts.

## II. Materials And Methods

### Microbial Isolation and Identification

*Escherichia coli*, *C. krusei* and *C. zeylanoides* isolates which used to biosynthesize AgNps were isolated from different food samples collected randomly from Baghdad markets; using MacConkey agar and Eosin Methylene Blue for *E. coli* and Potato Dexstrose agar for *Candida spp.* according to the instructions of the

Iraqi Standard Criterion No.3/2270 in Isolation, Enumeration and Identification of Microbiological Groups in Foods [9]. In addition to microscopic properties, colonial morphology, identification was carried out by using biochemical tests according to Bergey's Manual [10] Api20E system and Vitik complete system.

**Biosynthesis of AgNps**

Biomass production of both the bacteria and yeasts isolates was performed using the same protocol using nutrient broth; Shaker incubator (Gallenkamp /England) used to incubate the culture at 100 rpm for 18 h, thereafter the biomass were harvested by centrifugation at 12000 rpm for 10 min. Culture supernatants were collected for biosynthesis of AgNps. Then 10 ml of each supernatant sample were added to the reaction flasks, which contained 40 ml of 10-3 M (1%, v/v) silver nitrate, the incubation conditions were at pH 5, 37˚C for 24h, control flasks contained the supernatant without $AgNO_3$, were incubated at the same conditions. [4,11]

**AgNps analysis**

A first indicator of the formation of AgNPs was the change in color of the reaction mixture, after this change, the absorbance of the product was measured using a UV-visible spectrophotometer (UV-Visible; varian, Australia), scanning the 300- to 800-nm absorbance spectrum. Atomic Force Microscopy was used to complete the analysis.

**Antimicrobial activity analysis**

The silver nanoparticles synthesized from *E. coli*, *C. krusei* and *C. zeylanoides* were tested for their antimicrobial activity using well diffusion method against pathogenic microorganisms like *Klebsiella; E. coli; C. guilliermondii; C. albicans; Pseudomonas* [12].

**Statistical analysis**

In order to evaluate the isolates in each treatment (K AgNps, Z AgNps, E AgNps) using analysis of variance (ANOVA) in complete random design. Difference between means was analyzed by least significant difference (LSD) at $p<0.05$ using (SPSS) program 2010 and excel application to find the result and chart.

## III. Results And Discussion

Silver nanoparticles were biosynthesized by food-origin isolates of *E.coli , C. zeylanoides* and *C. krusei* culture supernatant and this was indicated rapidly after 24 h of incubation with silver nitrate by the colour change of reaction mixture from yellow to brown, while the control vessel without $AgNo_3$ remain unchanged as shown in figure [1].

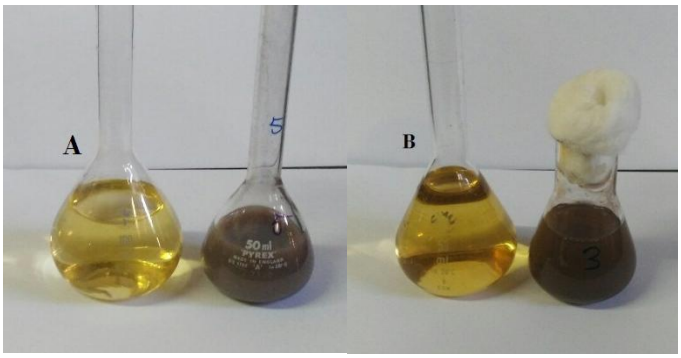


**Figure (1)**: Chromatic features of culture supernatant of *E. coli* (**A**), *C. zeylanoides* **(B)** indicating the biosynthesis of silver nanoparticles (Brown colour), the control flask without $AgNO_3$ (Yellow colour).

The figure above shows that both food-origin isolates of bacteria and yeast formed silver nanoparticles; as brown colour appeared in the culture supernatant of *E. coli*, *C. krusei* and *C. zeylanoides*, after incubation with $AgNO_3$ for 24h in 37˚C, while the control flask without $AgNO_3$ remain unchanged (yellow) when incubated at the same conditions.

