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Biosynthesis of MgO Nanoparticles Using *Klebsiella pneumoniae* and *Staphylococcus aureus* Supernatant

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A B S T R A C T

In the present work, a magnesium oxide nanoparticle production process that is inexpensive, ecologically benign, and repeatable is mediated by Klebsiella pneumoniae and Staphylococcus aureus. The purpose of this study was to produce magnesium oxide nanoparticles by employing the bacterial culture supernatant of (K. pneumoniae and S. aureus) and to characterize them.The magnesium oxide nanoparticles were created using an environmentally acceptable extracellular bio-synthetic technique. magnesium nitrate was used as a source of MgO NPs. The MgO NPs were examined by scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy dispersive X-ray (EDX), ultraviolet-visible (UV-Vis), and X-ray diffraction (XRD). The crystalline metallic MgO NPs' fifth main peaks were visible in the XRD pattern. The MgO K. pneumoniae NPs UV spectrum showed a sharp absorption peak at 330 nm and 334 nm for MgO S. aureus NPs. According to the current study's findings, K. pneumoniae and S.aureus bacteria can create MgO NPs extracellularly.

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Introduction

The study of incredibly tiny structures is the focus of nanotechnology, which is defined as the manipulation, characterization, exploration, and application of nanosized materials for scientific advancement. The prefix "Nano" denotes 109 of a meter or 10-9 m; the word is Greek in origin and means "dwarf or miniature," which means extremely small (1). Nanotechnology is a modern branch of science that combines nanotechnology and biotechnology to study phenomena at the atomic, molecular, and macromolecular dimensions. In the discipline of nanotechnology, structures were created, characterized, designed, produced, and used by manipulating their size and shape at the nanoscale scale. As the size of the nanoparticles decreases, the surface-to-volume ratio increases. Because surface energy increases with an increase in a nanoparticle's specific surface area, so does the particle's biological effectiveness (2). Nanoparticle applications were also covered (3). One significant advancement in nanotechnology is the synthesis of NPs. There are two different approaches to nanotechnology, are graphically "bottom-up" or (chemical and biological methods) and "top-down" or (physical methods) (4). Many people are interested in MgO nanoparticles because their fundamental characteristics and practical uses in numerous fields of physics, chemistry, and materials research (5). MgO NPs are non-toxic and odorless (6). They come in the form of a white powder and have great hardness, high purity, and high melting point (2852 Co) (7). Magnesium oxide (MgO), an inorganic compound with a wide band gap, is used in a variety of applications such as stiff components, anti-reflecting sheets, conductors, and toxic waste removal (8). MgO NPs have been used as antibacterial and anticancer agents (7). MgO NPs have unique medical applications like treating heartburn and regenerating bone (8). Bacteria are possibly the most suited candidates for nanoparticles because of their extraordinary capacity to decrease metal ions to their zero forms (9). The simplicity of handling and the needs of the medium culture are credited with facilitating the synthesis (10). Metal nanoparticles are produced by bacteria through biosynthesis. One of the tasks of heavy metal toxicity resistance mechanisms is the conversion of hazardous heavy minerals into non-toxic forms, which are then deposited as mineral groups (11). The nanoscale size and the unique form (1).

The bacteria used in this study, The Klebsiella pneumonia and Staphylococcus aureus. Pneumonia is caused by the Enterobacteriaceae family, of which Klebsiella is one of the most significant members (12). K. pneumoniae is gram-negative bacteria , rod-shaped, singly arranged in pairs or in short chains, opportunistic,non-motile, facultatively anaerobic, non-spore-forming (13). Not only dose K .pneumoniae colonize the human skin, gastrointestinal tract, and nasopharynx, but it was also chosen as a saprophyte bacteria (14). K. pneumoniae is oxidase negative, lactose-fermenting, having noticeable polysaccharide capsules that thick which provides colonies on agar plates with a mucoid look (15) . Under a microscope, Staphylococcus aureus, also known as Staph. aureus, is a gram-positive cocci that resembles blue-violet clusters. The word "aureus," which means "golden," refers to the colonies' yellow pigments, which are formed during the bacteria's growth (16). One of the most common pathogenic bacteria in humans, Staph. aureus causes a variety of infection-related sequelae in people of all ages and genders. It is a major source of nosocomial and community-acquired infections. Typically, the microorganism produces zones surrounding the colonies by causing hemolysis on enriched agar (blood agar containing 5% sheep or horse blood) (17, 18, 19). The synthesis of several enzymes known as hemolysins is what causes this hemolysis (18). Due to its salt tolerance, S. aureus is cultivated on selective media such mannitol salt agar, which contains 7.5–10% sodium chloride (20, 18). The medium turns yellow as the bacterium ferments the mannitol sugar, producing an acid and altering the medium's color from pink to yellow. This can be used to differentiate S. aureus from the non-mannitol fermenter S. epidermidis (21,22). This study aimed to synthesize and analyze magnesium oxide nanoparticles using the bacterial culture supernatant of K. pneumoniae and S. aureus.

