JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

BIOSYNTHESIS OF Mg AND Mn INTRACELLULAR NANOPARTICLES VIA EXTREMO-METALLOTOLERANT *Pseudomonas stutzeri,* $B_{4\ Mg/W}$ and *Fusarium nygamai,* $F_{4\ Mn/S}$

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doi: 10.15414/jmbfs.2017.6.5.1181-1187

ARTICLE INFO

Received 18. 7. 2016
Revised 3. 2. 2017
Accepted 15. 2. 2017
Published 3. 4. 2017

Regular article

OPEN ACCESS

ABSTRACT

Thirteen microbial isolates were evaluated for green synthesis of Mg and Mn nanoparticles. The isolates were come from soil and wastewater samples from detergent processing industry. Metallotolerance ability of isolates was assessed towards these metals. Bacterial isolate ***$B_{4\ Mg/W}$*** was selected as highly **extremo-tolerant for Mg^{+2}** and can grow between 800 to 15000ppm (80-1500%)and was identified as ***Pseudomonas stutzeri***, $B_{4\ Mg/W}$. Fungal isolate $F_{4\ Mn/S}$ was selected as **extremo-tolerant for Mn^{+2}** and grow in the range 800 to 45000ppm (80-4500%) and was identified as ***Fusarium nygamai,*** $F_{4\ Mn/S}$. Biosynthesis of the Mg and Mn nanoparticles was achieved in both cases extracellular and intracellular. The nanoparticles were characterized using atomic absorption spectrophotometer (AAS), dynamic light scattering (DLS) and transmission electron microscope (TEM). *Pseudomonas stutzeri*, $B_{4\ Mg/W}$ nanoparticle size was ranges from (229.3-553.2 nm) with different mean number for each size, the maximum mean number 33.7% was that of the particles with size 356.2 r. nm and atomic absorption spectrophotometer (AAS) revealed uptake percentage of the metal was 35.17%. *Fusarium nygamai*, $F_{4\ Mn/S}$ nanoparticles ranges from (61.21-127.5 to 412.5 nm) with different mean numbers for each size, the maximum mean number 23.1% was that of the particle size 82.09 nm and range from 23.1 to 28.1 % and AAS was 27.07%. Antimicrobial activity against *Streptococcus pyogenes* RCMB010015, 31.25 and 62.5 mm followed by *Candida albicans* RCMB05035, 15.63 and 62.5; then *Staphylococcus aureus* RCMB010027 and *Eschericia coli* RCMB010056 gave7.81and 62.5mm for both*;* while for *Aspergillus fumigates* RCMB02564 gave the least amount of inhibition 1.95 and 15.63mm*;* moreover *Pseudomonas aeruginosa* RCMB010043 was very resistant for both ***Pseudomonas stutzeri,*** $B_{4\ Mg/W}$ and ***Fusarium nygamai,*** $F_{4\ Mn/S}$ inracellular nanoparticles, respectively.

Keywords: Antimicrobial activity, extremo- metallotolerance, *Fusarium nygamai*, nanoparticles, *Pseudomonas stutzeri*

INTRODUCTION

Nanotechnology considered nowadays as essential basic science due its importance and involvements in all field of technology **(Rai *et al*., 2008)**. Every day there is more and more new achievements in the production and characterization of nanomaterials and its applications **(Sharma *et al*., 2009)**. Synthesis of nanoparticles can be done using several physical and chemical methods but these methods have limitations due to the use of toxic chemicals and controlled conditions as high temperature and pressure **(Rai *et al*., 2008; Birla *et al*., 2009; Sau and Rogach, 2010)**. Biological synthesis of nanoparticles has been proved using microorganisms including bacteria **(Husseiny *et al*., 2007; Shahverdi *et al*., 2007 & 2009)**, fungi **(Kumar *et al*., 2007a&b; Parikh *et al*., 2008; Gajbhiye *et al*., 2009; Govender *et al*., 2009)**, actinomycetes **(Ahmad *et al*., 2003a&b)**, lichens **(Shahi and Patra, 2003)**, algae **(Singaravelu *et al*., 2007; Chakraborty *et al*., 2009)**,…etc. It was found that, microorganisms have their own mechanism for production of nanomaterials as secretion of enzymes responsible for reduction of metal ions **(Thakkar *et al*., 2010)**. The field of nanotechnology improved rapidly and this require more reasonable thinking to make use of the extraordinary characteristics of the produced nanomaterials in the most required applications **(Heiligtag and Niederberger, 2013).**

The objective of our study was to synthesize magnesium and manganese nanoparticles by metallotolerant microorganisms as benign technique in chance to produce metal nanoparticles with unique properties. Also, characterization of the produced metal nanoparticles was essential. In addition, the produced metal nanoparticles were used as antimicrobial agents as required important application.

