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One-pot green synthesis of magnesium oxide nanoparticles using *Penicillium chrysogenum* melanin pigment and gamma rays with antimicrobial activity against multidrug-resistant microbes

Gharieb S. El-Sayyad *, Farag M. Mosallam, Ahmed I. El-Batal

Drug Microbiology Lab., Drug Radiation Research Dep., Biotechnology Division, National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt

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ABSTRACT

Melanin pigment is well-known as a common photo-protective polymer, insoluble in water. It drews considerable attention for many applications in nanotechnology, and medical fields. *Penicillium chrysogenum* was employed for the green synthesis of melanin after optimizing the media compositions. A method has been designed that included one-step synthesis of magnesium oxide nanoparticles (MgO NPs) by fungal melanin under the influence of different doses of gamma rays. Antimicrobial activity of MgO NPs was examined against some selected highly pathogenic microbes. The fungal melanin acted simultaneously as a photo-protector of the magnesium atom, and at the same time as a stabilizer towards the uncontrolled free radical attack resulting from gamma rays. Afterwards, gamma rays forced a condensation reaction to occur at room temperature. A proposed reaction mechanism for MgO NPs synthesis was discussed. MgO NPs were characterized and structurally identified by UV–Vis., XRD, DLS, TEM and FTIR. Results obtained from DLS and XRD with TEM images determined the mean diameter as 10.28 nm. In addition, MgO NPs were found to be promising antimicrobial agents against' *Enterococcus faecalis, Candida albicans*, and *Klebsiella pneumoniae* having activity of 22.0, 20.0, and 20.0 mm ZOI, respectively. Based on the capability of MgO NPs as effective antimicrobial agents, they possess a potential role – in different applications such as biomedicine, food control, pharmaceutics, and cosmetics.

1. Introduction

Melanin pigment has attracted notable consideration of many researchers because it is essentially found in all animals, worms, plants and several microbes with irregular deep brownish polymers used as a photo-protective agent [1]. In addition, it is involved in several fields as a promising polymer in food industry [2], pharmaceutical lotions, cosmetics, sun-light rays protections [3], bio-nanotechnology [4,5], biomedicine and additional purposes. In addition it is used as an antimicrobial agent-[6], radio-protective polymer [1], an antioxidant [7] and an antitumor factor.

Many fungal species produce melanin (a biologically significant dye) which is usually used as a protecting factor against ultraviolet rays released from the sun. Most of fungi synthesize – the pigment from their media substrate as 1, 8-dihydroxynaphthalene (DHN) intermediate. On the other hand, others incorporated melanin from L-3,4-dihydroxyphenylalanine (L-DOPA) in the presence of the tyrosinase enzyme [8]. *Penicillium chrysogenum* was tailored for melanin biosynthesis through the optimization of the culture and environmental conditions. The synthesized melanin (high reducing and capping agent) with the support of gamma radiation was used for metal oxide nanoparticle biosynthesis [5].

Dangerous infections caused by pathogenic microbes are among the principal reasons causing environmental hazards. The research for a novel antimicrobial agent capable of decreasing the resistance of the infectious microbes is of extreme importance. The development of multidrug-resistant (MDR) pathogens (Gram-negative and positive) is a common health issue that has gained increased attention in recent years. Such development of the microbial defense is due to the random use of antibiotics, giving the chance for fast growth and development of unusual MDR microbes [9].

Metal-oxide nanoparticles (MO NPs) are promising fighting tools (antimicrobial agents) towards a broad-range of infected microbes such as MDR isolates. The MO NPs properties are important for medical application, especially in the area of microbial resistance. This area needs the development of distinct antimicrobial agents required for microbial infection treatment, water purification, and textile applications [10].

* Corresponding author.
E-mail address: Gharieb.Elsayaad@eaea.org.eg (G.S. El-Sayyad).

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Magnesium oxide (MgO) is a widely available as inorganic substance including a broad band-gap [11]. It has been applied in various fields such as catalytic materials, rigid elements, removal of poisonous waste, adsorbents, recovering agents, anti-reflecting films, and superconductors [12].

In bio-medicine, MgO has been applied for bone repair, relies of heartburn, and in the treatment of sensitive stomachs [13]. Recently, MgO NPs have been used in cancer therapy besides – having significant activities as antibacterial agents [14].

The development of a novel efficient method for the improved MO NPs synthesis (including high-dispersed nano-crystals, resistant to aggregation as well as controlled shapes and sizes) is an identification of the primary purposes in the progression of nanotechnology [4,5,15].

Biological synthesis of nanoparticles presents both an eco-friendly and a cost-effective process. The biogenic method for nanoparticles production was achieved with the use of bio-active materials (plant extracts, and the microbial product like pigments) as producers, stabilizers and reducing agents [4,5,15].

In the work presented, for the first time, a cost-effective green method for MgO NPs synthesis has been tailored at room temperature. The procedure presented in the work has included fungal melanin as a capping agent for the MgO NPs production and as a protector from the uncontrolled free radical attacks [4,5].

It must be mentioned that the melanin pigment was highly soluble in alkaline solutions like KOH and NaOH. The soluble pigment in the presence of gamma rays increased the production of MgO NPs and supported the condensation reaction to occur at room temperature. The mechanism of MgO NPs formation is also discussed in this study.

