

## **SILVER NANOPARTICLES: BIOSYNTHESIS AND ITS ANTIMICROBIAL ACTIVITY**

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### **ABSTRACT**

Nanotechnology involves the production manipulation use of materials ranging in size from less than a micron to that of individual atoms. Nano' means very small in the range of  $10^{-9}$  to be precise 10 to 100 nm in size. Nanoparticles of noble metals are of great interest today because of their possible applications in microelectronics. Silver in colloidal state exhibits distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activity. An important area of research in nanotechnology is the biosynthesis of nanoparticles such as nanosilver. Biologically synthesized silver nanoparticles could have many applications, such as spectrally selective coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors, catalysts in chemical reactions, biolabelling, etc. The problem with most of the chemical and physical methods of nanosilver production is that they are extremely expensive and also involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks. There are three major sources of synthesizing silver nanoparticles: bacteria, fungi, and plant extracts. The antimicrobial nature of silver nanoparticles is the most exploited nature of silver nanoparticles in the medical field, though the anti-inflammatory nature is also considered immensely useful in the medical field.

**Key Words:** *Nanotechnology, Nanosilver, Biosynthesis, Antimicrobial Activity*

### **INTRODUCTION**

Nanotechnology due to its capability of modulating metals into their nanosize, which drastically changes the chemical, physical, and optical properties of metal, is gaining incredible impetus in the present century (Saha *et al.*, 2011). Nanotechnology contain the production manipulation use of materials ranging in size from less than a micron to that of individual atoms (Moharrer *et al.*, 2012). Of either simple or composite nature nanosized inorganic particles display unique physical and chemical properties and represent an increasingly important material in the development of novel nanodevices. That can be used in various physical, biological, biomedical, and pharmaceutical applications (Sondi *et al.*, 2004). Nanoparticles are serving as the fundamental building blocks of nanotechnology (Vahabi *et al.*, 2011). Because of their wide application in numerous areas such as electronics, catalysis, chemistry, energy, and medicine, the commercial requirement of nanoparticles is rising. By wet chemical techniques, metallic nanoparticles are synthesized where the chemicals used are toxic and flammable (Sivakumar *et al.*, 2011). 'Nano' means very small size the range between 10 to 100 nm (Thirumalai *et al.*, 2010). Nanoparticle is expressed as a measure of  $10^{-9}$  times of SI units (Venkataraman *et al.*, 2011). The term "Nanotechnology" has been coined by Norino Taniguchi which is researcher at the University of Tokyo, Japan (Taniguchi, 1974 and Venkataraman *et al.*, 2011). These crystallites exhibit novel material properties, due to their small size and that largely differ from the bulk properties (Wang *et al.*, 2005). There is great interest in the formulation of new pharmaceutical products of uniform nanosized drug particles with specific requirements in terms of size, shape, and physical and chemical properties (Sondi *et al.*, 2004). Nanoparticles have possible applications in microelectronics so nanoparticles of noble metals are of great interest today (Wang *et al.*, 2005). Biosynthesis of nanoparticles such as nanosilver is an important area of research in nanotechnology (Nithya *et al.*, 2009).

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The importance of silver for its curative properties has been known for centuries. Silver has been the most widely studied metal for purpose of fighting infections and preventing food spoilage. However the decline of its use as a consequence of the development of antibiotics, prophylaxis against gonococcal ophthalmia neonatorum with silver ions was considered the standard of care in many countries until the end of the 20th century (Galdiero *et al.*, 2011). There are various approaches are out there for the synthesis of silver nanoparticles for example, chemical reduction, photochemical, reverse micelles, thermal decomposition, radiation assisted, electrochemical, sonochemical, microwave assisted method and recently via green chemistry method (Sivakumar *et al.*, 2011). Due to its activity against mammalian tissues, silver is medically considered as one of the most powerful element where it acts as an antiseptic agent (Theaj Prakash *et al.*, 2012). In the living environment microorganisms, such as bacteria, molds, yeasts, and viruses, are often pathogenic and cause severe infections in human beings so there is a vital need to search for new antimicrobial agents from natural and inorganic substances (Gajbhiye *et al.*, 2009). In colloidal state silver exhibit distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activity (Kaler *et al.*, 2010).

The applications of silver nanoparticles are increased when the significances of the nanoparticles were discovered. Silver nanoparticles, they are used as antimicrobial agents in most of the public places such as elevators and railway stations in China (Venkataraman *et al.*, 2011).

In order to reduce the infections caused during surgery and are proposed to possess antifungal, antiinflammatory, antiangiogenic and antipermeability activities, silver nanoparticles are used as antimicrobial agents in surgically implanted catheters (Kalishwaralal *et al.*, 2009; Gurunathan *et al.*, 2009a, b and Sheikpranbabu *et al.*, 2009) (Venkataraman *et al.*, 2011). In its metallic as well as ionic forms, silver exhibits cytotoxicity against several microorganisms and hence used as an anti microbial agent (Theaj Prakash *et al.*, 2012). Development of reliable technology to produce nanoparticles is an important aspect of nanotechnology. The synthesis of silver nanomaterials /nanoparticles extensively studied by using chemical and physical methods, but the biological synthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum time required (Natarajan *et al.*, 2010). Biosynthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum time required (Natarajan *et al.*, 2010). Biosynthesized silver nanoparticles could have many applications, such as spectrally selective coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors, catalysts in chemical reactions, biolabelling etc (Nithya *et al.*, 2009). Among inorganic antimicrobial agents, silver has been employed most widely since ancient times to fight against infectious agent (Gajbhiye *et al.*, 2009).