MATERIALS AND METHODS

Isolation

The Supplemented Metal-Nutrient (SMN) agar medium which has the same composition as nutrient agar medium with the addition of different concentrations of the metals (Mg^{+2} and Mn^{+2}) separately *viz.* (50, 100, 200 and 500 ppm) was used for the isolation. The medium was poured under aseptic conditions in sterile plates. The plates were inoculated with either 0.1 ml of soil suspension or 0.1 ml of wastewater came from detergents processing wastes from (Savo Factory) in Alamereia, Cairo. The plates were incubated at 30°C for 2 days in case of bacterial isolates and 7 days for fungal isolates.

Extremo- Metallotolerance ability examination of the isolates

The isolates obtained were purified and then, tested for their ability to grow on SMN agar medium containing higher gradient concentrations of the same metal from which it was previously isolated.The maximum concentration was achieved after which no growth was determined.

Biosynthesis of the metal nanoparticles

Supplemented-Metal-Nutrient (SMN) broth medium was prepared and the selected metallotolerant isolates were allowed to grow on sub-lethal concentration of the metal. Incubation was carried out as usual.

Separation of intracellular and extracellular nanoparticles

At the end of the incubation period for bacterial and fungal isolates the extracellular filtrate was separated by centrifugation and filtration and cells were washed with distilled water. The intracellular contents were obtained by ultrasonic disruption of cells with an ultrasonic processor (Cole Parmer Ultrasonic Homogenizer CPX 400) over three 15 s periods, and with an interval of 45 s between periods. The sonicated samples were centrifuged at 15,000 rpm for 30 min at 4°C to remove cell-debris. The supernatants were then used for characterization of intracellular nanoparticles **(Kalimuthu *et al*., 2008)**.

Characterization of metal nanoparticles

The intracellular and extracellular nanoparticles were characterized and examined using atomic absorption spectrophotometer (AAS), dynamic light scattering (DLS). According to the comparison of all the results of metallotolerance ability and DLS measurements, the TEM examination was carried out for the samples giving the most relevant results.

Characterization of the selected bacterial and fungal isolates

The morphological and physiological characteristics of the isolates were studied. The selected fungal isolate was identified genetically based on 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2, and 28S ribosomal RNA gene sequences and the bacterial isolate was identified genetically also based on 16S ribosomal RNA.With A number of the biochemical tests were made using The GEN III MicroPlate test panel.

Studying the antimicrobial activity and MIC determination for the extracellular and intracellular nanoparticles

The antimicrobial activity was done by measuring the minimum inhibitory concentration MIC for the selected intracellular nanoparticles of ***Fusarium nygamai,* $F_{4\ Mn/S}$** and ***Pseudomonas stutzeri,* $B_{4\ Mg/W}$** using broth micro dilution method **(Saini *et al*., 2005)** against *Aspergillus fumigatus (RCMB 02564), Candida albicans* (RCMB 05035), *Staphylococcus aureus* (RCMB 010027), *Streptococcus pyogenes* (RCMB 010015), *Pseudomonas aeruginosa* (RCMB 010043) and *Eschericia coli* (RCMB 010056).

RESULTS AND DISCUSSION

Isolation on SMN agar medium lead to selection of six bacterial magnesium isolates, six bacterial and one fungal manganese isolate (Table 1). A metallo-tolerance ability examination for the selected isolates was performed by increasing metal concentration up to lethal concentration. The most resistant microbial isolates were selected and grow at sub- lethal concentration (Table 2 and 3).

Table 1 Isolation of different microorganisms on media containing Mg^{+2} and Mn^{+2}

Isolate code	Source of isolation	Concentration from which isolate selected (ppm)	Metal used
$B_{1\ Mg/W}$	water	500	Mg^{+2}
$B_{2\ Mg/W}$	water	500	Mg^{+2}
$B_{3\ Mg/W}$	water	500	Mg^{+2}
$B_{4\ Mg/W}$	water	500	Mg^{+2}
$B_{5\ Mg/S}$	Soil	500	Mg^{+2}
$B_{6\ Mg/S}$	Soil	500	Mg^{+2}
$B_{1\ Mn/S}$	soil	200	Mn^{+2}
$B_{2\ Mn/W}$	water	500	Mn^{+2}
$B_{3\ Mn/W}$	water	200	Mn^{+2}
$F_{4\ Mn/S}$	Soil	200	Mn^{+2}
$B_{5\ Mn/W}$	water	200	Mn^{+2}
$B_{6\ Mn/S}$	Soil	500	Mn^{+2}
$B_{7\ Mn/S}$	Soil	200	Mn^{+2}

Where: W means water, S means soil, B means bacteria, F means fungi.

