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STUDY ABILITY OF WILD *T.VIRIDE* COMPARED WITH MUTANT STRAINS FOR PRODUCTION NANOSILVER PARTICLES AND EFFECT ON SEED GERMINATION

Entesar Ali Mezeel

Department of Biology, College of Science, University of AL- Mustansarya, Baghdad, Iraq.

ABSTRACT: *The present work investigates the synthesis of silver nanoparticles using wild Trichoderma viride strain and induced mutant strain to compartment with each other. The cell filtrate of T. viride was used for the reduction of silver nitrate to silver nanoparticles. the results showed that success the wild and tow mutant strains that coded T. viride Fat13 and T.viride Has15 it were abile to synthesize nano silver particles . The results showed that the summit was the absorption after 12 hours in the mutant strain T. viride Has15 its have highest absoription band it was(0.674) followed by T. viride Fat 13 (0.6120) after 20 hours too, while in wild T.viride strain it was (0.511) after 72 hours and The durable surface plasmon resonance centered at ca. 412-420 nm, TEM micrograph providing comprehensive morphology of silver nanoparticles,The morphology of the nanoparticles is highly variable. Under observation of such images, these assemblies were found to be aggregates of silver nanoparticles in the size range 5–50 nm prouduced from T. viride Has15 ,while it was in the size range 6-60 and 8-80 in wild strain. The attained data refer to obviously revealed that experience to AgNPs had unimportant effect on seed germination as associated to the control treatment. representative that the engineered particles not posture any toxicological properties to the seeds throughout the germination so the germination rate was not pretentious.*

KEYWORDS: T.Viride, Mutant, Transmission Electron Microscope, Seed Germination, Silver Nanoparticles Synthesis.

INTRODUCTION

Nanoscale science is the punishment that study the exclusive behaviors and properties of materials that appear at the size variety of 1 to 100 nanometers. Nanobiotechnology is a sub-discipline of nanoscience that has ascended more lately. It operates unique performances and properties at the nanoscale to manipulate materials for numerous applications in biology , its previously impact in Many scientific applications. Synthesis of principled metals has become compulsory subject in the present period due to the irregular increase of their market standards (Binupriya, et al.,2010) . Metals such as gold, silver, and copper have been extensively used for the synthesis of steady dispersal of nanoparticles, which are existence beneficial in the part of photography, biological labeling, photonics optoelectronics, and surface-enhanced Raman sprinkling discovery (Kearns, et al.,2006). Nanosilver, a newfangled class of material with strangely diverse physicochemical and biological features from expedient silver-containing materials, has been exposed to have antibacterial, antifungal and antiviral belongings and it can decrease injury and fatalities caused by diseases (Choi et al. 2009; Lee et al., 2009). Different organisms have been used for the biosynthesis of nanoparticles like plants, bacteria, yeast and fungi , but fungi has advantage over other organisms like simple to handle, eco-friendly,

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requires very less time, produces the large amount of extracellular enzymes and can uptake the metal ion as compared to physical and chemical method (Samberget al.,2011; Forough, 2010).

Use of silver nanoparticles in its place of silver nitrate enlarged seed yield in borage (Borago officinalis L.) plants (Sahandi et al. 2011).Morevoer, disapproving belongings of overwhelming stress on growth of safroon plants can be reduced by foliar submission of nanosilver (Sorooshzadeh et al. 2012). In this study a comparison was made between, the cell filtrate of wild and mutant strain of T. viride was secondhand for the amalgamation of silver nanoparticles. However to study the effect of biosynthesized silver nanoparticles on germination of some seeds.

MATERIALS AND METHODS

Collection of Materials

Fungal Strains

Trichoderma viride strains were isolated from soil Samples collected from some Baghdad regains in Iraq , The isolated fungus was identified by lacto phenol cotton blue mounting by morphological and microscopic observation, they were selected for their ability to Biosynthesis of Silver Nanoparticles. Isolated strains were grown on PDAplates for 5 to 7 days at 28ºC. Cultures were stored at 4ºC and transferred weekly.

Seedes

Healthy Chickpea (Cicer arietinum L .) and broad bean (Vicia faba L) seed were collected from marketin Bahdad ,Iraq to check the effect of silver nanoparticles on seed germination.

Induction of mutants

Conidia from one-week-old PDA plates were suspended in 9 g/L sterile NaCl solution. A suspension containing 5x108 conidia/mL was treated with 200 µg/mL N-methyl-N-nitroN-nitrosoguanidine (NG, Sigma) for 30 min at 37ºC in a water bath shaker. At this dose, 90% mortality is achieved Surviving conidia were cloned on PDA plates with the addition of 0.1% Triton X-100 and 4 g/L L-sorbose as colony restrictors .Plates were incubated at 28ºC for four days. Isolated colonies were replicated onto the plate-screening medium .