The current chemical methods for synthesizing nanoparticles are energy intensive, contain toxic chemicals which are becoming outdated, expensive and inefficient (Saha *et al.*, 2011). Biosynthetic methods have been investigated as an alternative to chemical and physical ones. Depending on the place where the nanoparticles or nanostructures are created as many microorganisms can provide inorganic materials either intra-or extracellularly, biosynthetic methods can be divided into two categories (Sadowski *et al.*, 2008). New enzymatic approaches using microorganisms in the synthesis of nanoparticles both intra- and extracellularly have been expected to play key role in many conventional and emerging technologies. Live and dead microorganisms are gaining importance by virtue of their facile assembly of nanoparticles (Oza *et al.*, 2012).

The antibacterial and antiviral activity of silver, silver ions, and silver compounds has been thoroughly investigated. A recent literature survey showed remarkable findings on the bactericidal activity of silver nanoparticles (Ag-NPs) (Gajbhiye *et al.*, 2009). Because of the diversity richness and high tolerance, under ambient conditions of temperature, pressure and acidity, the microorganisms are greatly desired for the syntheses of different nanoparticles, among the biological systems. Various microorganisms including algae, bacteria and fungi have already been harnessed for the biosynthesis of silver nanoparticles (Saha *et al.*, 2011). Towards the synthesis of nanoparticles biotechnology approach has many advantages, such as process can be scaled up easily, economic viability, possibility of easily

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covering large surface areas by suitable growth of the mycelia, and its green chemistry nature provided the microorganism medium is safe (Vahabi *et al.*, 2011). Due to its metabolic activity, microbial source to produce the silver nanoparticles shows the great interest towards the precipitation of nanoparticles (Natarajan *et al.*, 2010). Bacteria *Pseudomonas strutzeri* isolated from silver mine materials is able to reduce Ag<sup>+</sup> ions and accumulates silver nanoparticles, ranging between 16–40 nm, with the average diameter of 27 nm (Sadowski *et al.*, 2008).

**Table 1: Silver- synthesizing bacteria fungi and plant (Prabhu S *et al.*, 2012)**

S. No.	Bacteria	Fungi	Plant
1	<i>P. stutzeri</i> AG259	<i>Verticillium</i> sp.	<i>Medicago sativa</i>
2	<i>Bacillus megaterium</i>	<i>Phoma</i> sp. 3.2883	<i>Azadirachta indica</i>
3	<i>Plectonema boryanum</i>	<i>F. oxysporum</i>	<i>Aloe vera</i>
4	<i>Enterobacter cloacae</i>	<i>Phanerochaete chrysosporium</i>	<i>Cinnamomum camphora</i> leaf
5	<i>Escherichia coli</i>	<i>Aspergillus fumigatus</i>	<i>Carica papaya</i> fruit
6	<i>B.licheniformis</i>	<i>Aspergillus flavus</i>	<i>Cinnamomum zeylanicum</i> bark
7	<i>Lactobacillus fermentum</i>	<i>Fusarium semitectum</i>	<i>Jatropha curcas</i>
8	<i>Klebsiella pneumonia</i>	<i>Coriolus versicolor</i>	<i>Desmodium triflorum</i>
9	<i>Proteus mirabilis</i>	<i>Fusarium solani</i>	<i>Coriandrum sativum</i> leaf
10	<i>Brevibacterium casei</i>	<i>Aspergillus clavatus</i>	<i>Piper betle</i> leaf

Filamentous fungi are more advantageous over the bacteria and algae because fungi having fungal mycelial mesh which can withstand flow pressure and agitation and other conditions in the bioreactors or other chambers. Other silver tolerant fungi like *Fusarium oxysporum*, *F. solani* USM 3799, *Aspergillus niger*, *Coriolus versicolor*, etc are capable of synthesizing silver nanoparticles of different sizes but with spherical shapes (Saha *et al.*, 2011). The extracellular production of metal nanoparticles by several strains of the fungus *Fusarium oxysporum* has been described by Duran Sadowski *et al.*, (2008). Extracellular synthesis reported that a reductase enzyme is released into solution which causes reduction of Ag<sup>+</sup> ions (Theaj Prakash *et al.*, 2012). Kim *et al.*, () reported that spherical silver nanoparticles (Ag-NPs) showed potent activity against *Trichophyton mentagrophytes*, *Trichosporon beigelii*, and *Candida albicans* compared with that of commercially available antifungal agents (amphotericin B and fluconazole). Stable colloidal solutions containing up to 35 ppm nanoparticles were found to have effective antifungal properties against *Aspergillus*, *Penicillium* and *Trichoderma* species (Gajbhiye *et al.*, 2009). There is two-step mechanism was suggested as a novel biological method for synthesis of silver nanoparticles using *Vericillum*. The first step involves trapping of Ag<sup>+</sup> ions at the surface of the fungal cells. *Aspergillus fumigatus* and *Phanerochaete chrysosporium* produced stable silver nanoparticles when challenged with silver nitrate in aqueous medium. The extra cellular synthesis of stable silver nanoparticles using the fungus *Aspergillus flavus* has also been reported and recently has reported the synthesis of silver nanoparticles using white rot fungus *C.versicolor* (Nithya *et al.*, 2009). *Bacillus licheniformis* is one such organism used to synthesize silver nanoparticle at 1 mM concentration (Venkataraman *et al.*, 2011).