Kaul *et al*. (2012) revealed that, *Pochonia chlamydosporium*, and *Aspergillus fumigatus* were tested to be grow on three different magnesium salts i.e. magnesium sulphate, magnesium chloride and magnesium oxide at two concentrations *viz*. 1000 and 10000 ppm of magnesium salts. The growth of all fungi and bacteria was very poor in the media containing magnesium compounds at 10000 ppm concentration. Comparing to the present study, the metallotolerance examination of the isolates at different gradient elevated concentrations of Mg^{+2} step by step has shown that, there was great ability of the selected four Mg^{+2} bacterial isolates *Viz*. (**$B_{2\ Mg/W}$**, $B_{4\ Mg/W}$, **$B_{5\ Mg/S}$** and **$B_{6\ Mg/S}$**) to tolerate Mg^{+2} at concentrations up to 15000 ppm (617.157 mM) which equals to 152.11 g/l salt of $MgSO_4.7H_2O$ (152110 ppm). As a result the growth of $B_{4\ Mg/W}$ isolate on magnesium represent as extremo metallotolerant.

On the other hand, one Mn^{+2} fungal isolate ($F4_{Mn/S}$) was selected to grow in a range 800 to 45000ppm (12.3%) as extrme-metalltolerant. Concerning the metalltolerance of Mn^{2+} a metal resistant *Bacillus* sp. strain, isolated from soil was used in an earlier study. The effect of manganese concentration on its growth was monitored at 100 mgl^{-1}, 150 mgl^{-1} or 200 mgl^{-1} manganese **(Sinha *et al*., 2011).** In addition, **Cheung *et al*. (1982)** reported that, the growth rate of *Bacillus stearothermophilus* cells in a chemically defined medium was inversely proportional to the concentration of Mn^{2+} between 15 and 300 µM. As the Mn^{2+} concentration was increased from 0 to 10 µM the growth was increased proportionally. It was found that, manganese inhibited growth at all concentrations above 15 µM and the optimal Mn^{2+} concentration for growth of vegetative cells was in the narrow range between 10 and 15 µM.

Selection was made according to metallotolerance ability of the microbial isolates, DLS measurements and the easier isolates to manipulate were preferred in selection. The two isolates (**$B_{4\ Mg/W}$** and **$F_{4\ Mn/S}$**) were allowed to grow at 10000 and 30000 ppm of Mg^{+2} and Mn^{+2}, respectively. Both isolates (**$B_{4\ Mg/W}$** & **$F_{4\ Mn/S}$**) were chosen for intracellular nanoparticles production.

Table 2 A summary of the metallo-tolerance ability of the selected Mg isolats

Isolate code	Growth of selected Mg^{+2} isolates												
	Mg^{+2} concentration (ppm)												
	800	1000	1200	1400	1800	2000	3000	5000	7000	9000	11000	13000	15000
$B_{1Mg/W}$	++	+	++++	-ve	++++	++++	-ve	-ve	-ve	-ve	-ve	-ve	-ve
$B_{2\ Mg/W}$	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	+++	++
$B_{3\ Mg/W}$	++++	++++	++++	++++	+++	++	-ve	-ve	-ve	-ve	-ve	-ve	-ve
$B_{4\ Mg/W}$	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	+++	++
$B_{5\ Mg/S}$	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	+++	++
$B_{6\ Mg/S}$	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	+++	++

Table 3 A summary of the metallo-tolerance ability of the selected Mn isolats

Isolate code	Growth of selected Mn^{+2} isolates																	
	Metal concentration (ppm)																	
	800	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000	15000	20000	25000	30000	35000	40000	45000
$B_{1\ Mn/S}$	++++	++++	++++	++++	++++	++++	+++	++	++	+	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
$B_{2\ Mn/W}$	++++	+++	+++	++	++	++	+	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
$B_{3\ Mn/W}$	++	++	-------	-------	-------	-------	-------	-------	-------	-------	--------	------	-------	-------	--------	--------	--------	--------
$F_{4\ Mn/S}$	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	++	++	+
$B_{5\ Mn/W}$	+++	++	-------	--------	-------	-------	-------	-------	--------	------	--------	-------	-------	------	--------	--------	--------	-------
$B_{6\ Mn/S}$	++++	++++	++++	++++	++++	++++	+++	++	+	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Studying the intracellular nanoparticles samples with AAS has shown that, the isolate **$B_{4\ Mg/W}$** is capable of uptake of 35.17% of the used concentration of the metal (12852ppm). The isolate **$F_{4\ Mn/S}$** is capable of uptake of only 27.07% of the used concentration of the metal (24860 ppm).

Studying the intracellular nanoparticles samples with DLS has shown that, the size distribution of the nanoparticles in the intracellular sample of isolate **$B_{4\ Mg/W}$** ranges from (229.3-553.2 nm) with different mean number for each size, the maximum mean number 33.7% was that of the particles with size 356.2 nm. The size distribution of the intracellular sample of isolate **$F_{4\ Mn/\ S}$** ranges from (61.21-995.1 r. nm) with different mean number for each size, the maximum mean number 23.1% was that of the particles with size 82.09 r. nm, table (4 & 5) , figure (1 &2).

