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Endophytes: Toward a Vision in Synthesis of Nanoparticle for Future Therapeutic Agents

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ABSTRACT

The development of reliable processes for the synthesis of Nanoparticles is an important aspect of nanotechnology. Biologically synthesized nanoparticles could have innumerable applications in different areas such as reception, catalyzers, biolabellers, etc. In the present review, we emphasize the richness of the microbial world which encompasses a plethora of endophytic entities as an emerging tool in the biosynthesis of nanoparticles. The present review is the first of its kind wherein it gives an idea and vision of unexplored applications of endophytes.

Keyword: Microorganisms, Endophytes, Nanoparticles, Biosynthesis, Bioconjugation.

1. INTRODUCTION

Microorganisms create huge biodiversity among the richness of which the microbial world encompasses a plethora of endophytic entities occupying utterly millions of unique biological niches (higher plants) in various, many times unusual, environments. The term *endophyte* (Gr. *endon*, within; *phyton*, plant) was first coined by de Bary (1866) and has become deeply embedded in the literature ever since. At present, endophytic organisms are defined as "microbes that colonize living, internal tissues of plants without causing any immediate, overt negative effects" [1]. Research on endophytes is of burgeoning importance due to its rich source of novel natural products which are known to have wide applications in the pharmaceutical and agriculture sectors [2]. Microorganisms (bacteria, yeast and fungi) play an important role in toxic metals remediation through the reduction of metal ions; this was considered an interesting fact and these microbes were employed as nanofactories for the synthesis of nanoparticles. Biosynthesis of nanoparticles is an important aspect of nanotechnology.

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Nanoparticles

Nanoparticles can be also defined as particles less than 100 nm in diameter that exhibit new or enhanced size-dependent properties compared with larger particles of the same material. Nanoparticles exist widely in the natural world: for example, as the products of photochemical volcanic activity, or as created by plants and algae. They have also been created for thousands of years as products of combustion and food cooking and, more recently, from vehicle exhausts [3]. Moreover, these nanoparticles have innumerable applications in different areas as reception, catalyzers, bio-labellers, etc[4]. Figure 1 represents various important applications of nanoparticles, owing to their wide applications. Nanoparticles with exotic physicochemical and optoelectronic properties are of prime importance, while nanoparticles of metals and semiconductors have extensive use in various other branches of sciences [5-6].

Biosynthesis of nanoparticles

With the recent development and implementation of new technologies, the nano-revolution unfolds, and the role of microbes in bio and green synthesis of nanoparticles seem to have drawn unequivocal attention with a view of reformulating the novel strategies to combat the threat to human life and ecosystems in future eras. The conventional methods for the synthesis of nanoparticles are bound with various implications such as expensive costs, toxicity risks on health from environmental contaminants, etc [7-8]. Hence, there is an ever-growing need to develop biomimetic, clean, non-toxic, and environmentally benign synthetic approaches for the synthesis of nanoparticles, and these procedures are being explored. Consequently, researchers have used biological synthesis, since this technique provides particles with good control over the size distribution. The main reason for this may be that the processes devised by nature for the synthesis of inorganic materials on nano- and micro- scales have contributed to the development of a relatively new and largely unexplored area of research based on the use of microbes in the biosynthesis of nanomaterials [9]. Ever-increasing pressure to develop environmentally benign techniques for nanoparticles synthesis has led to a

