REVIEW PAPER

The role of *Bacillus* species in the synthesis of metal and metal oxide nanoparticles and their biomedical applications: A mini review

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ABSTRACT
The biological synthesis of metallic nanoparticles (MtNPs) has increased greatly in the last few decades. Nanoparticles (NPs) are synthesized via different approaches like chemical and physical methods. However various drawbacks such as higher cost, high energy requirement, and the use of toxic chemicals limit the use of these approaches. It is thus important to look for an alternative method for the development of MtNPs. One such method, which has gained the most attention in recent years, is the bio-fabrication of MtNPs using microorganism like bacteria, fungi, actinomycetes and viruses. Amongst the microorganism used for NPs synthesis, bacteria are the most preferred candidate due to their diversity and better growth control. In this context, the present article concerns with association of genus bacillus with metal and metal oxide NPs. The current review thoroughly summarizes the mechanisms involved in the synthesis of MtNPs and their biomedical applications. The review also explains the major drawbacks associated with the synthesis of NPs via bacillus spp as well as the future prospects.

Keywords: *Bacillus, Bacteria, Metallic nanoparticles*

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INTRODUCTION

Nanotechnology is a field of science that encompasses the designing, production and use of objects at dimensions between 1 and 100 nanometers, where 1 nanometer is equal to 1/10,000,00 of a millimeter [1, 2]. Nanotechnology has emerged as an important research area in science over the past few years and has gained the attention of researchers across the globe [3, 4]. Nanotechnology has changed practically every aspect of human life, which is attributed to the unique physiochemical, electrical, and mechanical properties of Nano-scale materials. These properties make Nano-sized material ideal candidates for targeted drug delivery, gene therapy, diagnosis of different diseases, cellular repairing and medical devices etc. [5]. Bio-nanotechnology is a specialized application of nanotechnology, which refers to the intersection of biology and nanotechnology [6]. Nanobiotechnology deals with synthesis of biocompatible and environment friendly nanoparticles (NPs) using green-approaches, which then can be used for various medical purposes [7].

NPs can be fabricated via top-down approach and bottom-up approaches (Fig. 1) [8]. Top-down approach involves grinding of a bulk material via different wet milling techniques like high pressure homogenization, media milling and micro fluidization etc. to create Nano-sized particles. These techniques do not use any harmful solvents but the high energy requirements make these techniques

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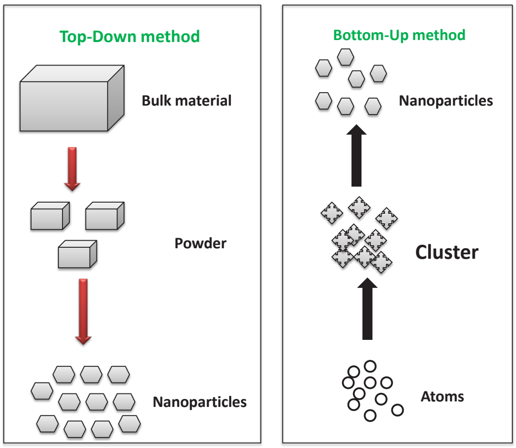

Fig. 1. Top-down and bottom-up approaches for the synthesizing nanomaterials

quite inefficient. During these operations a huge amount of energy is generated which ultimately make the handling of heat sensitive material difficult [9, 10]. On the other hand, "bottom-up approach" is a complete opposite approach, in which small atoms at nanoscale, assemble to synthesize NPs [11]. Biological and chemical syntheses of NPs are the examples of bottom-up approaches. Chemical methods include sol-gel, chemical reduction and pyrolysisetc. However these techniques involve the uses of toxic and hazardous chemical for the production of NPs [12]. On the other hand the biological method involves the synthesis of NPs via microorganisms such as fungi, bacteria and plants [13]. Among different biogenic sources, bacteria is considered the most suitable candidate for the fabrication of metallic nanoparticles (MtNPs) as bacteria have a remarkable metal resistance mechanism making them an ideal Nano-factories for metal ions into MtNPs [14]. Other advantages of using bacteria for NPs synthesis are; simple culturing, extracellular NPs fabrication, economical and less time consuming [15].