In an earlier study, *Bacillus sp.* (MTCC10650) was reported for the ability to produce manganese oxide nanoparticles intracellularly. The particle size distribution examination showed that the average size of the particles under investigation were 4.6 ±0.14 nm, with some particles, having 6–8 nm size and a very small percentage having diameter greater than 10 nm. The particles were isotropic in nature and monodispersed without any agglomeration. This was further confirmed by the TEM micrograph of the cell taken at higher magnification **(Sinha *et al*., 2011)**.

The nanoparticles samples were studied with TEM and it was found that, for the intracellular sample of isolate $B_{4\ Mg/W}$ the metal nanoparticles are spherical in shape. The size of the particles with respect to the spherical form ranges from from 2.90 nm to 9.44 nm. In addition, *Aspergillus flavus* strain TFR-12 was found to have potential to synthesize monodispersed MgO nanoparticles with an average diameter of 5.8 nm, MgO nanoparticles were characterized using dynamic light scattering (DLS). The crystal nature was confirmed by high resolution transmission electron microscope (HR-TEM) **(Raliya *et al*., 2014)**.

Hosseinkhani and Emtiazi (2011) reported in an earlier study the ability of *Acinetobacter* sp. isolate to produce extracellular Bixbyite-like Mn_2O_3 NPs. Characterization of morphology, size and chemical structure of these particles was determined by TEM, SEM, XRD and FTIR. The data showed that, this bacterium could produce NPs when grown aerobically in special medium supplemented with 1 mM $MnCl_2$. Studying the morphology and size distribution of the Mn-oxides showed clearly that, the biogenic Mn oxides nanoparticles were less than 500 nm.

Table 4 Dynamic light scattering measurements of intracellular sample of isolate $B_{4\ Mg/W}$ showing size distribution by number (%)

Size nm	Mean Number (%)	St. Deviation Number (%)
229.3	1.3	1.8
265.6	10.0	5.6
307.6	26.3	4.5
356.2	33.7	2.8
412.5	22.3	6.0
477.7	6.3	2.8
553.2	0.2	0.3

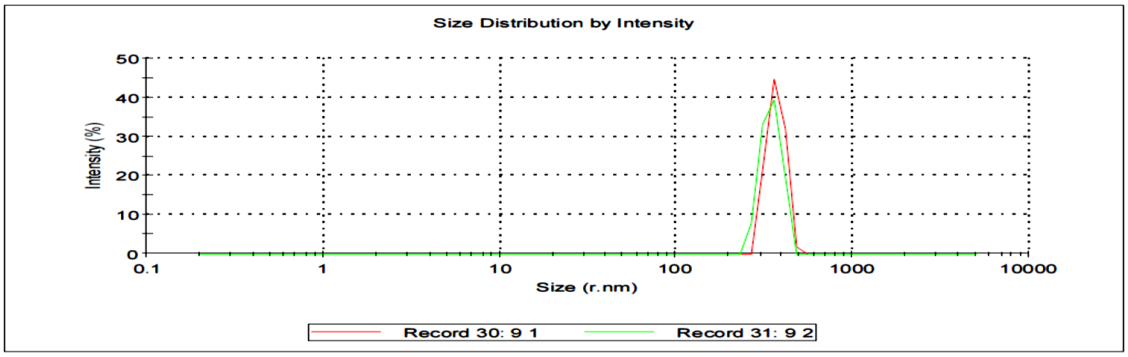


Figure 1 Dynamic light scattering measurements of intracellular sample of isolate $B_{4\ Mg/W}$ showing size distribution by intensity (%)

Table 5 Dynamic light scattering measurements of intracellular sample of isolate $F_{4\ Mn/S}$ showing size distribution by number (%)

Size nm	Mean Number (%)	St. Deviation Number (%)
61.21	3.6	3.2
70.89	13.9	7.2
82.09	23.1	3.9
95.07	21.8	2.2
110.1	12.5	3.6
127.5	4.1	1.9
147.7	0.6	0.4
553.2	4.8	1.7
640.7	5.0	1.9
741.9	3.5	1.2
859.2	1.3	0.4
995.1	0.1	0.0

Raliya and Tarafdar (2014) reported that, magnesium nanoparticles were synthesized using isolated soil fungi by employing various precursor salts of sulfate salts, nitrate salts, chloride salts and oxide salts *viz*. (MgO, $MgSO_4$, $MgCl_2$, $MgNO_3$). It was concluded that, 0.01 mM precursor salt concentration, 72 h of incubation at pH 5.5 and temperature 28 °C resulted smaller nanoparticles obtained. Bio-transformed products were analyzed using valid characterization technique i.e. dynamic light scattering, transmission electron microscopy, atomic force microscopy, energy dispersive X-ray spectroscopy. The average size of Mg nanoparticles was 6.4 nm. Result shows variability in morphological features of biosynthesized nanoparticles.In addition, earlier studies in the green synthesis of magnesium nanoparticles showed that, *Nepheliumlappaceum* L. peels was effectively used for the synthesis of magnesium oxide nanoparticles as a natural ligation agent. The XRD and SEM revealed the crystallinity and spherical morphology of the biosynthesized nanoparticles. The size of the particles was found to be 60-70 nm **(Suresh *et al*., 2014).**