In our study, the practical methodology introduces a novel and unique eco-friendly and cost-effective technique. It was noted that melanin was synthesized by the alternative L-tyrosine natural sources found in the environment. We used the optimized *P. chrysogenum* melanin pigment under the influence of different gamma radiation doses for the synthesis of MgO NPs in one active rapid step. The synthesized MgO NPs were examined for its antimicrobial activity against some common pathogenic bacteria, unicellular fungi and MDR pathogens. However, the synthesized MgO NPs can also be used as an alternative agent for combating and reducing the resistance of the infecting microbes, hence, MgO NPs were regarded as promising agents in different fields like food preservation, control of infectious diseases, medical devices and cosmetics.

2. Materials and methods

2.1. Chemicals and reagents

Media components were obtained from (Oxoid) and (Difco). Chemicals and reagents employed in the following experiments were obtained at standard level and applied without further refinement.

2.2. Fungal isolate

Based on the results of the screening process in our previous study [5], we chose the most potent fungus that owned the highest potential for melanin pigment formation. *P. chrysogenum* was kindly provided by the Drug Microbiology Lab., NCRRT, Cairo, Egypt. It was sub-cultured to fresh Potato Dextrose Agar medium (PDA) to support its adequate germination, and preserving its viability, so it was prepared for the subsequent pigment production. *P. chrysogenum* cultures used in these experiments were cultivated for 4–8 days prior to each experiment.

2.3. Fungal melanin production, extraction, and identification from the final optimized culture

Firstly, irradiated *P. chrysogenum* (2.5 kGy) was inoculated and cultivated in the optimized medium. It consists of potato starch (3.0%), yeast extract (5.0%), 0.25% L-tyrosine, 0.1% L-glycine, copper sulfate (0.2 mM), 2.0% banana peel, and 0.1% tween 20. Afterwards, it was incubated at 30 °C for 7.0 days using 180.0 rpm stirring speed and the pH was adjusted at 5.0 [5].

The extraction procedure was applied according to [16], with slight modification. The fungal filtrate was treated with Conc. HCl and allowed to precipitate overnight. Afterwards, the collected precipitate was centrifuged for 10.0 min at 5000 rpm, washed with distilled water and organic solutions (ethyl acetate, ethanol, and chloroform) to get rid of carbohydrates, amino acids, and lipids. Additionally, the received pellet was soaked in water, stocked in 4.0 M NaOH for 45.0 min, and centrifuged. The produced supernatant was treated with 5.0 M HCl to produce the precipitate. The presented pellet was centrifuged for 20.0 min at 5000 rpm, washed with distilled water and allowed to dry. The slightly purified pigment was stored at 4 °C for further identification studies.

The synthesized pigment was examined for its solubility in distilled water, organic solvents (benzene, chloroform, ethyl acetate, alcohols, and acetone), borate buffer, sodium hydroxide, and potassium hydroxide. The precipitating impact of ferric chloride and potassium ferricyanide was investigated. Finally, the decolorization effect resulting from the oxidizing factors such as potassium permanganate, and hydrogen peroxide was examined.

2.4. Synthesis and characterization of MgO NPs

The extracted fungal melanin (9.535 mg/ml) was mixed with magnesium nitrate solution (4.0 mM) in a proportion of 1:2 (v/v) adjacent by (0.2%) isopropanol as a free radical controller. The mixed suspension was exposed to various gamma rays doses (1.0, 3.0, 5.0, 10.0, 15.0, 20.0, 25.0, and 30.0 kGy). Finally, non-irradiated sample (positive control) and irradiated one that included the fungal melanin without Mg^{+2} (negative control) were used.

The characterization of MgO NPs was performed by UV–Vis. spectrophotometer (JASCO V-560. UV–Vis. Spectrophotometer) using the negative control for the auto-zero. Additionally, average particle size distribution of the synthesized MgO NPs was investigated by Dynamic Light Scattering (DLS-PSS-NICOMP 380-Barbara, California, USA). The shape and size of the synthesized MgO NPs were reported by considering TEM images (JEOL-JEM-100 CX).

X-ray Diffraction study was completed to investigate the MgO NPs crystallinity, using X-ray diffraction patterns-XRD-6000, Shimadzu device, Japan. Finally, FT-IR experiment was a suitable technique that determined the valuable chemical function groups occurring in the melanin pigment and responsible for the reduction and capping effects. The experiments were carried out using JASCO FT-IR 3600 spectrometer and operating KBr pellet design. It was measured at a wave number scale of 400–4000 cm^{-1}. All the validations and identification methods were examined at NCRRT, Cairo, Egypt.

2.5. Antimicrobial activity of MgO NPs

MgO NPs synthesized through radiolytic action with the assistance of *P. chrysogenum* melanin pigment, and Mg^{+2} was tested for their antimicrobial capacity, applying the agar-disc spread procedures [4,15].

They were screened against several isolates of MDR pathogens that were kindly provided by the Drug Microbiology Lab., Drug Radiation Research Dep., NCRRT, Cairo, Egypt. MDR included Gram-negative bacteria (*Escherichia coli, Enterobacter cloacae*, and,

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