Research Paper

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Biosynthesis of Metal Nanoparticles From Fungal Isolates of Soybean Rhizosphere

Agriculture

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ABSTRACT

The development of eco-friendly and economical technologies in material synthesis is of considerable importance to expand their biological applications. Nowadays, a variety of inorganic nanoparticles have been synthesized using chemical methods and their applications in many cutting-edge technological areas have been explored. The current reports and future prospects for the synthesis of metal nanoparticles by microorganisms are scanty due to the oligo dynamic property of metals. The present study highlights the bio-synthesis of metal nanoparticles from metal resistance fungal species isolates from soybean rhizosphere. Total 22 different fungal strains were isolated and identified belonging to; Aspergillus, Penicillium and Fusarium species respectively. All the isolates were tested for extracellular nanoparticle synthesis. The metal nanoparticles were detected by biological reduction process and further characterized adopting spectrophotometry for monitoring surface plasmon resonance. Out of 22 fungal isolates, 5 isolates showed the considerable synthesis of Ag, Zn, Mn and Cu nanoparticles respectively this indicates the potential of fungi to reduce the metal salts. The present study indicates the possibilities of fungal transformation method for the synthesis of nanoparticles as an effective and economical method over chemical synthesis.

Introduction

Recently bionanotechnology has emerged in developing green and ecofriendly technology for synthesis of nanoparticles of variable size, shapes, chemical composition and controlled dispersity. Nanoparticles show significant change in their physical and chemical properties, sometimes completely new phenomenon rather than their metal ions. The reduction in particle size of the material is an efficient and reliable tool for improving biocompatibility of metal nanoparticle (Mirkin and Taton, 2000; Kim et al., 2001). A wide variety of physical and chemical methods are used to synthesize nanoparticles; however their use is inappropriate in biological systems due to use of toxic solvents and generation of hazardous by-products. The disadvantages of physical and chemical method forced the use of green routes for synthesizing nanoparticles with high-yielding, low cost, non-toxic and environment friendly properties.

Eukaryotic organisms such as fungi are extremely good candidate for the synthesis of metal nanoparticles. The fungi give nanoparticles with good monodispersity and well-defined dimensions. Recently several studies have been shown that metal nanoparticles can be synthesized by using various fungi like, Aspergillus fumigatus and Fusarium semitectum (Basavaraja et al., 2007), Alternaria alternata (Gajbhiye et al., 2009), Aspergillus niger (Sadowski et al., 2008a; Sadowski et al., 2008b), Penicillium fellutanum (Kathiresan et al., 2009) and Cladosporium cladosporioides (Balaji et al., 2009).

The silver nanoparticles production capacity has been depended on the reductase/electron shuttle relationships. The reduction of metal ions occurs on the surface by the enzymes which are present in the cell wall of fungi (Mukherjee et al., 2001). Silver nanoparticles and silver based compounds are highly toxic to microorganisms (Slawson et al., 1992). It has been experimentally first proved by Waghmare et al., (2011), that Manganese nanoparticles (MnNPs) and Zinc nanoparticles (ZnNPs) with well-defined dimensions can be obtained by using Actinomycetes. Ahmad et al., (2005), reported the extracellular synthesis silver and gold–silver nanoparticles by using fungus Fusarium oxysporum. Pavani et al., (2009), synthesized stable copper nanoparticles (CuNPs) using culture filtrates of Aspergillus species, a commonly available fungus found in soil. Kathiresan et al., (2009), exploited the marine fungus, Penicillium fellutanum from mangrove sediment for synthesis of stable silver nanoparticles.

Primary requirements for the potential use of biologically synthesized metal nanoparticles include more information about green rout for synthesis of silver nanoparticles and the development of better strategies to increase the production of metal nanoparticles. The present study deals with the biosynthesis of Silver nanoparticles (AgNPs), Zinc nanoparticles (ZnNPs), Manganese nanoparticles (MnNPs) and Copper nanoparticles (CuNPs) using fungal isolates of soybean rhizosphere.