Therefore, *bacillus*, a genus of endospore-forming, rod-shaped and Gram-positive bacteria has gained widespread attention to fabricate MtNPs. The aim of this review is to provide an overview of the related published studies regarding the use of various strains of genus *bacillus* for the biogenic synthesis of metal and metal oxide nanoparticles. The article also unravels the possible mechanism behind the synthesis of these NPs and their biomedical applications.

Bioinspired synthesis of metal and metal oxide NPs via *Bacillus*spp.

Bacillus spp. are well-known Gram Positive bacteria used in the industry due to their ability to produce various therapeutic enzyme and active compounds and are also considered important sources from which new bioactive compounds can be used in the development of new therapeutic agents[16]. *Bacillus* spp. can effectively reduce dangerous metals like lead, zinc cadmium and arsenic to more stable/less harmful compounds [17]. A broad range of *Bacillus* strains have been reported to produce different bio-surfactants especially lipo-peptide, which helps and are responsible for the synthesis of NPs [18]. Several studies have successfully reported the bio-fabrication of MtNPs using different strains of *Bacillus* having potential medical applications [19-22], and therefore *bacillus* spp. can be a considered a potential bio-Nano-factory.

The synthesis of NPs via *Bacillus* can occur both intracellularly and extracellularly. During the intracellular synthesis of NPs, Metal ions are transported to the inside cell, where the enzymatic reduction of these ions to stable NPs occur [23]. On the other hand, the extracellular synthesis involves the inoculation of a *Bacillus* spp. into liquid media in an Erlenmeyer flask. Next the media incubated for 24hrs for bacteria which is then subjected to centrifugation. The supernatants (cell-free extract) obtained are mixed with silver nitrate solution ($AgNO_3$) and the mixture is incubated until NPs synthesis occur (Fig. 2) [24]. Despite having a better control of size and shape, NPs synthesized via intracellular method is not economical, as the process is extremely slow (24-120 hours) and extra steps for the recovery of NPs such as treatment with ultrasound or detergents are necessary [25, 26]. Therefore, the extracellular method for the synthesis of NPs is preferred since the process is easy and no additional treatment with detergents or ultrasound is required to recover the synthesized NPs [27]. However polydispersity of the NPs is a main issue in extracellular process, which can be overcome by improving the reaction condition [28].Therefore in this review we will mainly focus on extracellular biosynthesis of metal and metal oxide NPs by *Bacillus* spp.

Silver NPs

Silver nanoparticles (AgNPs), now days are the most widely used NPs in health sector. Being

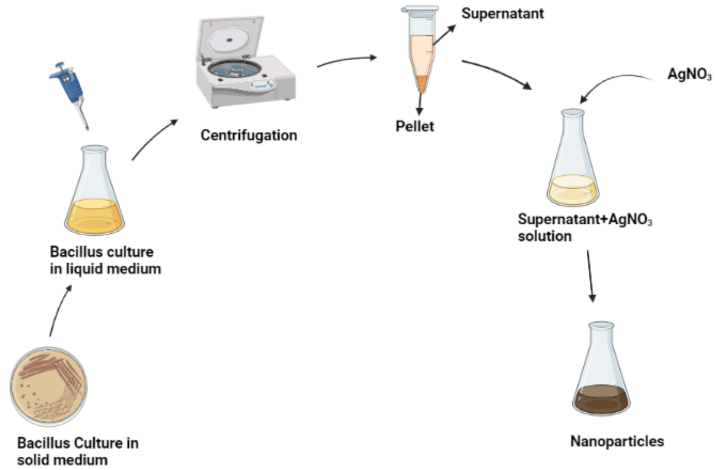

Fig. 2. Schematic representation of extracellular synthesis of AgNP using culture of Bacillus