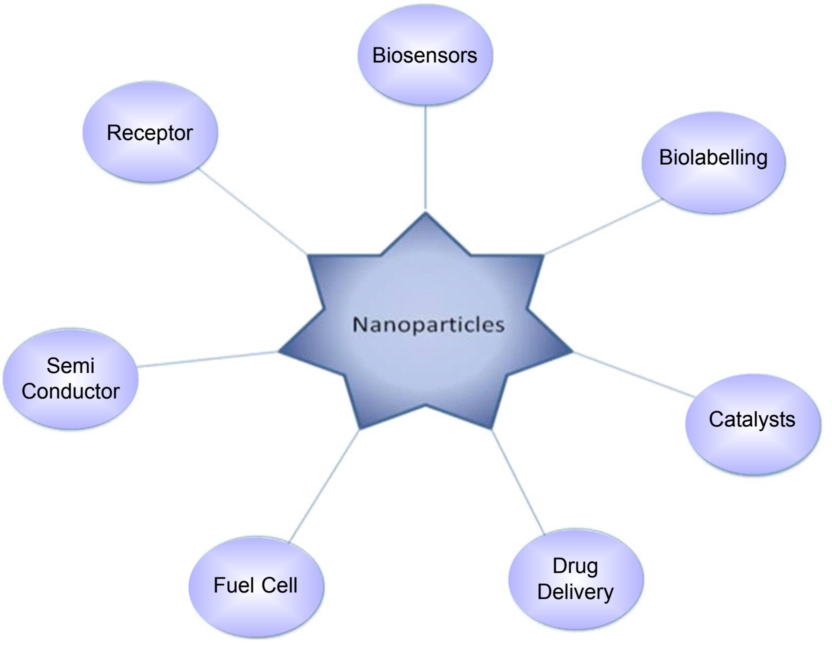

***Figure 1:** Applications of nanoparticles.*

renewed interest in biotransformation as a route to the growth of nanoscale structures. Biological systems have a unique ability to control the structure, phase and nanostructural topography of inorganic crystals [10].

Biological entities involved in synthesis of nanoparticles

Biological systems employed in nanoparticle synthesis vary from simple prokaryotic bacterial cells to eukaryotic fungi and even plants. It is well known that microbes such as bacteria, yeast, fungi, and algae [11-17] are able to absorb and accumulate metals and can be used in the reduction of environmental pollution and also for the recovery of metals from waste. Table 1 represents microorganisms in the synthesis of nanoparticles. Among these microorganisms, only a few groups have been confirmed to selectively reduce certain metal ions [18-24]. The potential of microbes to reduce metals has led to another new dimension of "Quantum Dots" or semiconductor bimetallic nanoparticles with extensive use in semiconductor devices [25]. Many microbes are known to produce highly structured metallic nanoparticles with very similar properties to that of chemically synthesized materials while having precise control over size, shape, and monodispersity. Thus, synthesis of nanomaterials using microorganisms is compatible with green chemistry principles, resulting in a surge of interest in scientists towards biological systems for inspiration [26-28].

Table 1: *Microorganisms employed in biosynthesis of nanoparticles [29].*

Microorganisms	Nanoparticles	Size of nanoparticles
Bacteria		
P. stutzeri	Ag	5- 15 nm
Klebsiellaaerogens	Au	20-200 nm
Clostridium thermoaceticum	CdS	-
hodopseudomonascapsulate	Gold Nanowires	-
Algae		
Diatoms		
Sargassumalga	SiO_2	50-100 nm
Yeast		
Candida glabrata	CdS	20 A
MKY3	Ag	2-5 nm
Schizosaccharomycespombe	CdS	1-1.5 nm
P. jadini	Au	100 nm
Fungi		
Aspergillusfumigatus	Ag	5-25 nm
Colletotrichum sp.	Au	20-40 nm
Fusariumoxysporum	Au, Ag	20-40 nm & 5-15 nm
Trichothecium sp.	Au	
Actinomycete		
Rhodococcus sp.	Au	5-15 nm
Thermonospora sp.	Au	8 nm

Probable mechanism in synthesis of nanoparticles

While a large number of microbial species are capable of producing metal nanoparticles, the mechanism of nanoparticle biosynthesis has not been established. The metabolic complexity of viable microorganisms complicates the analysis and identification of active species in the nucleation and growth of metal nanoparticles. Recent studies have demonstrated that proteins are the principal biomolecules involved in the synthesis of gold nanoparticles. Other researchers have postulated that microorganisms secrete enzymes that may be responsible for the reduction of metal ions, which results in the nanoparticles nucleation and growth. Figure 2 represents the probable mechanism involved in the microbial synthesis of nanoparticles. *Ahmad et al.* 2003 postulated that a NADH-dependent reductase is involved in silver nanoparticles synthesis by *Fusarium oxysporum*. However, the biochemical mechanism of metal ion reduction and subsequent nanoparticle formation remains unexplored [31].

