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Effect of carbon and nitrogen sources in the medium on silver nano conversion and assessment of silvernano conversion principle in culture filtrate

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Abstract
In order to assess the effect of carbon and nitrogen sources in the medium on nano convertibility, synthetic medium *viz*., Czepek Dox medium (modified) was used. Five different nitrogen sources *viz*., Ammonium nitrate, Casein, Glycine, Leucin and Sodium nitrate and five different carbon sources *viz*., Chitin, Carboxy Methyl Cellulose (CMC), Sucrose, Glucose and Lactose were assessed for their effect on the growth of *Trichoderma* ET-1 and the ability of culture filtrate in nano conversion. In order to have an initial idea about the principle responsible for nano conversion, three different solvents *viz*., diethyl ether, n-butanol and ethyl acetate and were used to extract the principle from *Trichoderma* ET-1 culture filtrate prepared in PDB. Silver bionano particles synthesized using diethyl ether solvent fraction at 0.0154% concentration were analysed for their stability up to 14 days in terms of absorption peaks using UV-VIS spectrophotometer. It may inferred from the experiment that cellulases play a role in nano conversion as filtrate from chitin as sole C source (and there by chitinases in culture filtrate) failed to yield satisfactory yield of bionano silver. On the other hand, addition of casein in the broth, the only protein derived N source among the different N sources evaluated, resulted in satisfactory conversion of silver in to bionano silver indicating specificity of proteins in nanoconversion. The dilution end point for converting silver in to nano silver by the solvent extracts was found to be 0.0154% dilution for diethyl ether extract and 0.0298% dilution for ethyl acetate extract. Dilutions more than that did not yield satisfactory nano conversion. The stability of biosilver nano particles was declined after 8 days due to the agglumaration of particles.

Keywords: Chitin, Casein, Ethyl acetate, Biosilver nano particles and Cellulases

Introduction
Nanotechnology is the rapidly developing field in recent era. Silver nanoparticles were synthesized by using different methods such as physical, chemical and biological methods. Silver nano particles synthesis by biological method provides an advantage of environmental friendly, low cost and rapid method. Nanotechnology literally means any technology on a nanoscale material that has applications in the real world. Nanotechnology encompasses the production and application of physical, chemical, and biological systems at scales ranging from small individual atoms or molecules to submicron dimensions, as well as the integration of the resulting nanostructures into larger systems. Nanotechnology is likely to have a profound impact on our economy and society in the early 21st century, comparable to that of semiconductor technology, information technology, or cellular and molecular biology (Prakasha *et al*., 2013). Science and technology research in nanotechnology promises breakthroughs in areas such as materials and manufacturing, nanoelectronics, medicine and healthcare, energy, biotechnology, information technology, and national security. It is widely felt that nanotechnology will be the next Industrial Revolution. Several chemical methods have been developed for the synthesis of silver nanoparticles including chemical reduction, aqueous solution chemical reduction, nona queous chemical reduction, the template method, electrochemical reduction, ultrasonic-assisted reduction, photo-induced or photo-catalytic reduction, microwave assisted synthesis, irradiation reduction, the microemulsion method, biochemical method etc., but these chemical methods have been reported along with various drawbacks, including the use of toxic solvents, generation of hazardous by-products, and high energy consumption, which pose potential risks to human health and to the environment (Sathyavathi *et al*., 2010; Awwad *et al*., 2013 and Bar *et al*., 2009). Currently, there is a growing need to develop an environment friendly nanoparticle synthesis that does not use toxic chemicals in the process of its synthesis. The microbial-mediated biological synthesis of metallic nanoparticles has recently been recognized as a promising source for mining

nanomaterials (Badr *et al*., 2008) The microbial recovery of precious metals with the formation of their nanoparticles is a green alternative to the conventional method. Biosynthesis of silver nanoparticles using bacteria, fungi, and plants are already well-documented. However, the exploration of actinomycetes has recently gained interest for the efficient biological synthesis of metallic nanoparticles. The bonding reaction between the antibiotic and nanoparticles enhances the inhibition effect against the test organisms. The antibiotic molecules contain many active groups such as hydroxyl and amide groups, which react easily with nanosilver by chelation, and helps in effective inhibition (Brust *et al*., 1994 and Faure *et al*., 2003). In the present investigation, we examined extracellular biosynthesis of silver nanoparticles (AgNPs) from fungal bio control agent *Trichoderma* spp strain ET-1, also assessed the effect of carbon and nitrogen sources in the medium on nano convertibility and in order to have an initial idea about the principle responsible for nano conversion, three different solvents *viz*., diethyl ether, n-butanol and ethyl acetate and were used to extract the principle from *Trichoderma* ET-1 culture filtrate prepared in PDB.