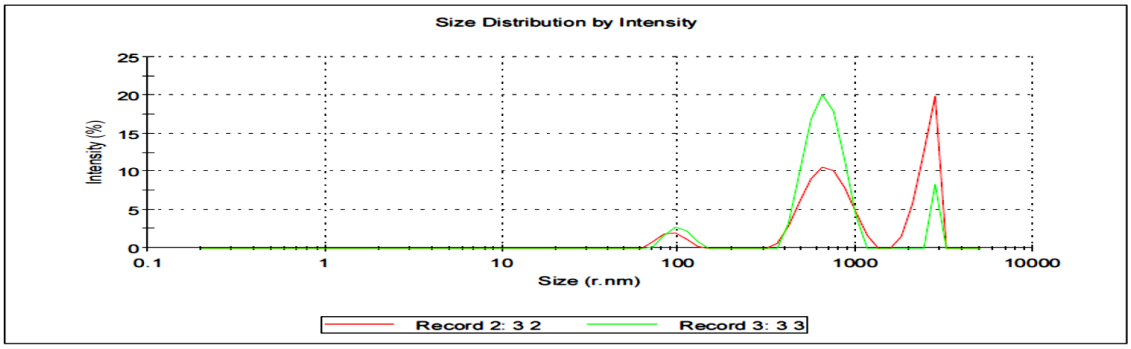
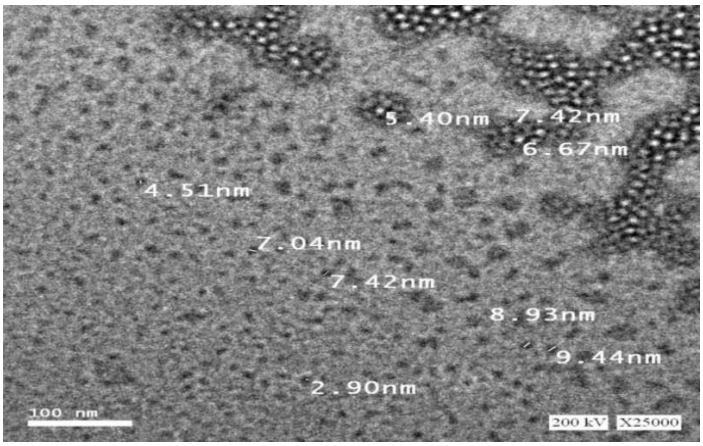


Figure 2 Dynamic light scattering measurements of intracellular sample of isolate $F_{4\ Mn/S}$ showing size distribution by intensity (%)

Waghmare *et al*. (2011) reported the synthesis of manganese nanoparticles by actinomycetes (*Streptomyces* sp.HBUM171191) when exposed to 50 ml of sterilized aqueous solution of manganese sulphate. The formation of whitish yellow to yellow colour indicated the formation of manganese nanoparticles. Transmission electron microscopy results clearly showed the polymorphic nanoparticles with 10 to 20 nm. **Indira and Tarafdar (2015)** reported the synthesis of magnesium nanoparticles by *Aspergillusbrasiliensis* TFR 23 protein. The produced nanoparticles were characterized using appropriate techniques and were having size (< 5.9 nm).

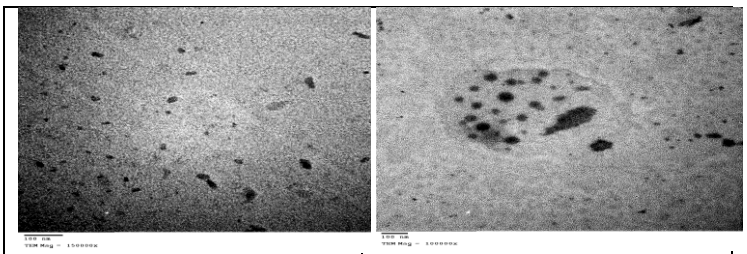
The synthesis of manganese dioxide nanoparticles (MnO_2 NPs) by microorganisms from reducing potassium permanganate was investigated in recent study. The microbial supernatants of the bacterium *Saccharophagus degradans* ATCC 43961 and of the yeast *Saccharomyces cerevisiae* showed positive reactions to the synthesis of MnO_2 NPs. Transmission electron microscopy micrographs revealed the presence of uniformly dispersed hexagonal and spherical particles with an average size of 34.4 nm **(Salunke *et al*., 2015).**



(a)

Figure 3 a TEM image of the intracellular Mg^{+2} nanoparticles of *Pseudomonas stutzeri*, $\mathbf{B}_{4Mg/W}$- Bar scale 100 nm .