the most marketed nanomaterial, Nano-silver has gained the attention which is attributed to its unique physical and chemical properties such as chemical stability, conductivity, antibacterial, anticancer and antiangiogenic activities as well its uses in food industry [29].According to a report, every year 500 tons of silver are produced around the globe [30]. AgNPs can be prepared via different methods such as chemical physical and biological methods [31]. The bio-fabrication of AgNPs via microorganism has gained the most attention as the process is environmentally friendly [32]. Therefore, researchers have made attempts to make *Bacillus* spp environmental friendly bio-Nano-factories for the synthesis of AgNPs.

AgNPs have been synthesized via *Bacillus* strainC11, both intracellularly and extracellularly in the same study. First, the researchers inoculated the bacterial culture into a flask followed by incubation (48hrs) and centrifugation (at 12000 rpm for 10 mints). The pallet (containing biomass) was taken for the intracellular while the supernatant was taken for extracellular synthesis of NPs. Interestingly; NPs were successfully synthesized by both biomass and supernatant as indicated by the color change of solutions from pale yellow to brown [33].

A recent study reported the extracellular synthesis of AgNPs using the culture supernatant of *Bacillus cereus.* The bacterial culture was first sonicated over 3 fifteen seconds periods via an ultrasonic processor, followed by centrifugation. The debris was removed and the supernatant was used for the synthesis of AgNPs. It was observed that synthesized NPs were irregular in shape, poly dispersed with 62.8 nm in size [34]. Another similar study also reported the bio-fabrication of AgNPs using culture supernatant of mutant *Bacillus licheniformis* M09. The synthesized NPs were spherical in shape having size between 10-30 mm. The synthesized NPs exhibited excellent antibacterial, cytotoxic activities as well as photocatalytic degradation of methylene blue [35].

The exact mechanism involve in the synthesis of AgNPs by *bacillus* spp is not yet fully known, however previous reports have shown that Nicotinamide adenine dinucleotide (NADH), NADH-dependent reductase enzyme and other components in the cell free supernatant of bacillus spp (Fig. 3), are the crucial factors for the reduction of Silver Ions (Ag+) into stable AgNPs, which is the most widely accepted mechanism behind the synthesis of AgNPs by bacteria [36, 31]. Nitrate reductase accepts an electron from NADH and transfers it to the metal ions (Fig. 4) [37]. It has been reported that silver NPs were prepared from the culture supernatant of *B. licheniformis.* It was shown that nitrate reductase was involved in reduction of Ag+ to AgNPs. The role of nitrate reductase in the bio-fabrication of AgNPs was

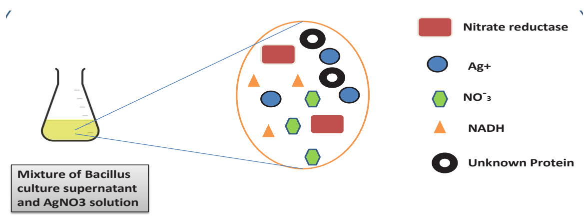

Fig. 3. Fig showing the presence of various components in the mixture of $AgNO_3$ and Bacillus culture supernatants. Nitrate reductase transfers an electron from NADH to Ag+ converting it to a stable NP. The synthesized NPs are further stabilized by proteins present in the mixture