Endophytic system in biosynthesis of nanoparticles and its future prospects

Endophytes are microorganisms that reside in the inner tissues of healthy living plants and form various associations with their hosts. The present review focuses on endophytes and their role as emerging and promising alternative entities in synthesizing these nanoparticles with a better application for prospective future drugs. Research on endophytes has yielded potential drug lead compounds with antibacterial, antiviral, anti-“oxidant, insulin mimetic, anti-neurodegenerative, and immunosuppressant properties [32]. The present review emphasizes the synthesis of nanoparticles by employing endophytes. The interface between endophytic systems and nano-materials is a relatively new and unexplored area and may open avenues in the future to push the frontier forward in coming decades.

Endophytic system so far reported in synthesis of nanoparticles

Keeping the lacunae of biosynthesis of nano-particles in view, an attempt has been made in this

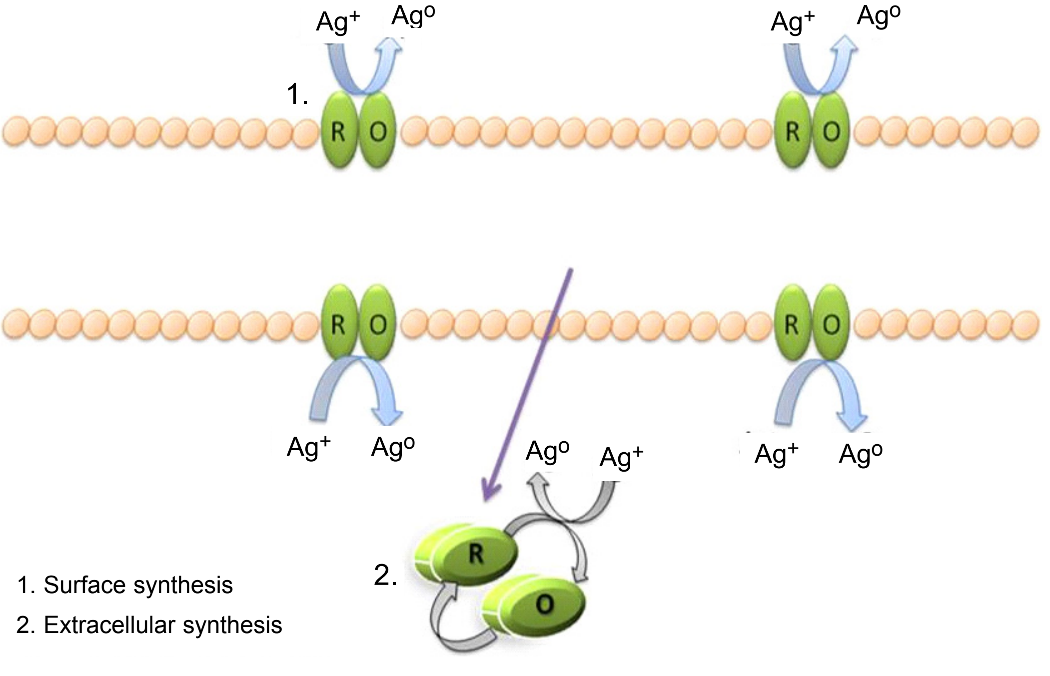

Figure 2: *Mechanism for nanoparticle synthesis by microorganism.*

review to focus on endophytes. There are a few literatures already reported and proven that endophytes and their hosts can easily synthesize nanoparticles. *Sankar* and *co-workers* (2003) reported use of geranium leaves (*Pelargonium graveolens*) and its endophytic fungus in the extra-cellular synthesis of gold nanoparticles. Sterilized geranium leaves and an endophytic fungus (*Colletotrichum sp.*) growing in the leaves were separately exposed to aqueous chloroaurate ions. In both cases, rapid reduction of the metal ions was observed, resulting in the formation of stable gold nanoparticles of variable size. In the case of gold nanoparticles synthesized using geranium leaves, the reducing and capping agents appear to be terpenoids, while they are identified to be polypeptides/enzymes in the case of *Colletotrichum sp.* The biogenic gold nanoparticles synthesized using the fungus were essentially spherical in shape, while the particles grown using the leaves exhibited a variety of shapes that included rods, flat sheets, and triangles. This report confirms the monodispersity of nanoparticles with endophytic fungus as compared to its host [33].