Material & Method

Isolation of Fungus:-

Isolation of fungal strain was done using composite soil sample collected from soybean rhizosphere of healthy plant. The soil sample was serially diluted in sterilized distilled water and transferred aseptically to PDA containing 0.1 mM of $AgNO_3$, $ZnSO_4$, $MnSO_4$ and $CuSO_4$, separately and incubated for 96 h at room temperature followed by sub culturing for pure culture on PDA. Further all the isolated strains of fungi were examined by slide culture method (Saeed et al., 2012).

Preparation of fungal cell free filtrates.

Isolated fungi were separately cultured in 250 ml flask containing 100ml MYPG medium, composed of malt extract (3gr) peptone(5gr), glucose (10gr) and yeast extract (3gr) per litter respectively (Ahmad et al., 2003). The fungal cultures were incubated on orbital shaker with 150-rpm at 28^0C for 96 h. After 96h incubation, fungal biomass were separated from MYPG broth by filtration followed by centrifugation at 3500 rpm at 10^0C for 20 minutes and the supernatant were used for the synthesis of metal nanoparticles.

Synthesis Metal Nanoparticles

10 ml supernatant from each fungi was mixed with 10 ml of 1 Mm aqueous solution of AgNO3, ZnSO4, $MnSO_4$ and $CuSO_4$, separately by adjusting pH 6.5 using 1N, HCL and agitated at 28^0C for 72h along with control (without the supernatant). The formation of nanoparticles was indicated by colour change from yellow to dark brown. Further centrifugation at 15000 rpm for 30 min was carried out to remove non protein part. After centrifugation the metal nanoparticle were collected from upper layer of aqueous solution.

Result & Discussion

Characterization of Metal Resistant fungal isolates:

The characterisation of metal resistance fungal cultures was done adopting conventional method from the result (Table 1) it was observed that, among (22) metal resistance fungi isolated,

total (11) isolates were Aspergillus species and was predominance as metal resistance fungi in rhizospheric soil of soybean followed by (6) Penicillium and (5) Fusarium species respectively. Similar reports on existence of metal resistance fungi viz; Aspergillus, Penicillium and Fusarium species respectively in rhizospheric soil samples was reported by Saeed et al., (2012); Kathiresan et al., (2009) and Basavaraja et al., (2007).

Synthesis and characterization of silver nanoparticles:

In the present study, different metal nanoparticles were synthesized using a reduction of respective aqueous metal solution with the culture supernatant of isolated fungus at room temperature. The colour changes from yellow to dark brown indicated synthesis of metal nanoparticles as compared to control without supernatant in which no colour changes at same conditions was observed (Plate 1). It was generally recognized that metal nanoparticles produced brown colour due to the surface plasmon resonances (SPR) effect followed by reduction of metal ions (Wiley et al., 2006). The ultraviolet-visible spectra of the cell filtrate with AgNPs showed λ max at 425 nm, with ZnNPs at 385 nm, with MnNPs at 360nm where as with CuNPs the λ max recorded at 315 nm which indicated the presence of respective metal nanoparticles. The results are in accordance with the reports of (Saeed et al., 2012; Waghmare et al., 2011; Pavani et al., 2013). The colour intensity of the cell filtrates was sustained even after 48 h incubation, which indicated that the particles were well dispersed in the solution and without no obvious aggregation. From the result it was also observed that (Table 2 & Plate 2), among (22) metal resistance isolated fungi, total five isolates (3) of Aspergillus species showed the ability to transformed the multiple nanoparticles and was predominant in total population as metal resistance nanoparticles synthesizing fungi in rhizospheric soil of soybean followed by Penicillium and Fusarium (1) each species respectively, showed the ability to transformed the multiple nanoparticles viz; Ag, Zn, Cu, & Mn nanoparticles respectively.

Conclusions

The present study demonstrated the biosynthesis of Ag Zn Mn and Cu nanoparticles by using fungal isolates of soybean rhizosphere. The research helps in finding better biosynthesis methods for production of metal nanoparticles. The present investigation also gives the green method for synthesis of various metal nanoparticles as safe, economical and ecofriendly method over conventional methods like chemical and thermal synthesis which show adverse effects on the environment. However, further research should focus on optimisation of condition for large scale production of metal nanoparticles. It is also a point of quest to check whether the extra-cellular machinery of the fungi involved in nanoparticle synthesis, contribute any special properties to metal nanoparticles by imparting any additional anti-microbial properties or is it just a mere reduction of the ionic metal.