Production of Biomass

To prepare the biomass for biosynthesis studies the fungus was grown aerobically in liquid broth containing (g/L) KH2PO4, 7; K2HPO4, 2; MgSO4·7H2O, 0.1; (NH2)SO4, 1; yeast extract, 0.6; glucose, 10 The culture flasks were incubated on an orbital shaker at 27 °C and agitated at 150 rpm. The biomass was harvested after 72 h of growth by sieving through a plastic sieve followed by extensive washing with sterile double-distilled water to remove any medium components from the biomass.

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Biosynthesis of Silver Nanoparticles

Glucose nutrient broth medium (GNB) was used for biomass preparation of T. viride . 25gm of clean fresh fungal biomass was again inoculated in 100 mL of double distilled water for 3 days at 30°C and agitated again at 120 rpm The cell filtrate was obtained by filtering it through Whatman filter paper No. 1 and the cell free filtrate was collected for experiment. The 10 mL filtrate was treated with 10 mL of 1 mM AgNo3 solution in an Erlenmeyer flask and incubated at room temperature in dark. Control containing cell-free filtrate without silver nitrate solution was run simultaneously as standard with the experimental flask. All experiments were done in duplicate.

Characterization of Silver NanoparticlesUV-visible spectroscopy analysis:

Color of the cell free filtrate changes after the incubation of silver nitrate solution was visually observed. Silver ion bio-reduction was monitored by sampling of aliquots (1 mL) at different time intervals. Absorption measurements were carried out on UV-visible spectrophotometer and absorbance was measured between 300-600 nm.

Transmission electron microscope (TEM)

Synthesized AgNPs drop was placed on the carbon coated copper grids and kept for dry After dryness of sample grid loaded on to a specimen holder. TEM images of the sample were taken using the TEM instrument .

Effect of biosynthesized silver nano-particles on seed germination:

Seeds of soybean and safflower were surface sterilized with 1% mercuric chloride solution for 1min. and rinsed several time in sterile distilled water. Clean seeds were per-soaked in 3 days old silver nanoparticles solution of T. harzianum for varying period of time (2hr and 4hr) in undiluted solution. Control containing water in which seeds were pre-soaked was run simultaneously as standard with the experimental flask.

RESULT AND DISCUSSION

Mutagenesis of T.viride

The study relied on a chemical mutation for the wild strain of T.viride to get new strain best of wild strain in some details such as the production of enzymes or other metabolic products, After mutagenic step it has been selected 10 new strains randomly and tested for the purpose of comparable ability to synthesize nano silver particles with wild strain, wild type strain, isolated by its ability to study the synthesis of nanosilver particles was subjected to successive mutagenic treatment with NG and the results showed that success tow mutant strains that coded T. viride Fat13 , T.viride 15 it were abile to synthesize nano silver particles .

Extracellular Synthesis of Silver Nanoparticles

In this investigation, we have exposed for the first time the use of T. veride in the extracellular synthesis of silver nanoparticles. After cell-free filtrate of Wild T. viride and mutants strains T. viride Fat13 , T. viride Fat14, T. viride Has15 were incubated with silver nitrate salt, the color of cell filtrate was showed a gradual change to brown color below dark condition. The color of the Wild T. viride culture filtrate with silver nitrate salt changed to brown after 72hr of incubation whereas the mutants strains T. viride Fat13 and T. viride Has15 showed a

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gradual change to brown color after 4and 3 hour respectively, while T. viride Fat14 culture cell filtrate was not showed a any change to color that could have led to the development of more diverse genetically changes in T. viride ,control (deprived of silver nitrate salt did not show any color change. Figure-1 shows a conical flask of the fungal cells after elimination from the culture medium and before involvement in 1 mM AgNO3 solution. The entrance of brown color in solution containing the biomass is a pure indication of the development of silver nanoparticles in the reaction mixture. The color of the solution is owing to the excitation of surface plasmon vibrations (fundamentally the vibration of the assembly conduction electrons) in the silver nanoparticles. In the biosynthesis of metal nanoparticle by a fungus, enzymes are formed which decrease a salt to its metallic solid nanoparticles through the catalytic effect. Associated to other filamentous fungus, the *Trichoderma* is considered to be the most efficient extracellular enzyme producer, and has a extended history in the manufacture of manufacturing enzymes (Oksanen et al.,2000). The silver nanoparticles were formed very rapidly within 30 min Fayaz et al. (2010) have reported the extracellular synthesis of silver nanoparticles by T. viride after 4 h of incubation .

B **A**

Figur1: (A) Chloroauric acid solution (B) crude cell filtrate after immersion of 1mM aqueous solution of AgNo3

Ultraviolet-Visible (UV-Vis) Spectroscopy

UV-Vis spectroscopy to shadow up with the reaction procedure. The spectra recorded from the wild and mutant strains of T. viride reaction container at diverse reaction times are described in Figure 2 3,4 and 5, no absorption band absorbed in the control treatment. we have been measuring the absorbance of each isolation every 4 hours. The results showed that the summit was the absorption after 12 hours in the mutant strain T. viride Has15 its have highest absoription band it was(0.674) followed by T. viride Fat 13 (0.6120) after 20 hours too, while in wild T.viride strain it was (0.511) after 72 hours. The durable surface plasmon resonance centered at ca. 412-420 nm, This peak increased from 412 to 420 nm as the reaction continued.