Musarrat et al in 2010 have isolated and used an endophytic filamentous fungus, *Amylomyces rouxii*, for silver nanoparticle synthesis. *A. rouxii* is a major component of starter cultures for traditional fermented foods in Southeast Asia, China, and the Indian sub-continent. The silver nanoparticles were characterized by determining the time-dependent increase in surface plasmon resonance through UV-Vis spectrophotometry, X-ray diffraction, transmission electron microscopy, and atomic force microscopy. Silver nanoparticles synthesized with extract from *A. rouxii* KSU-09 exhibited broad spectrum antimicrobial activity against Gram-negative and Gram-positive bacteria as well as human and plant pathogenic fungi. The efficient antimicrobial property of silver nanoparticles compared to silver salts is due to their extremely large surface area, which provides better contact with the microorganisms. The silver nanoparticles release silver ions into the bacterial cells, which enhances their bactericidal activity. Hence, this report confirms syntheses of antimicrobial silver nanoparticles were produced with water extracts from the fungus *A. rouxii* strain KSU-09. This fungal system has, therefore, the potential for low-cost and environmentally friendly production of silver nanoparticles [34].

Verma et al 2010 report the endophytic fungus *Aspergillus clavatus* can be isolated from sterilized stem tissues of *Azadirachta indica*. When the biomass of fungus was challenged with 1 mM HAuCl4 aqueous solution, results showed that triangular gold nanoparticles were formed along with some spherical as well as hexagonal morphology. It was also observed that the synthesis of gold nanotriangles is extracellular and shows a high aspect ratio. The study reported herein serves as a unique single-step green protocol for the generation and stabilization of nontoxic gold nanotriangles, exploitable in a myriad of diagnostic and therapeutic applications. *A. clavatus* induced synthesis of gold nanotriangles will provide unprecedented opportunities for the design and development of engineered "green" gold nanotriangles that can be widely utilized in biomedical applications. Characterization of nanoparticles was carried out with the help of UV-Vis spectrophotometry, X-ray diffraction, transmission electron microscopy, and atomic force microscopy [35].

Raheman et al 2012 reported an endophytic *Pestalotia sp.* isolated from healthy leaves of *Syzygiumcumini* (L.) for the extracellular synthesis of silver nanoparticles to evaluate their anti-bacterial activity against human pathogens. The study confirmed the formation of spherical and polydispersed nanoparticles in the range of 10-40 nm having an average size of 12.40 nm when the endophyte was treated with silver nitrate[36].

Devi et al 2012 reported an endophytic fungus, *Penicillium* sp, isolated from the Centellaasiatica. Silver nanoparticles were synthesized using the filtrate of cell mass of an isolated *Penicillium* sp. The SEM studies confirmed the formation of silver particles in the size of 100 nm, a clear indication of the formation of silver nanoparticles. The silver nanoparticle synthesized exhibited an antimicrobial effect on various human pathogens [37].

Perspective of endophytes in synthesis of nanoparticles and new antimicrobial drug formulation