Materials and methods

Extracellular synthesis of nanoparticles by using microorganisms

The work was performed according to (23,24) with minor changes Separately:.

Preparation isolates of bacteria:

The isolates came from the microbiology division of the Azadi Teaching Hospital, where the disease had previously been recognized using the vitek2 system and a biochemical test. The synthesis of magnesium oxide nanoparticles used two isolates, Staphylococcus aureus and Klebsiella pneumonia.

Preparation of Supernatant Solution of bacteria:

Under sterile conditions, 100 ml of sterile Luria Bertani broth for K. pneumoniae and nutrient broth medium for S. aureus were inoculated with pure bacterial isolates. These media were then incubated for 24 hours at 37 °C. Following the incubation period, the bacterial culture is centrifuged at 6000 rpm for 12 minutes, after which the cell supernatants are separately collected for each isolate in a sterile 250 ml conical flask for the creation of nanoparticles.

preparation of magnesium oxide nanoparticle:

Biological formation of MgO nanoparticles by dissolving 2.5 g of magnesium nitrate Mg(NO3)26H2O in 90 ml deionized water using a magnetic stirrer for 30 min to prepare 0.1M from solution. Then the bacterial culture supernatant (S. aureus or K. pneumoniae) was mixed with the precursor solution of magnesium nitrate by magnetic stirrer for 1 h and another bacterial culture without magnesium nitrate is used for control. The cultures were then maintained for 15–20 minutes at 40°C in a water bath, after which 2 M of sodium hydroxide (NaOH) was added drop-wise until a precipitate formed, For the last 10 hours, the cultures were incubated at room temperature without being disturbed. the cultures were centrifugedat 5000 rpm for 15 min and the pellet was washed with water and ethanol. Then, it dried in the oven at 80 °C.Then, the powder was treated at 450 °C for 2 h with the formation of a fine powder.

Transmission electron microscopy (TEM), energy-dispersive X-ray analysis (EDX), field emission scanning electron microscopy (FESEM), ultraviolet-visible spectroscopy (UV-Vis), and X-ray diffraction (XRD) were used to characterize the produced MgO NPs [23].

These analyzes were conducted at the Phi Nanoscience center in Baghdad Governorate, Iraq.

Statistical analysis

Using the statistical analysis program SPSS version 26, the data from the current study are examined using One-Way ANOVA, Least Significant Difference (LSD), and independent t tests.

Results and discussion

Bacterial identification

The microorganisms recovered from plates were fully identified by; Colony morphology (hemolysis, pigment, and size) and Gram stain. Morphological examination of K. pneumoniae isolates grown on MacConkey agar medium gave the pink glamorous colonies with mucus texture, due to the ability to lactose fermenting, more purified by biochemical tests (Oxidase negative, catalase positive and urease production). Isolates that grey pigmented, smooth, convex, and haemolytic colonies on blood agar suspected to be S. aureus, more purified by biochemical tests (Positive result for Catalase, Coagulase test and slide coagulase test and Oxidase negative). All the Staph.aureus isolates can grow on Mannitol Salt Agar (MSA) and form large, Round, creamy-gold colonies that shift the medium's color from pink to yellow and are encircled by broad yellow zones, about Baird-Parker Tellurite can be reduced to telluride by Staphylococci, resulting in shiny, convex, dark gray to black colonies with complete borders and clear zones, either with or without an opaque zone surrounding the colonies due to the addition of egg yolk.