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The spectra obviously show the upsurge in intensity of silver solution with period, representative the formation of augmented number of silver nanoparticles in the solution.

The silver nanoparticles were designed very fast within 30 min Fayaz et al. [2010] have described the extracellular synthesis of silver nanoparticles using T. viride after 4 h of incubation. This marvel of blue shift of absorption advantage has been credited to a decrease in particle size This results in a shift in the absorption advantage to a lower wavelength area. Also, nanoparticles are highly sensitive and functionally effectual because of minor grain size and high surface to capacity ratio as likened to the conservative materials in micrometer range where in event of nano-scale materials most atoms, ions and flaws would be on the surface (Gherbawy et al.,2013). The reaction It should be pointed out it was permissible to continue for about one month fascinatingly, the solution was tremendously steady even after a month of reaction, with no signal of combination of particles because the figures showed that there is no considerable change in the UV-Vis spectra of the reaction produce after 72 hours revealing of the detail that reaction originated to symmetry at about 72 hours. Fayaza, et al.,(2010) found that the absorption from the spectra of T. viride synthesized silver nano solution, that the silver surface plasmon band occurs at 405 nm in addition to prominent band at around 260 nm. Chitra and Annadurai(2013) show that The absorption peak in UV spectrum corresponding to the surface plasmon resonance, the peak originate at 400 nm, and the maximum synthesis of silver nanoparticles happened at 96 h of incubation to T. viride cell filtrate.

Transmission Electron Microscopy

TEM micrograph providing comprehensive morphology of silver nanoparticles. The figures attained from micrograph showed dissimilar shape and size of nanoparticles , TEM picture detailed from the silver nanoparticle film dropped on a carbon coated copper TEM grid. This picture shows different silver nanoparticles as well as a number of collections. The morphology of the nanoparticles is highly variable. Under observation of such images, these assemblies were found to be aggregates of silver nanoparticles in the size range 5–50 nm prouduced from T. viride Has15 ,while it was in the size range 6-60 and 8-80 in T. viride Fat 13 and wild T.veride strain. Chitra and Annadurai (2013) study found that the silver nanoparticles demonstrations their appearance centered cubic structure, and the synthesized silver nanoparticles are in crystalline countryside. This is the first study to explosion on silver nanoparticle synthesis by T.viride resultant in nano bowl-shaped nanoparticles. silver nanoparticles synthesized by richoderma viride presented production in the range of 320–520 nm wavelength (Fayaza, et al.,2010). The morphology of the nanoparticles manufactured byTrichoderma reesei was very mutable and these assemblies were originate to be aggregates of silver nanoparticles in the size range 5–50 nm (Vahabi, et al.,2011). AgNPs synthesized by dissimilar species of Trichoderma were create single or aggregated with round and uniform in shape and 6–80 nm in size (Devi, et al.,2013). Effectiveness of AgNPs biosynthesis was described owing to reductase action or by electron shuttle quinones or together (Duran, et al.,2005).

Influence of silver nanoparticles on Chickpea and broad bean germination:

The results suggest in the table below as positively impacting clear each of the strain parental T.veride as well as mutagenic strains, as it resulted in the treatment of seeds of Chickpea and Broad bean with a solution of silver nanoparticles for a period of 4 hours to amazing results at the speed of seed germination in comparison with control by seed treatment has been the water sterile distilled Just. Results signposts that increasing the soaked

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period of silver nanoparticles solution, will intensification the germination of soybean and sunflower which specifies that's seed germination is directly comparative to soaking period of silver nanoparticles solution (Figure-7).

Table 1: Influence of silver nanoparticles on seed germination:

treatmentes	%Chickpea germination	%broad bean germination
control	7.8	9.5
Wild T.veride	7.5	8.9
T. viride Has15	6.9	7.5
T. viride Fat13	7.7	9.5

The results indicate no clearly effect to influence the solution of silver nanoparticles to break the soaking period in both the seeds of broad bean and chickpea seeds, Which led to differences were not significant increase seed germination rate when treated with a solution of silver nanoparticles dramatically in comparison with control.

Xiu et al.,(2012) found that the AgNPs may aggregate or be completed by ligands which can foundation a reduced in toxicity and would clue to subordinate experience to seeds and seedlings. Lin and Xing(2007) found that the two nanoscale metal oxides of Al and Zn had, commonly, no understandable influence on seed germination of six species usually charity in phytotoxity research. This is consistent with other studiesof Castiglione and Cremonine (2009) , Yin et al ., (2012) that report NPs of (Ag, Zn and Al) had less of an effect on seed germination.

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