Material and Methods

Effect of carbon and nitrogen sources in the medium on silver nano conversion

To assess the effect of carbon and nitrogen sources in the medium on nano convertibility, synthetic medium *viz*., Czepek Dox medium was used as a basal medium and carbon and nitrogen sources of the medium were replaced by the five different nitrogen sources *viz*., Ammonium nitrate, Casein, Glycine, Leucin and Sodium nitrate and five different carbon sources *viz*., Chitin, Carboxy Methyl Cellulose (CMC), Sucrose, Glucose and Lactose. *Trichoderma* isolate ET-1 were inoculated in the prepared broth medium (50 ml, three replications maintained) in 250 ml conical flask and allowed to grow for five days. Then, the mycelium was harvested and fresh weight and dry weights were assessed to know the effect of different sources on the growth of *Trichoderma* ET-1. Finally the culture filtrate was filtered through whattman filter paper 1 and bacterial proof filter, filtered culture filtrate was used to assess the ability in nano conversion.

90 ml of aqueous solution of 1mM Silver nitrate ($AgNO_3$) (equivalent to 170 ppm) was mixed with 10 ml of culture filtrate for the extra cellular synthesis of silver nanoparticles in a 250 ml conical flask. The whole mixture was incubated at room temperature for 24 hrs. The color change of silver nitrate from colorless to brown color was considered as indicator of formation of silver nanoparticles through reduction of silver ions from Ag^+ to Ag^0 and nanoconversion was confirmed by UV-VIS spectrophotometer.

Elucidation of nano conversion principle using solvent extraction method

Isolation and identification of nano conversion principle from *Trichoderma* spp. culture filtrate was done using solvent extraction method.

Solvents used for the extraction

1. Di ethyl ether
2. Ethyl acetate
3. Butanol

Method: one week old 50 ml culture filtrate of *Trichoderma* and 50 ml solvent was poured in to the 500 ml separating funnel and closed the cap of separating funnel. Then the separating funnel was shaken for 1 hour. The pressure built in the separating funnel was released by gently loosing the cap. After shaking, venting and stabilization of solvent and aqueous layers were collected separately in to the conical flasks. This process was repeated with all the solvents separately and the solvent portion was poured in to the Petri plates and kept 24 hours to evaporate the solvent under fume hood. The left over solid material was collected and used for nano conversion ability using U.V. Spectrophotometer.

Finding minimum dilution end point of nano conversion principle from antagonist culture filtrate

The extracted solid material was quantified and serial diluted to 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001, 0.0000001 dilutions. Ten ml of each dilution was mixed with 90ml of 170 ppm $AgNo_3$ and observed for nano conversion using UV-VIS Spectroscopy.

Results and Discussion

Effect of carbon and nitrogen sources in the medium on silver nano conversion

In order to assess the effect of carbon and nitrogen sources in the medium on nano convertibility, synthetic medium *viz*., Czepek Dox medium was used. Five different nitrogen sources *viz*., Ammonium nitrate, Casein, Glycine, Leucin and Sodium nitrate and five different carbon sources *viz*., Chitin, Carboxy Methyl Cellulose (CMC), Sucrose, Glucose and Lactose were assessed for their effect on the growth of *Trichoderma* ET-1 and the ability of culture filtrate in nano conversion.

When fresh and dry weight of *Trichoderma* ET-1 in 50 ml of Czepek Dox broth (amended or not) was recorded, among different C sources tested, lactose was found better in supporting the growth of *Trichoderma* ET-1 in terms of fresh weight (3.00g) and dry weight (0.61g). Chitin (1.13g fresh weight and 0.43g dry weight) and CMC (1.63g fresh weight and 0.40g dry weight) supported least. Among the N sources, ammonium nitrate supported *Trichoderma* ET-1 growth maximum (2.66g fresh weight and 0.56g dry weight). Least support among the N sources was by leucine (1.20g fresh weight and 0.43g dry weight) Fig 1.