Vigneshwaran and his colleagues explained that the reason behind the brown color in the mixture vessel may be due to the surface plasmon vibrations excitation, which is characteristic of AgNps [13]. When an electromagnetic field in the visible range is coupled to the collective fluctuations of conduction electrons; dipole fluctuation arises, leading to these surface plasmon vibrations [14].

Biosynthesized AgNps were analyzed using techniques like UV-visible spectrophotometer and Atomic Force Microscopy (AFM). The results of UV-Vis spectral analysis showed a peak at 423 nm, 415 nm and 426 nm at range between 300 to 800nm wavelength were detected for the silver nanoparticles prepared from *E. coli,*

*C. zeylanoides*and *C. krusei*. This signifies AgNps formation via $Ag^+$ reduction to elemental silver in the culture supernatant.

In a previous study UV-Vis spectra was recorded for the liquid AgNps biosynthesized by *C. albicans* and a characteristic strong peak was noticed at near 400 nm [13].

The results obtained from Atomic Force Microscopy showed that the biosynthesized silver nanoparticles in this study have an average diameters of found equal to 64.93 nm for *E. coli* ,102.86 nm *C. zeylanoides*and 95.37 nm for *C. krusei* [Table 1].

**Table (1):** The average diameter of biosynthesized silver nanoparticles as analyzed by AFM

| Producer microorganism | average diameter (nm) |
|---|---|
| *E. coli* | **64.93 nm** |
| *C. zeylanoides* | **102.86 nm** |
| *C. krusei* | **95.37 nm** |

Thus these results obtained by UV-Vis spectroscopy and AFM analyses exhibited that food-origin *E.coli* isolates biosynthesized AgNps smaller than that synthesized by *Candida sp*. [Figures 2-4].

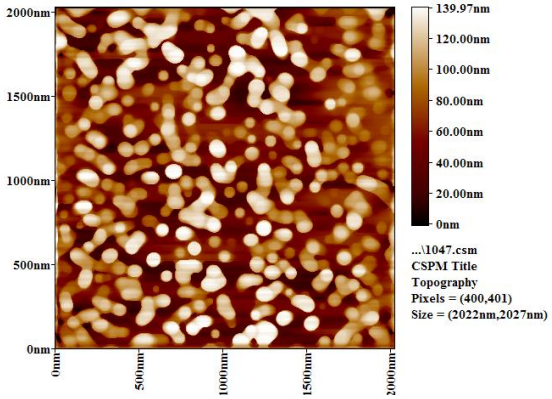


**Figure (2):** Atomic Force Microscopy analysis of silver nanoparticles biosynthesized by food-origin *E. coli* isolate

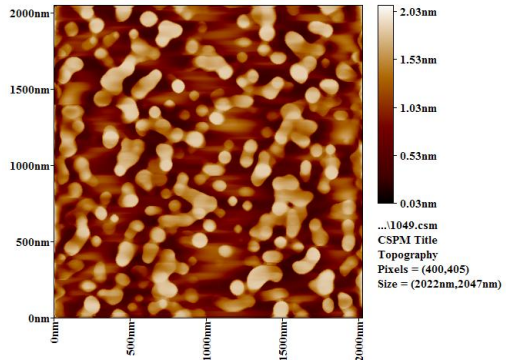


**Figure (3):** Atomic Force Microscopy analysis of silver nanoparticles biosynthesized by food-origin *C. zeylanoides* isolate

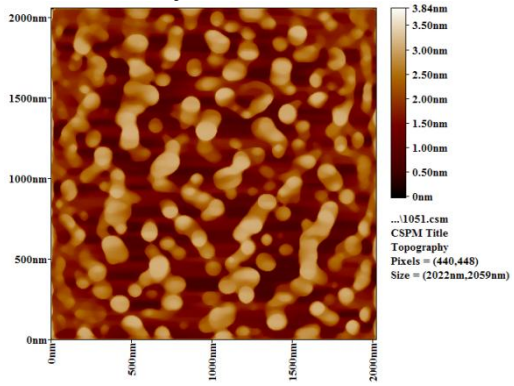

**Figure (4):** Atomic Force Microscopy analysis of silver nanoparticles biosynthesized by food-origin *C. krusei* isolate

The AFM images clarified the shape of the AgNps as longitudinal and spherical.