Table 1: Characterization of Metal Resistant Fungal Isolates.

Sr. No	Surface	Reverse	Size	Shape	Nature	Conidiosphere	Hyphae	Elevation	Growth Rate	Possible Species
1.	Black	Yellow	21-30 mm	Circular	Cottony	Unbranched	Absent	Sepiod	Flat	Fast	*Aspergillus sp.*
2.	Pale Yellow	Brownish orange	21-23 mm	Irregular	Velvety	Branched	Present	Sepiod	Raise	Fast	*Penicillium sp.*
3.	Brown	Yellow	20-23 mm	Circular	Cottony	Unbranched	Absent	Sepiod	Rise	Fast	*Aspergillus sp.*
4.	Greenish green	Yellowish brown	20-22 mm	Irregular	Velvety	Branched	Present	Sepiod	Flat	Fast	*Penicillium sp.*
5.	White	Pale yellow	21-30 mm	Irregular	Cottony	Branched	Present	Sepiod	Raise	Slow	*Fusarium sp.*
6.	White	Greenish green	21-28 mm	Irregular	Velvety	Branched	Present	Sepiod	Rise	Fast	*Penicillium sp.*
7.	Brown	Brown	20-25 mm	Circular	Cottony	Unbranched	Absent	Sepiod	Flat	Fast	*Aspergillus sp.*
8.	Greenish white	Golden	17-20 mm	Irregular	Velvety	Branched	Present	Sepiod	Rise	Fast	*Penicillium sp.*
9.	Yellowish green	Golden brown	35-40 mm	Circular	Cottony	Unbranched	Present	Sepiod	Flat	Fast	*Aspergillus sp.*
10.	Creamish pink	Red	30-40 mm	Circular	Cottony	Branched	Present	Sepiod	Rise	Slow	*Fusarium sp.*
11.	Greenish black	Yellowish brown	24-28 mm	Irregular	Cottony	Unbranched	Absent	Sepiod	Rise	Fast	*Aspergillus sp.*
12.	White	Brown	40-45 mm	Circular	Cottony	Branched	Present	Sepiod	Rise	Slow	*Fusarium sp.*
13.	Black	Yellowish brown	22-28 mm	Circular	Cottony	Unbranched	Absent	Sepiod	Rise	Fast	*Aspergillus sp.*
14.	Yellowish green	Golden brown	32-38 mm	Circular	Cottony	Unbranched	Present	Sepiod	Flat	Fast	*Aspergillus sp.*
15.	Creamish	Brown to Red	34-40 mm	Circular	Cottony	Branched	Present	Sepiod	Rise	Slow	*Fusarium sp.*
16.	Yellowish white	Greenish yellow	11-14 mm	Irregular	Velvety	Branched	Present	Sepiod	Raise	Fast	*Penicillium sp.*
17.	Brown	Yellowish brown	20-23 mm	Irregular	Cottony	Unbranched	Absent	Sepiod	Raise	Fast	*Aspergillus sp.*
18.	Yellowish brown	Golden red	28-34 mm	Circular	Cottony	Unbranched	Present	Sepiod	Rise	Fast	*Aspergillus sp.*
19.	Cinnamon	White	28-42 mm	Circular	Powdery	Unbranched	Absent	Sepiod	Flat	Fast	*Aspergillus sp.*
20.	Yellowish white	Golden	17-20 mm	Irregular	Velvety	Branched	Present	Sepiod	Rise	Fast	*Penicillium sp.*
21.	White to Yellow	Yellowish brown	30-38 mm	Circular	Cottony	Branched	Present	Sepiod	Rise	Slow	*Fusarium sp.*
22.	Cinnamon	Brown	28-33 mm	Circular	Powdery	Unbranched	Absent	Sepiod	Rise	Fast	*Aspergillus sp.*

Table 2: Fungal isolates synthesizing multiple Nanoparticles.