Hence, the use of the endophytic system for nanomaterial biosynthesis has come in vogue in the recent past as an effective alternative to chemical synthesis. The ability to synthesize nanoparticles rapidly with morphology control by eco-friendly biological methods is exciting and represents an important advance in making them viable alternatives to the more popular chemical methods. Especially, extracellular synthesis offers the advantage of obtaining significant quantities in a relatively pure state and can be easily processed. These studies also exemplify the ability of microbes to tolerate metal and also support the hypothesis of endophytes playing a major role in the defense mechanism of their hosts by forming a symbiotic relationship and protecting their hosts from toxic metals and harsh environments. Exploiting endophytic flora in the synthesis of nanoparticles against other biological entities can lead to significant advances, especially in the area of antimicrobial studies. Table 2 represents some of the potent antimicrobial agents isolated from various endophytes. Problems related to human health, such as the development of drug resistance in human pathogenic bacteria, fungal infections, and life-threatening viruses; make a claim for new therapeutic agents in the effective treatment of diseases in human, plants, and animals that are currently unmet. In this regard, functional metabolites of endophytic origin can be tailored with nanoparticles as a potent antimicrobial complex which can be presumed to have an effective therapeutic impact on antimicrobial research. New antimicrobial complexes with novel mechanisms of action should have advantages over known available drugs, especially in the fight against multi-drug resistant bacteria and emerging pathogens. Endophytes are reported to secrete various antimicrobial bioactive compounds as their secondary metabolite; when such endophytes are employed in the synthesis of nanoparticles, the antimicrobial bioactive principles can capped or tailored with synthesized nanoparticles, which can be a very effective antimicrobial complex compared to an antimicrobial bioactive compound or nanoparticles alone [2]. These hybrid antimicrobial compounds unravel novel antimicrobial approaches against infectious diseases and have a large variety of biomedical and pharmaceutical applications. Advantages of bioconjugated antimicrobial agents, when compared to conventional antimicrobial agents of low molecular weight, are their non-volatile character, their chemical stability, and the improved efficacy of some existing antimicrobial agents, in addition to their prolonged lifetime. They can also be embedded onto a bandage to cure a wound or used in the packaging material of food to reduce microbial spoilage and increase the shelf life of perishable food products.

Bioconjugation

In the biomedical literature, bioconjugates, as a

Table 2: *Antimicrobial agents isolated from endophytes[38-41].*

Organism	Host	Bioactive Compounds	Activity
Pseudomonas viridiflava	Grass	Ecomycins B and C	Antimicrobial
Streptomyces griseus	Kandeliacandel	p-Aminoacetophenonic acids	Antimicrobial
Streptomyces NRRL 30562	Kennedianigriscans	MunumbicinsMunumbicin D	Antibiotic Antimalarial
Streptomyces NRRL 30566	Grevilleapteridifolia	Kakadumycins	Antibiotic
Serratia marcescens	Rhyncholacispenicillata	Oocydin A	Antifungal
Paenibacillus polymyxa	Wheat	Fusaricidin A-D	Antifungal
Cytonaema sp.	Quercus sp. 103	Cytonic acids A and D	Antiviral
Streptomyces sp.	Monstera sp.	Coronamycin	Antimalarial, Antifungal

rule, are considered to mean the structure consisting of a metal core and adsorbed or chemically-attached bio macromolecules. The mechanism in formation of conjugates and inorganic nano-particles and biological molecules remains incompletely understood and can involve electrostatic interactions between a negatively charged particle and positively charged regions of biomolecules, hydrophobic interactions and covalent bonding of the nanoparticle to sulfhydryl groups (-SH) of the biomolecule. The data on the role of the free amino group (-NH) in the biomolecule for the effective conjugation can be found in the literature [42-45]. In the use of peptides, the amino acid sequence in the peptide chain has significance, because the intra chain interactions substantially affect the formation of the compact ligand shell near the nanoparticle [46]. In each specific case, the technique for effective conjugation is determined by the structure and characteristics of the used biomolecules and inorganic nanoparticles. Recently, nanoparticles have developed that are covalently linked to biological molecules such as peptides, proteins, nucleic acids, or small-molecule ligands [47-49]. Medical applications have also appeared, such as the use of super paramagnetic iron oxide nanoparticles as a contrast agent for lymph node prostate cancer detection and the use of polymeric nanoparticles for targeted gene delivery to tumor vasculatures [50]. Figure 3 provides a schematic representation of bioconjugation with nanoparticles; in step 1 the bioactive compounds having various functional groups are tailored with the nanoparticles, and step 2 results in the engineered nanoparticles bio-conjugated with bioactive compounds resulting in the potent drug molecule of interest.