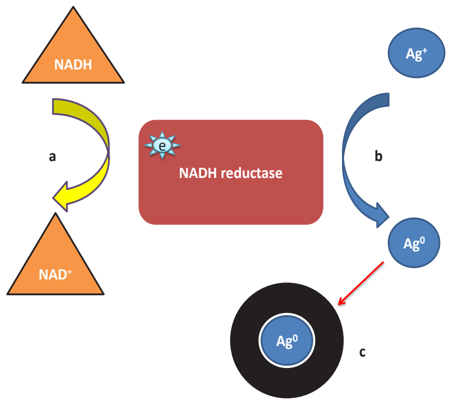

Fig. 4. The most widely accepted mechanism behind the synthesis of nanoparticles. a) First the NADH reductase accepts an electron from NADH, generating NAD+. b) Nitrate reductase transfers the electron toan Ag+, converting it to a more stable state Ag^0. c) The Ag^0 (nanoparticle) is capped by protein present in the reaction mixture

confirmed by using an enzyme inhibitor, sodium azide. When sodium azide was employed, no AgNPs production was observed however when the enzyme was precipitated via acetone and then silver ions were added, the production of NPs was observed. In addition the effect of different variables (Glucose, Peptone, Yeast extract and KNO3) on nitrate reductase production was evaluated. The highest nitrates activity i.e. 452.206 U/ml, was observed when medium contain Glucose: 1.5, Peptone: 1, Yeast extract: 0.35 and KNO was used [38]. Furthermore, the potential of *Bacillus clausii* culture supernatants for the production of AgNPs have been explored. The cell-free culture supernatant was mixed with AgNO3, leading to the color change of solution indicating the production of AgNPs. The possible mechanism of NPs synthesis was revealed via in silico studies on nitrate reductase. With the help of software Hex 8.0.0, the successful docking of reductase (which was taken as receptor) and silver (taken as Ligand) was reported and 10 different ligand binding regions with different energies were predicted [39].

In addition to enzymes, exopolysaccharide (EPS) secreted by bacteria could also help in the production of NPs as their sugar components might reduce metal ions to stable NPs [40]. EPS secreted by *Bacillus subtilis* have been shown to biofabricat AgNPs. First, EPS was recovered from the bacteria followed by addition to AgNO3 solution. Next, the solution was agitated until the production of NPs was achieved. The synthesis of AgNPs, entrapped in polysaccharide Nano-cages was reported. Fourier transform infrared spectroscopy (FTIR) analysis revealed that silver interact with C-O-C and OH group of the EPS which resulted in the formation of stable and spherical EPS entrapped AgNPs [41]. A majority of the microbial EPS are poly-anionic in nature due to the presence of uronic acids (like D-glucouronic acid, D-galactouronic acid, D-mannuronic acid etc.), metallinked pyruvate, inorganic phosphates and sulphates [42]. This poly anionic nature of the EPS leads to electrostatic attraction of cations and forms complexes with it [43].

In addition, the spores from *Bacillus stratosphericus* have been reported to reduce Ag+ to AgNPs. Transmission electron microscopy (TEM) analysis revealed the presence of small AgNPs aggregates on the spore surface as well as outside the spores having size from 2 to 20 nm and 2 to 15 nm respectively. Further, NPs with different shapes such as spherical, triangular, cubic, and hexagonal were noted. They also assayed the role of different enzyme like nitrate reductase, catalase and laccase in AgNPs synthesis by adding an enzyme specific substrate solution on the spore mass separately. Also the role of amount of Dipicolinic acid (DPA) and its role in AgNPs production was investigated. Interestingly the spores did not show any enzymatic activity indicating that another mechanism might be at work. In addition the vegetative cell of *B. stratosphericus* could not survive in $AgNO_3$ solution indicating the lack of any enzymatic activity to reduce Ag+. Therefore, the possible factor behind the synthesis of AgNPs by spores might be DPA, since it's only found in the spores but not the vegetative cells [44]. Therefore, it becomes clear that there is no single method involved in synthesis of AgNPs in *Bacillus* spp. The mechanism behind NPs synthesis may vary from strain to strain.