Sr. No.	Isolate Nomination No:	Possible Species	Positive For AgNPs Synthesis	Positive For ZnNPs Synthesis	Positive For MnNPs Synthesis	Positive For CuNPs Synthesis	Efficient species
1	DKF 1	*Aspergillus sp.*	+VE	+VE	+VE	+VE	4
2	DKF 2	*Penicillium sp.*	+ VE	+VE	- VE	+VE	3
3	DKF 3	*Aspergillus sp.*	+VE	- VE	- VE	- VE	1
4	DKF 4	*Penicillium sp.*	+VE	+VE	+VE	+VE	4
5	DKF 5	*Fusarium sp.*	-VE	+VE	-VE	- VE	1
6	DKF 6	*Penicillium sp.*	+ VE	- VE	- VE	- VE	1
7	DKF 7	*Aspergillus sp.*	+VE	+VE	+VE	+VE	4
8	DKF 8	*Penicillium sp.*	+ VE	- VE	- VE	- VE	1
9	DKF 9	*Aspergillus sp.*	+VE	+VE	+VE	+VE	4
10	DKF10	*Fusarium sp.*	+VE	- VE	- VE	+VE	2
11	DKF11	*Aspergillus sp.*	+VE	- VE	-VE	+VE	2
12	DKF12	*Fusarium sp.*	- VE	- VE	- VE	- VE	0
13	DKF13	*Aspergillus sp.*	+VE	- VE	+VE	- VE	2
14	DKF14	*Aspergillus sp.*	+VE	- VE	- VE	- VE	1
15	DKF15	*Fusarium sp.*	+VE	+VE	+VE	+VE	4
16	DKF16	*Penicillium sp.*	+VE	- VE	- VE	- VE	1
17	DKF17	*Aspergillus sp.*	+VE	+VE	- VE	- VE	2
18	DKF18	*Aspergillus sp.*	+VE	- VE	-VE	+VE	2
19	DKF19	*Aspergillus sp.*	-VE	- VE	- VE	+VE	1
20	DKF20	*Penicillium sp.*	+VE	- VE	- VE	- VE	1
21	DKF21	*Fusarium sp.*	+VE	- VE	-VE	- VE	1
22	DKF22	*Aspergillus sp.*	+VE	- VE	- VE	- VE	1
Total	22		19	08	06	10	05

Value in parenthesis is per cent value.

Plate 1. Colour test for detection of nanoparticles after 48h.

AgNP ZnNP MnNP CuNP

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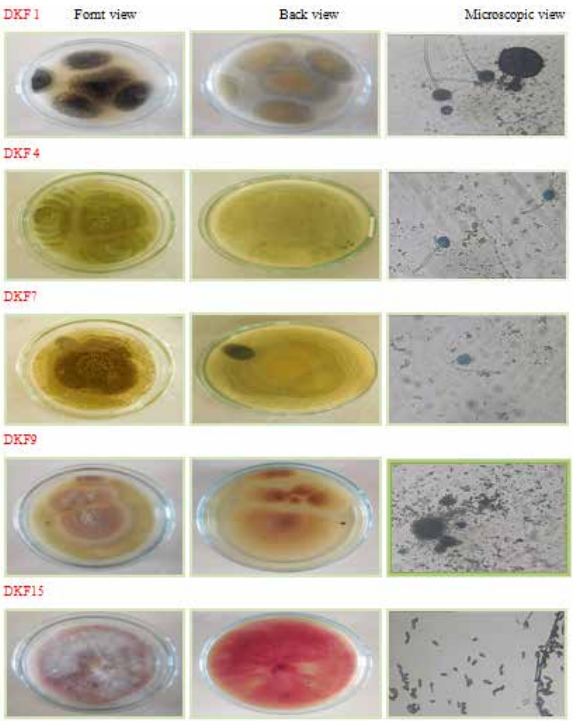
Plate 2. Fungal isolates synthesizing multiple nanoparticles.



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