When nanoparticles synthesized by endophytes can be bio-conjugated with the secondary bioactive metabolite secreted from the same potent endophyte, they can be used as nanodrugs for various diseases, especially in targeted drug delivery. Furthermore, some of the nanoparticles have photo thermal properties that can be exploited for localized heating resulting in drug release, thus

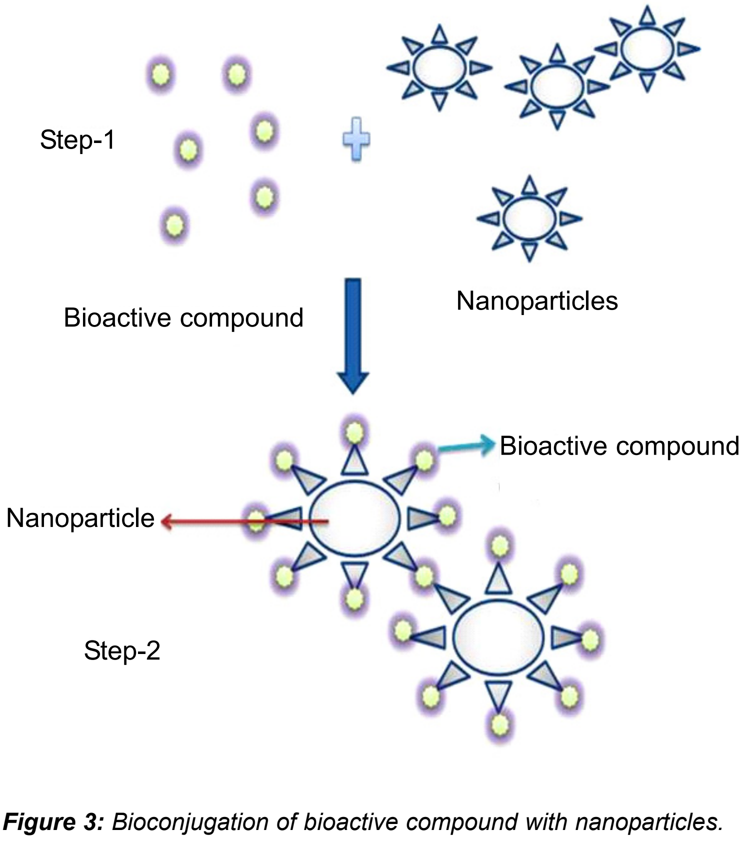

Figure 3: *Bioconjugation of bioactive compound with nanoparticles.*

increasing their potential for therapeutic applications. As a whole, these bioconjugated bioactive compounds with nanoparticles can act as effective therapeutic agents and can be useful as potent drug molecules in curing a disease and reformulating the drugs that can be like missiles in combating various emerging drug-resistant microorganisms and emerging infectious diseases. The increase in drug-resistant microorganisms has been one of the major concerns across the globe. Bioconjugated drugs can open a new avenue as an effective antimicrobial agent by their mode of mechanism. If a drug-resistant microorganism is resistant to a particular drug molecule, then the nanoparticle can display its effect against the microorganism by targeting the site and mode of action. Hence, bioconjugated drugs will be more significant in coming decades and can give a new vision to the pharmaceutical era which is in search of novel drugs.

Mode of action of bioconjugated antimicrobial complex

Nanoparticles have been used to improve remedial efficacy or to fight against multi-drug-resistant bacteria. They can also act as an effective drug carrier through cell membranes. Potentially, nanoparticles could be efficient through the polyvalent effect [51-53]. Figure 3 represents the mode of action of bioconjugated nanoparticles on multi-drug-resistant bacteria. The antimicrobial activity of silver nanoparticles has been reported to interact with cytoplasmic components and nucleic acids, to inhibit respiratory chain enzymes, and to interfere with the membrane permeability Dehydrogenase of complex I [54].