With all the C and N sources tested, colour change was observed in silver nitrate solution indicating nanoconversion. However, among the different nitrogen sources, casein alone could produce distinct peak at 450 nm (UV-Spectrophotometer) Fig. 2 representing nano conversion while in all others only peaks representing silver nitrate at 220 nm could be detected. Though with other nitrogen sources peaks were obtained but they were very faint and distributed from 300 to 600 nm. Among the carbon sources, CMC was found to give a distinct peak at 420 nm Fig 3 while in all others only peaks representing silver nitrate at 220 nm and another at around 250 nm were visible. Though with other carbon sources peaks were obtained but were very faint and distributed from 350 to 650 nm. Thus the present investigation revealed no correlation between growth and ability of nanoconversion as evidenced by the fact that lactose and ammonium nitrate that supported maximum growth of *Trichoderma* ET-1 failed to yield satisfactory nanoconversion. (Kabasiyan *et al*., 2008; Janardhan *et al*., 2009 and Elgobran *et al*., 2015) reported variation in nano conversion due to different media. (Yamanaka *et al*., 2005 and Vahabi *et al*., 2011) stated that certain agriculturally important micro organisms were exposed to metal salt solution which may prompts to produce enzymes and metabolites which may play a role in nano conversion.

It may be noted here that among the N sources substituted for

N in Czepek Dox broth, only casein could induce nano conversion. Further, casein is the protein used as an N source in the present investigation. All others, *i. e.*, glycine and leucine (aminoacids), ammonium nitrate and sodium nitrate (inorganic N sources) failed to effect nano conversion of silver nitrate, indicating involvement of protein rich nutrients in nanoconversion. (Vahabi *et al*., 2011 and Afreen and Vandana, 2011) through FTIR analysis indicated role of proteins in nanoconversion.

The present investigation indicated that glucose, sucrose and lactose failed to yield substantial nanoconversion of silver nitrate. Further, chitin also failed to yield nanosilver compared to CMC. This indicated that proteins such as cellulases may play a better role in nano conversion than chitinases. Accordingly, the present investigation highlights the importance of proteins in nanoconversion. With PDB and NB, proteins present in potato and beef extract may be responsible for nanoconversion. However, nanoconversion with CMC as sole C source indicated that protein from the test *Trichoderma* ET-1 such as celluloses are responsible for nanoconversion.

Assessment of silvernano conversion principle in culture filtrates using solvent extraction method

In order to have an initial idea about the principle responsible for nano conversion, three different solvents *viz*., diethyl ether, n-butanol and ethyl acetate and were used to extract the principle from *Trichoderma* ET-1 culture filtrate prepared in PDB. Individual solvent extract was dried to obtain the principle in solid form.

When the solid obtained after solvent extraction was mixed in 10 ml of water and then added to 90 ml of 170 ppm silver nitrate solution, brown coloured solution was obtained only with diethyl ether extract and ethyl acetate extract which signified conversion of silver nitrate in to silver nano. In case of n-butanol extract, no crystals or amorphous form was obtained and hence n-butanol was deleted for further studies.

In order to further assess the dilution end point of diethyl ether extract and ethyl acetate extract, known quantity of the solid extracts were mixed in 10 ml water and used for silver nano conversion.

When 0.0154 g of diethyl ether extract was mixed with 10 ml of water and diluted upto 10^{-14}, only 0.154% and 0.0154% dilutions gave a distinct peak at 420 nm while in other dilutions a faint peak was obtained. Hence, for diethyl ether 0.0154% dilution was considered as dilution end point for converting silver nitrate into silver nano.

When 0.029 g of ethyl acetate extract was mixed with 10 ml of water and diluted up to 10^{-14}, only 0.298% and 0.0298% dilutions gave a distinct peak at 420 nm while in other dilutions a faint peak was obtained. Hence, for ethyl acetate 0.0298% dilution was considered as dilution end point for converting silver nitrate into silver nano.

Stability of silver bionano particles

Silver bionano particles synthesized using diethyl ether solvent fraction at 0.0154% concentration were analysed for their stability up to 14 days in terms of absorption peaks using UV-VIS spectrophotometer.

Result obtained indicated that the absorption peak lied between 408nm (on day1) to 450nm (on day 8). Beyond eight days, the peak moved between 500 to 600nm indicating lack of stability of colloidal silver bionano synthesized and agglomeration of silver bionanoparticles. (Pinto *et al*. 2008) reported that nanoparticle stability depended on storage conditions and the stability is quickly lost at room temperature compared to 4^{O}C.