The Magnesium nanoparticles were spherical in shape. The size of the NPs ranges from 11.90 nm to 43.29 nm.

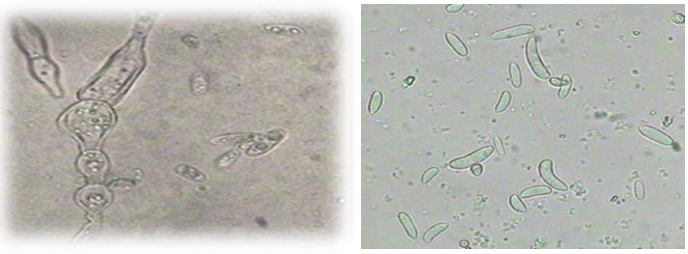


A **b**

Figure 4 TEM image of the intracellular Mn^{+2} nanoparticles of the isolate $F_{4\ Mn/S}$, (a, b) - Bar scale 100 nm

The manganese nanoparticles showed diversity in shape mostly were spherical. The size of the NPs ranges from 11.90 nm to 43.29 nm.

The $\mathbf{F}_{4\ Mn/S}$ isolate identification was based on using Compendium of soil fungi **(Domsch, *et al*., 1980)**, Atlas of clinical fungi **(de Hoog *et al*., 2000)** &using an Image Analysis System. It was identified also base on genetic characteristics and it was found to be more closely related to *Fusarium nygamai* according to the collected data (Fig. 5), Table (8).



(a) (b)

Figure 5 photographs of Mn^{+2} ($F_{4\ Mn/S}$) on microscopic examination

On the basis of collected data of morphological, physiological and biochemical studies and the comparative study of the properties of $B_{4Mg/W}$ isolate **Bergey's manual of systematic bacteriology, (Krieg, 1984 & Holt *et al*., 1994) table (6 &7)** and genetic identification also made based on 16S rRNA sequencing, it could be stated that, the present isolate $\mathbf{B}_{4Mg/W}$ suggested to be belongs to *Pseudomonas stutzeri***.** The present bacterial isolate ($\mathbf{B}_{4\ Mg/W}$) suggested to be belongs to *Pseudomonas stutzeri* as a new extremotolerant variety. Euclidean distance was 95% between the isolate $\mathbf{B}_{4\ Mg/W}$ and *Pseudomonas stutzeri*, While Mn^{+2} fungal isolate $F_{4\ Mn/S}$ extremotolerant variety of *Fusarium nygamai*, *Euclidean distance was* 89% between the isolate ($\mathbf{F}_{4\ Mn/S}$) and *Fusarium nygamai* (Fig. 6 & 8).

Table 6 Morphological and biochemical characteristics of the bacterial isolate $B_{4\ Mg/W}$

Characteristic	Result
Morphological characteristics: Shape	rod-shaped have a single polar flagellum
Colonies shape and color	Colonies are disc shaped with ridges radiating from the center
	reddish brown, typically hard, dry and tenaciously coherent
Motility	motile
Aeration	aerobic
Biochemical characteristics	
Gram reaction	gram negative
Catalase	+
Oxidase	+
Utilization of carbon sources	
Dextrin	+
D-Maltose	-
D-Trehalose	-
D-Cellobiose	-
Gentiobiose	-
Sucrose	-
D-Turanose	-
Stachyose	-
D-Raffinose	-
α-D-Lactose	-
D-Melibiose	-
β-Methyl-D-Glucoside	-
D-Salicin	-
N-Acetyl-D-Glucosamine	-
N-Acetyl-D-Galactosamine	-
N-Acetyl Neuraminic Acid	-
α-D-Glucose	+
D-Mannose	-
D-Fructose	+
D-Galactose	-
3-Methyl Glucose	-
D-Fucose	+
L-Fucose	+
L-Rhamnose	-
Inosine	-
D-Sorbitol	-
D-Mannitol	-
D-Arabitol	-
myo-Inositol	-
Glycerol	-
D-Glucose- 6-PO4	-
D-Fructose- 6-PO4	±
Characteristic	**Result**
Pectin	+
D-Galacturonic Acid	-
L-Galactonic Acid Lactone	-
D-Gluconic Acid	+
D-Glucuronic Acid	-
Glucuronamide	+
Mucic Acid	-
Quinic Acid	-
D-Saccharic Acid	-
p-Hydroxy-Phenylacetic acid	-
Methyl Pyruvate	-
D-Lactic Acid Methyl Ester	-
L-Lactic Acid	-
Citric Acid	+
α-Keto-Glutaric Acid	+
D-Malic Acid	-
L-Malic Acid	+
Bromo-Succinic Acid	+
Tween 40	+
γ-Amino-Butryric Acid	-