Gold nanoparticles

Gold nanoparticles (AuNPs), synthesized via various biological routes are nontoxic to the human body, compared to other NPs which are more or less toxic [45, 46]. For over 400 years, AuNPs have been used for various purposes such as treatment of different diseases, staining of glass [47]. A wide range of properties such as high electrical conductivity, shape and size dependent surface plasmon resonance, affinity with organic compounds make AuNPs one of the most favorite candidates in many areas like canalization, drugs delivery, chemical sensing and medical therapy [48]. AuNPs can be synthesized into a miscellaneous of forms included gold nano spheres, nano rods, nano belts, nano cages, nano prisms, and nanostars [49].

Different studies have found that *B. subtilis* was able to reduce gold ions (Au^{3+}) to AuNPs [50, 51]. The bioinspired synthesis of AuNPs using supernatant of *Bacillus marisflavi* was reported. The synthesized NPs were found to be crystalline and spherical with an average size of ~14 nm. It was found that the biomolecules especially proteins in the cell free supernatant performed both reduction and stabilization of AuNPs. The Ultraviolet visible spectroscopy analysis showed that the synthesized AuNPs were stable for one month without shift in the peak over time [52]. However none of the above mentioned studies revealed the exact mechanism behind AuNPs synthesis.

For the first time the mechanism behind the reduction of Au+3 to stable AuNPs was elucidated. The mechanism of extracellular synthesis AuNPs was investigated using *B. subtilis*. It was proposed that the mechanism of extracellular AuNPs synthesis using the supernatant of *B. subtilis* is a 2-step process: 1) an initial gold-Sulfur bond formation via methionine present in Catalase A (Cat A) and the Au^{3+} in solution, followed by 2) AuNPs stabilization via "capping" of the denatured of Cat A at lower concentrations of Au^{3+} and the higher dilution factors of supernatant [53]. Li Y and co-workers documented the production of AuNPs by the extracellular secretion of *Bacillus niabensis*. They showed the role of cyclic peptide, with a molecular weight of 1122Da, in reducing the Au^{3+} to AuNPs via possible electron transfer [54]. Another study exploited the AuNPs manufacturing potential of *B. Subtilis*. They reported the successful production of AuNPs after mixing chloroauric acid aqueous solution with culture supernatant of *B. subtilis*. Since *B. Subtilis* is known to secrete cofactor NADH and NADH dependent enzymes, which may be responsible for the bio-reduction of Au^{3+} to AuNPs. The reduction seems to be initiated by electron transfer from the NADH by NADH-dependent reductase as electron carrier. Then the Au^{3+} obtain electrons and are reduced to AuNPs [55].

Cadmium Sulfide nanopartilcles

Cadmium sulfide nanoparticles (CdS-NPs) are conventional semiconductors with artificially controllable photoluminescence, optical characteristics [56, 57] and having potential applications in solar energy conversion, nonlinear optics, photo electrochemical cells and heterogeneous photo catalysis[58, 59]. For the production of stable CdS-NPs, surfictin (cyclic lipo-peptide bio-surfactant) has been extracted from *Bacillus amyloliquifaciens* strain KSU-109. Surfactin solution was mixed with cadmium nitrate solution (Cd $(NO_3)_2$) and a few drops of sodium sulfide (Na_2S) were added to make CdS-NPs. The presence of stable CdS-NPs was determined by the mixture's transition color. Furthermore, FTIR research revealed a probable interaction between CdS-NPs and surfictin, resulting in CdS-NPs production and stability [60]. Using culture supernatants of *Escherichia coli* ATCC 8739, *B. subtilis* ATCC 6633, and *Lactobacillus acidophilus* DSMZ 20079T, a quick bioinspired synthesis of CdS-NPs was reported. After 24 hr of incubation, all of the bacterial cultures efficiently transformed cadmium chloride ($CdCl_2$) solution and aqueous Na2S solution to CdS-NPs with sizes ranging from 2.5 to 5.5 nm. In addition NPs aggregates were also recovered, indicating that the NPs produced were not monodispersed [61].