Although silver nanoparticles are known to be a very potent and rapidly acting antibacterial agent, silver-resistant bacteria do exist [55]. In such cases, these bioconjugated drugs come in to play wherein if a bacterium is resistant to one component of the

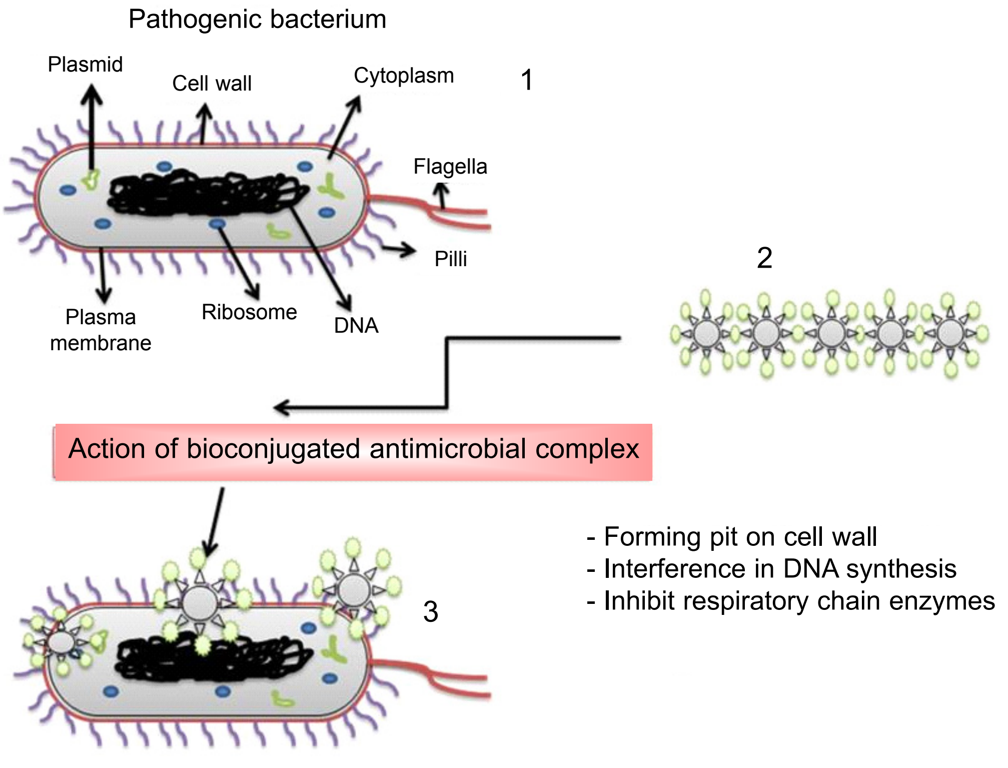

Figure 4: *Mode of action of bioconjugated nanoparticles on multidrug resistant bacteria.*

bioconjugated drug then other component will act against it. It is reported that silver nanoparticles exert anti-HIV activity at an early stage of viral replication, most likely as a virucidal agent or as an inhibitor of viral entry. Silver nanoparticles bind to gp120 in a manner that prevents CD4-dependent virion binding, fusion, and infectivity, acting as an effective virucidal agent against cell-free virus (laboratory strains, clinical isolates, T and M tropic strains, and resistant strains) and cell-associated virus. Besides, silver nanoparticles inhibit post-entry stages of the HIV-1 life cycle. The antiviral properties of silver nanoparticles against HIV-1 and found by invitro assays are active against a laboratory-adapted HIV-1 strain at non-cytotoxic concentrations. Silver nanoparticles of 30-50 nm were tested against a panel of HIV-1 isolates using indicator cells in which infection was quantified by a luciferase-based assay. Silver nanoparticles inhibited all strains, showing comparable antiviral potency against T-tropic, M-tropic, dualtropic, and resistant isolates [56].

2. CONCLUSIONS

In summary, a brief overview of endophytes in biosynthesis nanoparticles has been envisioned in the present review as a Viable route for low-cost and environmentally-friendly production of nanoparticles as an alternative to the more popular physical and chemical methods currently in vogue. These biologically synthesized nano-particles can be exploitable in a myriad of diagnostic and therapeutic applications, providing unprecedented opportunities for the design and development of engineered "green" nanoparticles. The present review also attempts to explore the endophytic plethora for functional bioactive metabolites and bio-conjugating it with synthesized nanoparticles, resulting in a potent drug complex with future prospects. This can be widely utilized in biomedical applications, especially in targeted drug delivery and formulating nanodrugs to combat various multi-drug-resistant micro-organisms.

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