α-Hydroxy- Butyric Acid	-
β-Hydroxy-D,L- Butyric Acid	-
α-Keto-Butyric Acid	-
Acetoacetic Acid	+
Propionic Acid	-
Acetic Acid	+
Formic Acid	-
Utilization of nitrogen sources	
D-Serine	-
Gelatin	-
Glycyl-L-Proline	-
L-Alanine	+
L-Arginine	-
L-Aspartic Acid	-
L-Glutamic Acid	+
L-Histidine	-
L-Serine	-
L-Pyroglutamic Acid	+
D-Aspartic Acid	-
Growth at different NaCl concentrations (%)	
1	+
4	+
8	-

Table 7 A comparative study of the characteristics of bacterial isolate B4Mg/W, in relation to reference strain *Pseudomonas stutzeri* **(Burgey's manual of systematic bacteriology), (Krieg, 1984)**

Characteristics	Bacterial isolate B4Mg/W	Reference strain
Morphological characteristics shape	rod-shaped	rod-shaped
Colonies shape and color	Reddish brown, typically hard, dry and tenaciously coherent.	Yellow colonies, typically hard, dry.
motility	Motile	Motile
Aeration	aerobic	aerobic
Biochemical characteristics		
Gram reaction	Gram negative	gram negative
Catalase	+	+
oxidase	+	+
Utilization of		
Glucose	+	+
trehalose	-	-
glycerol	-	+
L- alanine	+	+
lactate	-	+
L-glutamate	+	+
L-malate	+	+
L-arginine	-	-
citrate	+	+
L-histidin	-	-
Citric acid	+	+
D-Sorbitol	-	-
L-Arginine	-	-
L-Histidine	-	-
Growth at 40 & 41 °C	+	+
Hydrolysis of gelatine	-	-
Growth at 4 °C	-	-
Growth at NaCl (12-15%)	-	-
Growth at pH 3.6	-	-

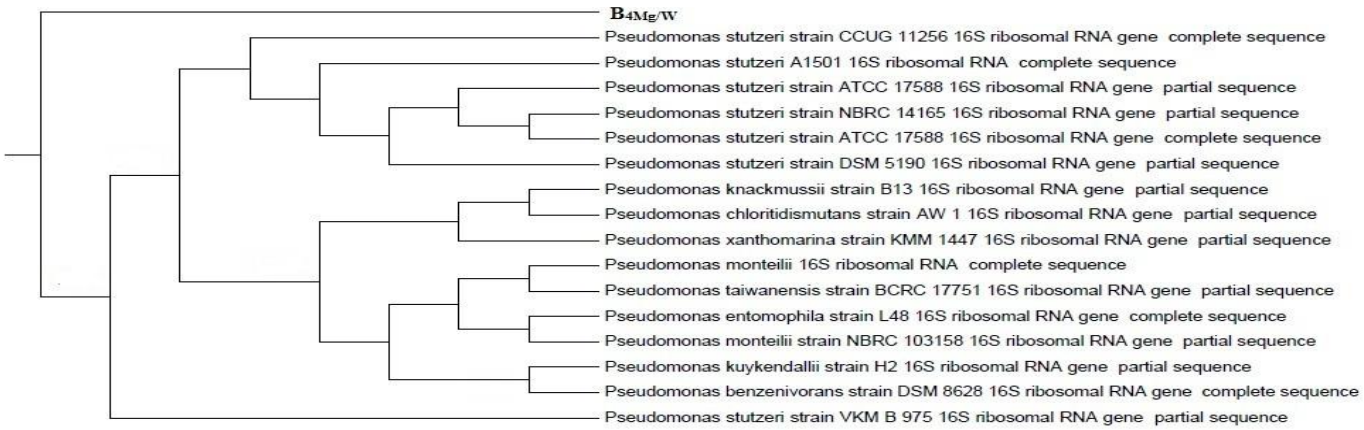


Figure 6 Dendrogram of Mg^{2+} bacterial isolate ($B_{4\ Mg/\ W}$) and reference *Pseudomonas* strains based on the similarity matrix of phonetic data

Table 8 Morphological and cultural characteristics of the fungal isolate $F_{4\ Mn/\ S}$

Character	Examination
Culture Exam.:	
Growth characteristics	Colonies on PDA growing rapidly. Mycelium white becoming violet with brown to violet reverse.
Microscopic Exam.:	
Micro-conidia	Micro-conidia, abundant, in short chain or in heads, one celled, fusiform 12X3 µm.
Macro-conidia	Fusiform, 3-5 septate, not abundant, 25X4.0 µm.
Chlamydospores	Chlamydospores, abundant, almost intercalary, single or in chain.