Other NPs

The intracellular synthesis of Palladium nanoparticles (PdNPs) has been reported using *Desulfovibrio desulfuricans* and *Bacillus benzeovorans*. The production of monodispersed PdNPs was observed in the cytoplasam of both strains from Sodium tetrachloropalladate ($Na2PdCl_4$) using hydrogen and format as electron donors. It was hypothesized that; in case of hydrogen as an electron donor, hydrogenase may have played a vital role in the production of PdNPs. In addition, since *B. benzeovorans* was cultivated anaerobically, therefore another mechanism could have been involved. On the other hand, when format was employed as an electron donor, the breakdown of format, resulting in the release of an electron reducing palladium ion (Pd+2) to PdNPs could be the possible mechanism [62]. Another study reported the production MtNPs such as lead, cadmium, and silver NPs using *Bacillus megaterium*. The synthesized NPs were observed to be accumulated on the bacterial cell wall [63].

Metal oxide NPs

Metal oxides are one of the most well

studies class of inorganic compounds owing to their structural diversity, characteristics, and remarkable phenomena displayed by its NPs [64]. In addition, metal oxide nanoparticles (MtONPs), are of great interest due to their exceptional optical, electrical, and magnetic capabilities [65]. Due to these exceptional properties, MtONPs find wide range of industrial applications, including catalytic processes, electronics, sensors, magnetic storage media, and solar energy conversion, due to their unique features [66]. The biofabrication of MtONPs has been widely reported using various *Bacillus* strains [67-69]. The extracellular synthesis of magnetic iron oxide nanoparticles via *B. cereus* strain HMH1 has been reported recently. Large particles due to aggregation of NPs were observed, indicating that the NPs were not monodispersed. The study further proposed that reductase enzyme was responsible for the synthesis of magnetic iron oxide nanoparticles [70]. Thus, the mechanism of metal and metal oxide NP reduction appears to be the same.

Biomedical applications

Current review briefly explains *Bacillus* strains a potential bio-Nano-factories for synthesis of different metal and metal oxide NPs and their potential uses in th medical field. NPs, especially AgNPs have variety of medical applications such as antibacterial, anticancer, antifungal, antiviral and wound healing activities [71]. Many studies have revealed the importance of NPs derived from *Bacillus* species to have bactericidal, antifungal, antiviral and anticancer properties. MtNPs synthesized by *Bacillus* spp, their shapes, sizes and medical uses are shown in Table1.

Antibacterial activity

Over the last few years, the emergence of antibiotic resistance strains has increased, rendering antibiotics to be less effective in fighting against microbial infections. Utilization of NPs could be a potential solution, which has gained the attention of many researchers, in combating pathogenic bacteria without the risk of generating antibiotic- resistant strains [72]. A study reported the antibacterial activities of AgNPs derived from *B. cereus*. The synthesiezd NPs were found to be active against wide range of bacteria, including *Staphylococcus aureus*, *Klebsiella pneumonia, Salmonella typhi* and *E. coli* [34].

Another similar study reported the antibacterial potential of *B. cereus* A30 mediated AgNPs against Methicillin resistant staphylococcus aureus, *E. coli, K. pneumoni and pseudomonas aeruginosa* [73]. The exact mechanism of antibacterial activity of AgNPs is not yet fully known [74]. However, a study for the first time elucidated the possible mechanism behind the antimicrobial effect of AgNPs against *B. substillis*. It was proposed that AgNPs release Ag+, which enters the bacterial cell and are oxidized to silver oxide (Ag_2O). Ag_2O subsequently exert toxic effect on bacteria via Growth arrest, chromosomal degradation, damaging cellular membrane, decreasing the activity of reducatse and reduction in proteins expression [30]. Similary, it has been shown that there could be various mechanisms of action of AgNPs to inhibit bacteria growth such as; 1) By affecting the cell wall synthesis, 2) By affecting the synthesis of nucleic acids, 3) By inhibiting metabolic pathways, 4) And inhibition of protein synthesis [74].