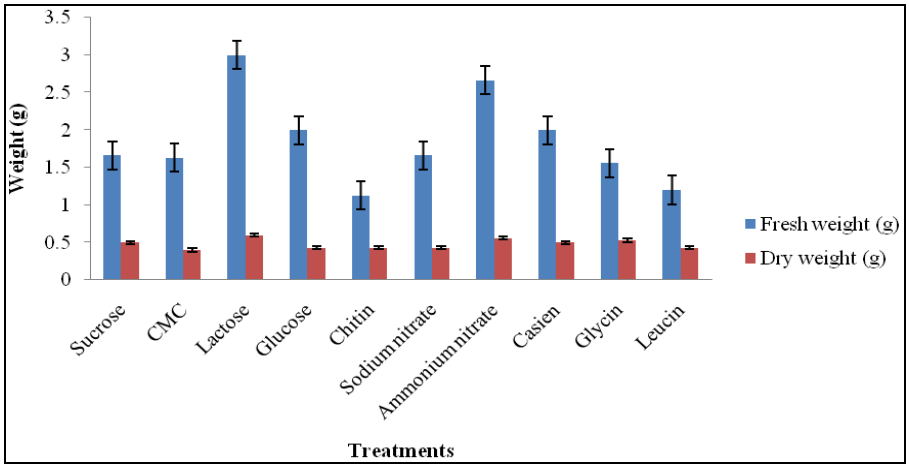

Fig 1: Fresh weight, Dry weights of *Trichoderma* in Modified Czepekdox agar (different C and N sources)

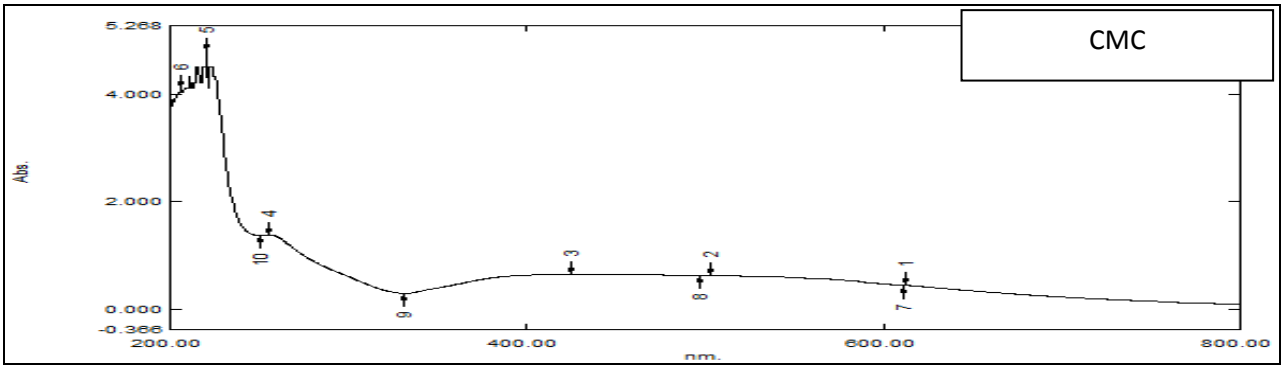

Fig 2: Effect of carbon source CMC in the medium on nanoconversion by *Trichoderma* ET-1

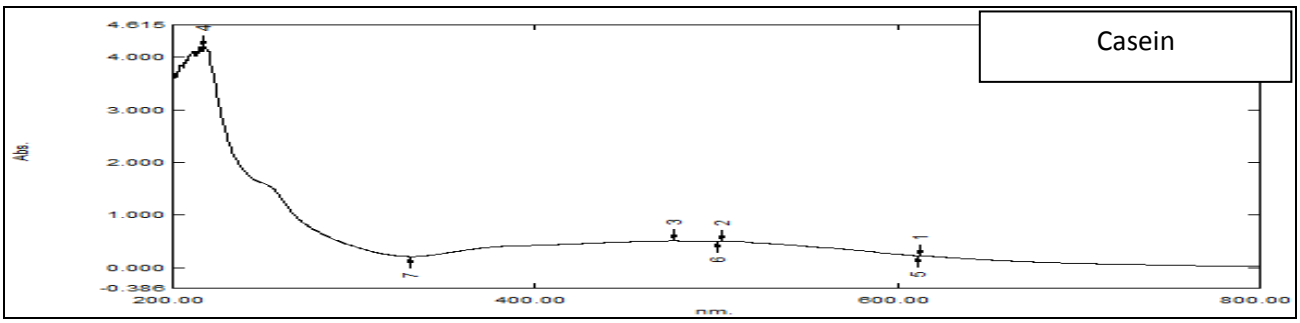

Fig 3: Effect of nitrogen source casein in the medium on nanoconversion by *Trichoderma* ET-1