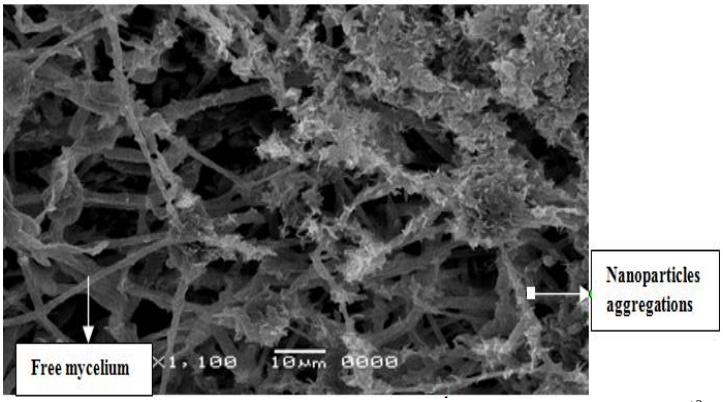


Figure 7 SEM image of *Fusarium nygamai*, $F_{4\ Mn/S}$ showing the Mn^{+2} nanoparticles on the mycelium of the fungus

Figure 8 Dendrogram of Mn^{+2} fungal isolate $F_{4\ Mn/S}$ and reference *Fusarium* strains based on the similarity matrix of phonetic data

The antimicrobial activity study of the nanoparticles samples has shown that, all samples have antimicrobial activity against certain test organisms indicated by the inhibition of their growth (table 9).

Table 9 Minimum inhibitory concentration measurements of the produced microbial Mn^{2+} and Mg^{2+} nanoparticles by the most potent metal nanoparticles producers

	Nanoparticle sample		Standard (ppm)
Tested microorganisms	**1**	**2**	
Fungi	**Minimum inhibitory concentration of nanoparticles (ppm)**		amphotericin
Aspergillus fumigatus (RCMB 02564)	15.63	1.95	0.98
Candida albicans (RCMB 05035)	62.5	15.63	3.9
Gram positive bacteria			Ampicillin
Staphylococcus aureus (RCMB 010027)	62.5	7.81	0.015
Streptococcus pyogenes (RCMB 010015)	62.5	31.25	0.06
Gram negative bacteria			Gentamycin
Pseudomonas aeruginosa (RCMB 010043)	NA	NA	62.5
Eschericia coli (RCMB 010056)	62.5	7.81	0.12

Where (1): Intracellular NPs of isolate *Fusariumnygamai*, $\mathbf{F}_{4\ Mn/S}$, (2): Intracellular NPs of isolate *Pseudomonas stutzeri*, $\mathbf{B}_{4\ Mg/W}$
(NA): means no activity

Antimicrobial activity against *Streptococcus pyogenes* RCMB010015, 31.25 and 62.5 mm followed by *Candida albicans* RCMB05035, 15.63 and 62.5; then *Staphylococcus aureus* RCMB010027 and *Eschericia coli* RCMB010056 gave7.81and 62.5mm for both*;* while for *Aspergillus fumigates* RCMB02564 gave the least amount of inhibition 1.95 and 15.63mm*;* moreover *Pseudomonas aeruginosa* RCMB010043 was very resistant for both ***Pseudomonas stutzeri,*** $\mathbf{B}_{4\ Mg/W}$ and ***Fusarium nygamai,*** $\mathbf{F}_{4\ Mn/S}$ intracellular nanoparticles, respectively.
Also, **Moustafa *et al.* (2015)** reported that, ***Fusarium nygamai*** was a Zn metallotolerant and nanoparticles producer. The results revealed that, Zn nanoparticles have antimicrobial activity against *Aspergillus fumigatus*, *Candida albicans*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Eschericia coli*.

Tindall *et al.* (1980) stated that, magnesium concentration as low as 10 mM exerts an inhibitory effect on microorganisms isolated from the Dead Sea It has also found that, varied results were obtained among G +ve and G –ve bacteria which may referred to the size of the produced nanoparticles as reported by **Sundrarajan *et al.* (2012).**

CONCLUSION

Manganese (II) nanoparticles have been synthesized by *Fusarium nygamai*, $\mathbf{F}_{4\ Mn/S}$ extremo-metallotorent fungus and it was found that, it can produce nanoparticles intracellularly and extraceluellarly. Also, *Pseudomonas stutzeri*, $\mathbf{B}_{4\ Mg/W}$ was proved as magnesium (II) nanoparticles producer intracellularly and

extraceluellarly. Only the samples with the best results were chosen. They showed different morphology and size on DLS and TEM examinations. All nanoparticles samples have different high power abilities of antimicrobial activity against test organisms. The green synthesis of the metal nanoparticles was achieved with the possibilities of using these nanoparticles in other serious applications and may have more important impact in other fields.

Acknowledgement: I would like to express my attitude to the whole team of the work with exceptional thanks to the professor doctor ***Nagwa Sidkey*** for her help and experience giving thought the whole work. In addition, special thanks to the Botany and Microbiology Department, Faculty of Science, Al Azhar University, Girls Branch.

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