In addition to AgNPs, other metal and metal

Table 1. Table showing the summary of different types of NPs obtained from Bacillus strains and their biomedical applications

*Bacillus*Spp	Nanoparticle	Size (nm)	Shape	Medical use	Reference
Bacillus cereus PMSS-1	Zinc Oxide	10-70	Spherical and Cylindrical	Anti-cancer	12
Bacillus C11	AgNPs	42–92	Spherical	NA	33
Bacillus cereus	AgNPs	62.8	Irregular	Antimicrobial	34
*Bacillus licheniformis*M09	AgNPs	10-30	Spherical	Anti-bacterial, Cytotoxic	35
Bacillus Clausii	AgNPs	150	Glitter Spherical	NA	39
Bacillus subtilis	AgNPs	NA	NA	Antibacterial	41
Bacillus stratosphericus	AgNPs	2-20	Spherical, Triangular, Cubic, Hexagonal	Cytotoxic	44
Bacillus marisflavi	AuNPs	14	Spherical	NA	52
Bacillusniabensis	AuNPs	10-20	Spherical	Anti-bacterial	54
Bacillus subtilis	AuNPs	NA	NA	Anti-bacterial, Anti-bacterial	55
Bacillus subtilis	CdS-NPs	2.5-5.5	Spherical	NA	61
Bacillus benzeovorans	PdNPs	0.2-8	Icosahedrons	NA	62
Bacillus Megaterium	Metallic NPs	7-12	Spherical	Antifungal	63
Bacilluscereus HMH1	MtONPs	25-70	Spherical	Antibacterial	70
Bacillus cereus A30	AgNPs	44	Spherical	Antibacterial	73
Bacillus KFU36	AgNPs	5-15	spherical	Anti-cancer	83
Bacillus amyloliquefaciens	AgNPs	20-40	spherical	Anti-cancer, Cytotoxic	84
Bacillus pumilus	AgNPs	77–92	Triangular, Hexagonal, Spherical	Anti-viral	89
Bacillus amyloliquifaciens	AgNPs	15.9-80	Spherical	Antimicrobial	91

oxide NPs like Gold [55], Cr [75], zinc oxide [76], obtained from various *Bacillus* spp, have been reported to be active against a wide range of bacterial species.

Anti-cancer activity

Uncontrolled cell division followed by the invasion of other healthy cells and tissues is known cancer [77]. Cancer is one of the main cause of mortality in both men and women across the world and approximately 6 million people suffer from this disease each year [78]. Different treatment methods like radiation therapy, chemotherapy, surgery, immunotherapy, cancer vaccinations, photodynamic therapy and stem cell transformation have been used to treat cancer, however various drawbacks limit the use of these approaches [79]. Some of these drawbacks are toxicity, un-specificity, low bioavailability, fast clearance and restriction in metastasis [80-81, 82]. Alternative approaches are thus need to be adopted are more effective and have less or no side effects.

One such approach which is efficient, economical, environment friendly and have less side effecsts could be the use of NPs derived from bacterial cell.

The anticancer activity of AgNPs produced from the culture supernatants of *Bacillus* spp KFU36 has been reported. The NPs showed anticancer activity by inducing apoptosis in breast cancer MCF-7 cells. Furthermore as shown by the results of flow cytometry, the cell viability drastically decreased when the concentration of NPs was increased [83].

Another similar report also investigated the anticancer activity of AgNPs derived from *B. amyloliquefaciens.* The results of the study showed that synthesized AgNPs exerted cytotoxic effect on A549 cell line through the stimulation of reactive oxygen species (ROS) production [84]. Similarly the cytotoxic activity of magnetic iron NPs obtained from *B. cereus* strain HMH1 supernatants was also documented. The synthesized NPs exhibited anticancer activity in MCF-7 and 3T3 cell lines [85].