Silver nanoparticles produced by *E. coli*, *C. zeylanoides* and *C. krusei* antimicrobial activity against various pathogens: *Pseudomonas flourescence, Klebsiella pneumonia, E. coli, Candida guilliermondii, and C. albicans,* which were clinically isolated was analyzed. Inhibition zone diameter was measured after 24h. and the figures [5-8] clarify the results.

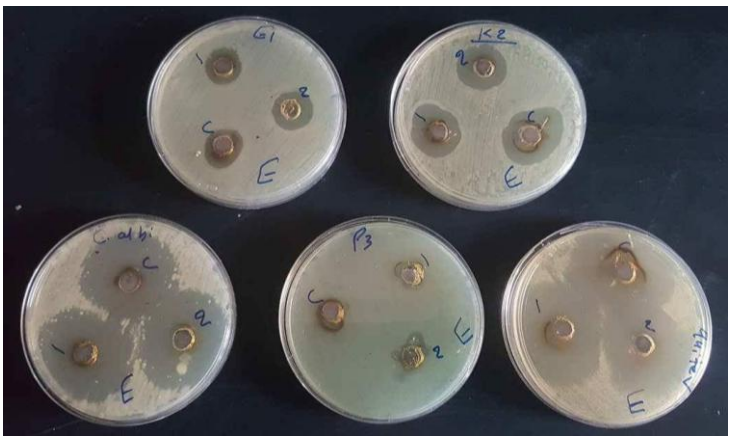

**Figure (5)**: Inhibition zones of silver nanoparticles biosynthesized by food-origin *E. coli* for various pathogens: *P. flourescence, K. pneumonia, E. coli, C. guilliermondii, and C. albicans*

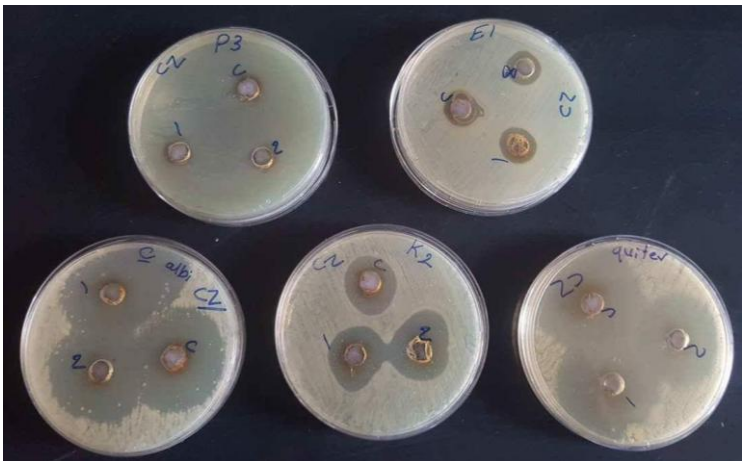

**Figure (6)**: Inhibition zones of silver nanoparticles biosynthesized by food-origin *C. zeylanoides* for various pathogens: *P. flourescence, K. pneumonia, E. coli, C. guilliermondii, and C. albicans*

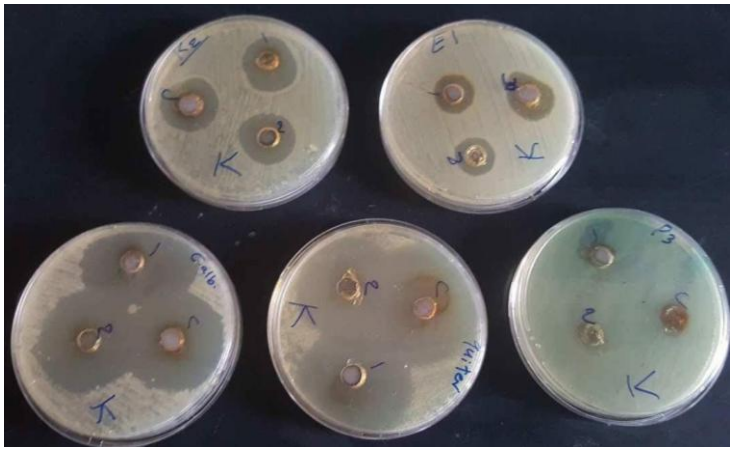

**Figure (7)**: Inhibition zones of silver nanoparticles biosynthesized by *C. krusei*for various pathogens: *P. flourescence, K. pneumonia, E. coli, C. guilliermondii, and C. albicans*