Biosythesis of MgO nanoparticles

Pure bacterial isolates were inoculated into sterilized nutrient broth container for S. aureus and Luria Bertani broth media for K. pneumoniae under sterile conditions, for biomass production, Bacterium filtrate used as a reducing agent and stabilizing factor in as appeared in biosynthesis method, the result showed a white precipitate formation at the bottom of the container, figure (1-a) which is an indecency of magnesium oxide formation due to magnesium ion reducing by proteins and enzymes existing in the filtrate, lead to the formation of white aggregates from magnesium oxide nanoparticles (23). This outcome was consistent with several findings that demonstrated the importance of microbe-derived proteins, enzymes, and other biomolecules in processes like NP reduction. Multiple organic constituent secreted with in suspension and growth medium were essential for the formation of NPs of multiple sizes with mono- and poly-dispersed NPs. Moreover, the protein that microorganisms naturally make could function as a capping agent to give stability to the NPs' creation. magnesium oxide nanoparticles were obtained as a white powder after drying (24).

Characterization

UV-Vis Absorpation Spectroscopy

By using UV-Vis analysis, the optical characteristics of MgO NPs were discovered in the 200–500 nm range. At 330 nm, the UV spectrum of the K. pneumoniae MgO NPs was captured, confirming the production of MgO nanoparticles. According to (25), the sharp absorbance peak at 330 nm in the UV-visible spectroscopy suggested the development of tiny-sized particles of MgO, (Fig.1-b). While the peak absorption at 334 nm to S.aureus MgO NPs Figure (1-c) which is near from previously reported result (25).

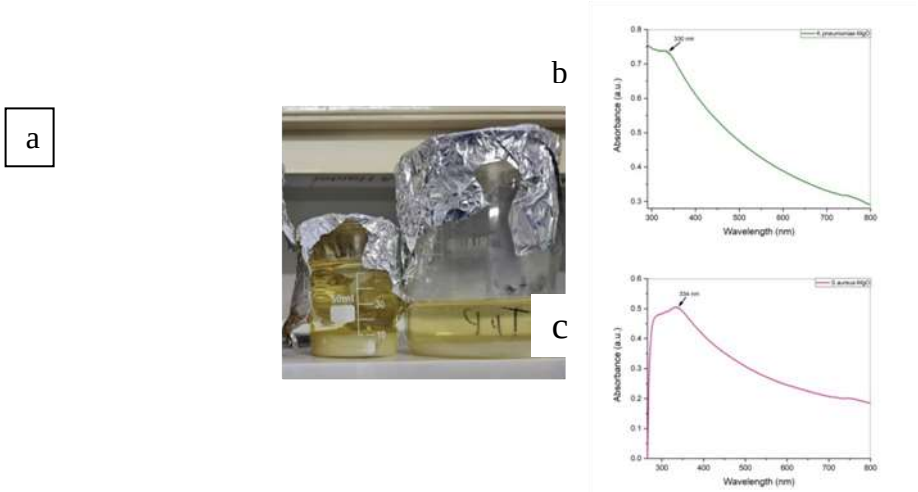

Figure 1. a: Formation of MgO NPs ; show white precipitate formed,b- UV-visible spectrophotometer illustrate absorbance of MgO K.pneumoniae NPs and c- absorbance of MgO S.aureus NPs.

XRD Characterization

MgO cubic crystal shape reported in reference code (01-078-0430) from the data obtained for K.pneumoniae as ~36.8°,42.8°,62.1°,74.5°and 78.4°, with orientation (111),(200),(220),(311)and(222) planes (Fig. 2-a). Furthermore, that MgO NPs obtained from S.aureus were in a code (01-075-0447) is listed peak as ~ 36.8,42.8,62.2,74.5 and 78.5,that is corresponding to (111), (200), (220), (311) and (222), (Fig.2-b). the results are agree with that registered from (23,26).