Antifungal activity

Fungal diseases are controlled through different chemical fungicides. The use of chemical fungicides might adversely affect the environment, human health and important microorganism present in soil [86]. Thus the production of fungicide that are more effective and less/not harmful is therefore the need of time. One such study has made an attempt to synthesize zinc oxide NPs using *Bacillus* sp. Fcl1. The NPs exhibited excellent antifungal activity against *Pythium aphanidermatum* [90]. Another report showed the extracellular production of AgNPs from *Bacillus* spp. GP-23.The results further revealed that the synthesized NPs were extremely effective against a plant pathogen fungus *Fusarium oxysporum* at the concentration of 8μgml-1 [63]. However the exact mechanism of antifungal activity of MtNPs is yet to be understood.

Antiviral activity

Viruses cause wide range of dangerous diseases in human, many of which are fatal and its treatment is challenging [87]. Even though for some viral diseases vaccine is available, but there are still various infections which require effective treatment [88]. MtNPs act as antiviral agent, either inside (suppressing viral replication) or outside (blocking the entry of virus) of the host cell [87].

AgNPs derived from culture supernatants of *Bacillus pumilus*, *Bacillus persicus,* and *B. licheniformi* were documented to be effective against the Bean Yellow Mosaic Virus [89]. A report has shown that the entry of Vaccinia virus (VACV) was successfully halted by using AgNPs having 25nm size at non-cytotoxic concentrations. The AgNPs were able to block the macropinocytosis-dependent entry as well as direct fusion entry of VACV into the host cell. The results further showed that, AgNPs bind directly to the entry fusion complex of VACV thus exhibiting potential antiviral activity [87]. Very few studies have been carried out regarding the antiviral potential of *Bacillus* spp mediated MtNPs. Thus it is recommended to perform more research in this area

Challenges and future prospects in the synthesis of metal nanoparticles using *Bacillus* Spp.

Lack of understanding the proper mechanism involved in the biosynthesis of NPs using *Bacillus* is a major limitation. Even though NADH enzymes are regarded as the main factor in reduction of MtNPs, but more detailed molecular studies that could provide a deep insight into the biofabrication of MtMPs are unavailable. The unavailability of such studies thus limit the production monodispersed MtNPs having ideal shape and size. NPs having spherical shape and small size are more stable having effective antimicrobial and anticancer activities. In addition, aggregation may also reduce the surface area of NPs, ultimately decreasing stability in solution and surface reactivity [31]. The synthesis of NPs using bacillus is sometime a slow process as compared to physiochemical routes.

According to a report, AgNPs were prepared from *Bacillus mojavensis* strain 32A after 7 days of incubation [20]. Another similar study reported the complete synthesis of AgNPs from *B. cereus* A30 strain after six days of incubation [73]. In contrast, contrast it was revealed that the synthesis of AgNPs from *B. amyloliquefaciens* and *B. subtilis* was achieved within 24 hr [91], while another study documented the synthesis of AgNPs from *Bacillus safensis* LAU 13 within 8 min of reaction [92]. Therefore, it seems that using different strains of *Bacillus* can result in NPs having different shapes and size and different rate of synthesis. Therefore the selection of right candidate is important. In addition, right reaction parameters like ph, temperature and incubation time should be optimized to insure the maximum NPs synthesis.

CONCLUSION

A brief description of the role of genus *Bacillus* in the synthesis of MtNPs has been provided. Furthermore, the synthesis mechanism and its various applications have been described. *Bacillus* species play an important role in the production of a variety of NPs. According to available reports, the use of *Bacillus* spp for the synthesis of MtNPs with potential antibacterial, antifungal, and anticancer activities seems to be a very promising approach. However the synthesis of NPs via *Bcaillus* species is slow process and sometime it may take up to several days to completely synthesize NPs. In addition, till date very few strains of *Bacillus* have been explored and it is important to look for new efficient strains

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COfFLICTS OF INTEREST

The authors declare that they have no personal interest.

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