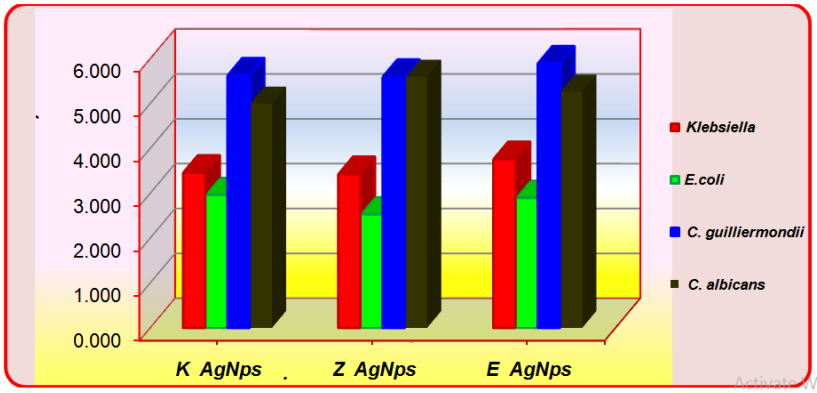


**Figure (8)**: Assessment of the antimicrobial effect of the biosynthesized AgNps from *E. coli* (E AgNps), *C. zeylanoides*(Z AgNps) and *C. Krusei* (K AgNps) against: *K. pneumonia, E. coli, C. guilliermondii, and C. albicans*

**Table (2)** illustrate the results of the antimicrobial analysis of the biosynthesized AgNps

| Treatments / Isolates | K AgNps | Z AgNps | E AgNps |
|---|---|---|---|
| *K. pneumoniae* | 3.467 c ± 0.088 | 3.433 b ± 0.145 | 3.767 c ± 0.233 |
| *E.coli* | 2.967 c ± 0.120 | 2.533 c ± 0.033 | 2.900 d ± 0.058 |
| *C. guilliermondii* | 5.633 a ± 0.186 | 5.600 a ± 0.200 | 5.900 a ± 0.058 |
| *C. albicans* | 5.000 b ± 0.306 | 5.600 a ± 0.306 | 5.267 b ± 0.133 |
| LSD P ≤ 0.05 | 0.632 | 0.643 | 0.458 |

*P. fluorescence* resisted all the treatments, while *K. pneumonia, E. coli, C. guilliermondii, and C. albicans* were inhibited by the biosynthesized AgNps from *E. coli*, *C. zeylanoides* and *C. krusei*. The results concerning the antimicrobial activity of AgNps obtained from the present study may vary according to the nanoparticle producer microbe and the predict size of the produced nanoparticles [15].

In a previous study, it was concluded that, Ag Nps effect on the bacterial cells may be related to leakage forming in the outer membrane that leading to the loss of cellular materials, thereafter the AgNps enters and inactivates dehydrogenases enzymes of the respiratory chain, thus causing growth inhibition, some proteins and phosphate lipids may be affected by AgNps which leads to cell decomposition and decline finally (16).

The mechanism of silver bactericidal and fungicidal activity is still undefined, but the researchers proposed that the bactericidal effect may be due to morphological changes in the cell like nucleic acid condensation, cytoplasm shrinkage and cell wall segregation upon exposure to silver ions [15].

Martinez-Gutierrez and his colleagues reported that reactive oxygen species generation by silver ions causes silver sulphur granules accumulation on the microbial cell wall [17].

Due to their abundant surface area which offers better interaction with microorganisms; silver nanoparticles exhibit effective antimicrobial potential compared with other metals [18].

It was reported the medical importance of silver nanoparticles as “natural” antibacterial and antifungal agents in the drug resistance era, where AgNPs exhibited enhanced antifungal and antibacterial effectiveness towards *Candida spp*. and *E. coli* [19,20].

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