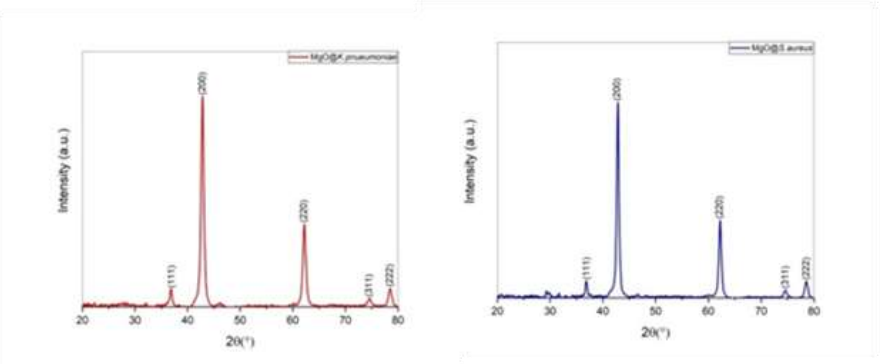

Figure 2 . XRD plot a-MgO K.pneumoniae,b-MgO S.aureus.

Characterization by TEM

Magnesium oxide nanoparticles biosynthesized from K.pneumoniae are depicted in a TEM picture in figure (3-a,b), MgO K.pneumniae nanoparticles size has a size distribution with a range of 30 to 80 nm with an average particle size of 22 nm. While MgO NPs from S.aureus, TEM images reveal that the synthesized

MgONPs formed were mainly irregular and spherical shapes with an average particle size of 26 nm, and size distribution with a range between 10-80 nm. The results are near to previously reported results (26,27).

SEM and EDS Characterization

Fig. (3-c,d) shows the surface morphology of MgO NPs that were obtained using the SEM image, it shows that the synthesized MgO NPs were approximately spherical and hexagonal structure, the size of the synthesized MgO K.pneumniae NPs ranging from 29.03 to 60.29 nm and (17.86 to 53.59) for MgO S.aureus NPs. According to the EDX spectrum, the product was primarily made up of Mg and O, MgO K.pneumniae NPs have 69.8% of magnesium and 30.2% of oxygen and MgO S.aureus NPs have 61.2% of magnesium and 38.8% of oxygen, These results are almost identical to (23,28-31).

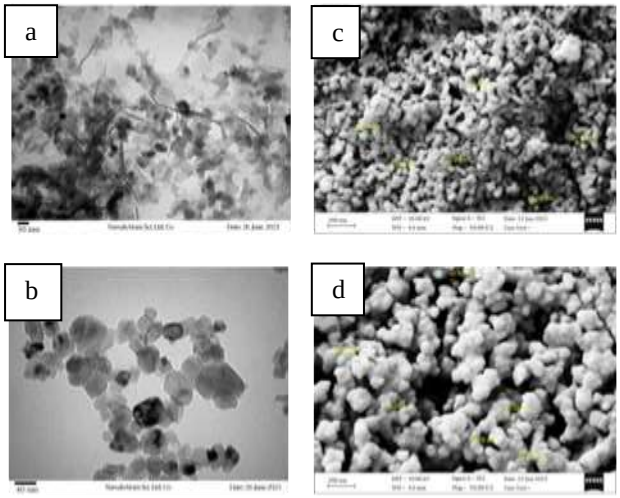

Figure 3- TEM images a: MgO K.pneumoniae NPs,b: MgO S.aureus NPs, , SEM images c:MgO K.pneumniae NPs,d: MgO S.aureus NPs.

CONCLUSION:

This work was developed as a result of the biological synthesis of MgO NPs by K. pneumoniae and S. aureus. Results of analyses using UV-Vis Absorption Spectroscopy, EDX, TEM, SEM, and XRD provided evidence for the effective synthesis of MgO nanoparticles. The XRD and SEM analysis validates the biosynthesized nanoparticles surface form and crystallinity. The average size of the biosynthesised MgO K. pneumoniae and MgO S. aureus nanoparticles, as shown by TEM images, was 22 nm and 26 nm, respectively. The results of this investigation indicate that K. pneumoniae and S. aureus bacteria are both able to produce MgO NPs extracellularly. We also suggest investigating how these NPs interact pharmacologically with various human and animal disorders.

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