Summery and Conclusion

When five carbon and five nitrogen sources added separately in Czepek Dox broth and assessed for their impact on nano convertibility by ET-1, five day old culture filtrates from casein as the sole N source and cellulose as the sole C source were found to yield higher concentrations of bionano silver as observed from the distinct absorbance peaks formed at 450nm and 420nm respectively. With others though colour change of silver nitrate solution was observed, the peaks were very faint and distributed from 300 to 600 nm. It may also be inferred from the experiment that cellulases play a role in nano conversion as filtrate from chitin as sole C source (and there by chitinases in culture filtrate) failed to yield satisfactory yield of bionano silver. On the other hand, addition of casein in the broth, the only protein derived N source among the different N sources evaluated, resulted in satisfactory conversion of silver in to bionano silver indicating specificity of proteins in nanoconversion.

When the 5 day old PDB culture filtrate of ET-1 was extracted using three different solvents separately, ethyl acetate and diethyl ether extract gave crystalline particles that could convert silver nitrate in to nano silver. Further, the dilution end point for converting silver in to nano silver by the solvent extracts was found to be 0.0154% dilution for diethyl ether extract and 0.0298% dilution for ethyl acetate extract. Dilutions more than that did not yield satisfactory nano conversion.

The stability of synthesized bionano silver was found to be up to eight days when silver bionano particles synthesized using diethyl ether solvent extract at 0.0154% concentration was analyzed for their stability in terms of absorption peaks (408nm on day1 to 450nm on day 8) using UV-VIS spectrophotometer. Beyond eight days, the peak moved between 500 to 600nm indicating lack of stability of colloidal bionano silver synthesized resulting in agglomeration of bionano silver particles.

References

1. Afreen B, Vandana R. Synthesis and characterization of silver nanoparticles by *Rhizopus stolonier*. International Journal of Biomedical and Advance Research. 2011, 148-158.
2. Awwad AM, Salem NM, Abdeen AO. Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity. International Journal of Indusustrial Chemistry. 2013; 4(5):1-6.
3. Badr Y, Wahed E, Mahmoud MG. Photo catalytic degradation of methyl red dye by silica nanoparticles. Journal of hazardous materials. 2008; 154(7):245-253.
4. Bar H, Bhui DK, Sahoo GP, Sarkar P, Priyanka S. Green synthesis of silver nanoparticles using seed extract of Jatropha curcas. Colloids and Surfaces A: Physicochemical and Engineering. 2009; 348:212-216.
5. Brust M, Walker M, Bethell D, Schiffrin DJ, Whyman R. Synthesis of thiol derivatized gold nanoparticles in a two-phase liquid-liquid system. Journal of the Chemical Society, Chemical Communications. 1994; 8:801-802.
6. Elgorban AM, Samawaty AEME, Yassin MA, Sayed SR, Adil SF, Elhindi M *et al*. Antifungal silver nanoparticles: synthesis, characterization and biological evaluation. Biotechnology & Biotechnological equipment. agriculture and environmental biotechnology.1-7 online journal. 2015.
7. Faure C, Derre A, Neri W. Spontaneous formation of silver nanoparticles in multilamellar vesicles. Journal of Physical Chemistry. 2003; 107:4515-4526.
8. Janardhanan R, Karuppaiah M, Hebalkar N, Rao TN. Synthesis and surface chemistry of nano silver particles. *Polyhedron.* 2009; 28:2522.
9. Kabasiyan L, Sang-Woo Kim, Jin Hee Jung, Yun Seok Kim, Kyoung Su Kim, Youn. Application of silver nanoparticles for the control of *Colletotrichum* species *in vitro* and pepper anthracnose disease in field. *Mycobiology*. 2008; 39(3):194-199.
10. Prakasha P, Gnanaprakasama P, Emmanuela R, Arokiyarajb S, Saravanan M. Green synthesis of silver nanoparticles from leaf extract of *Mimusops elengi*, Linn. For enhanced antibacterial activity against multi drug resistant clinical isolates. *Colloids Surf B: Biointerfaces*. 2013; 108:255-259.
11. Sathyavathi R, Krishna MB, Rao SV, Saritha R, Rao DN. Biosynthesis of silver nanoparticles using Coriandrum sativum leaf extract and their application in nonlinear optics. *Advanced Science Letters*. 2010; 3(4):138-143.
12. Vahabi K, Mansoor A, Karimi S. Biosynthesis of Silver nanoparticles by fungus Trichoderma reesei. Insciences Journal. 2011; 110:65-79.