### Biosynthesis of Silver Nanoparticles

In recent years resistance of bacteria to bactericides and antibiotics has increased due to the development of resistant strains. Some antimicrobial agents are extremely irritant and toxic and there is vital need and much interest in finding ways to formulate new types of safe and cost-effective biocidal materials (Sondi *et al.*, 2004). The chemical and physical methods of nanosilver production is extremely expensive and also involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks, which is big problem. The silver nanoparticles synthesized have to be handled by humans and must be available at cheaper rates for their effective utilization; thus, there is a need for an environmentally and economically feasible way to synthesize these nanoparticles and it is an unavoidable fact (Prabhu *et al.*, 2012). Due to the growing need to develop environmentally benign technologies in material synthesis, biosynthesis of nanoparticles has received considerable attention (Oza *et al.*, 2012). There are few reports

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in literature on the biosynthesis of silver nanoparticles using microorganisms, such as fungi and bacteria (Jain *et al.*, 2012). There are three major sources of synthesizing silver nanoparticles: bacteria, fungi, and plant extracts. Biosynthesis of silver nanoparticles is mostly involves reduction/oxidation reactions. The microbial enzymes or the plant phytochemicals with antioxidant or reducing properties that acts on the respective compounds and give the desired nanoparticles (Prabhu *et al.*, 2012).

### Silver Synthesizing Bacteria

The first time bacterial synthesizing silver nanoparticles was made up by using the *Pseudomonas stutzeri* AG259 strain which was isolated from silver mine (Prabhu *et al.*, 2012). Bacteria *Pseudomonas stutzeri* isolated from silver mine materials is able to reduce  $\text{Ag}^+$  ions and accumulates silver nanoparticles, the size of such nanoparticles being in the range 16–40 nm, with the average diameter of 27 nm (Sadowski *et al.*, 2008). Due to its metabolic activity microbial source to produce the silver nanoparticles shows the great interest (Natarajan *et al.*, 2010).

Klabunde and co-workers recently demonstrated that highly reactive metal oxide nanoparticles exhibit excellent biocidal action against Gram-positive and Gram-negative bacteria (Sondi *et al.*, 2004). There are some microorganisms that can survive metal ion concentrations and can also grow under those conditions, and this phenomenon is due to their resistance to that metal. The mechanisms involved in the resistance are efflux systems, alteration of solubility and toxicity via reduction or oxidation, biosorption, bioaccumulation, extracellular complex formation or precipitation of metals and lack of specific metal transport systems. The presence of the nitrate reductase enzyme is the most widely accepted mechanism of silver biosynthesis. The enzyme converts nitrate into nitrite. Nitrate is converted into nitrite during the reduction and the electron is transferred to the silver ion; hence, the silver ion is reduced to silver ( $\text{Ag}^+$  to  $\text{Ag}^0$ ). This has been said to be observed in *Bacillus licheniformis* which is known to secrete NADPH and NADPH-dependent enzymes like nitrate reductase that effectively converts  $\text{Ag}^+$  to  $\text{Ag}^0$  (Saha *et al.*, 2011).

### Silver Synthesizing Fungi

The metabolic activity of microorganisms can lead to precipitation of nanoparticles in external environment of a cell, the fungi being extremely good candidates for such processes. The extracellular synthesis of silver and gold nanoparticles by the fungus *Colletotrichum* sp. or *Aspergillus fumigatus* has been reported. A novel biological method for synthesis of silver nanoparticles using *Vericillium* was proposed by Mukherjee *et al.* He suggested two-step mechanism for synthesis of silver nanoparticles (Sadowski *et al.*, 2008). The biological process more advantages which include filamentous fungal tolerance towards metals, their high binding capacity and intracellular uptake of metals. The mass production of fungi is easy, for synthesis of nanoparticles. Due to their physicochemical properties,  $\text{Ag}$  nanoparticles have been widely employed and are currently used as anti-bacterial agents in food storage, textile and health industries, for biolabeling and as biosensors. The anti microbial activity of silver nanoparticles has now been well established and they possess anti inflammatory, anti viral, anti platelet and anti fungal activity (Theaj Prakash *et al.*, 2012).

Filamentous fungi are more advantageous over the bacteria and algae because fungal having fungal mycelial mesh which can withstand flow pressure and agitation and other conditions in the bioreactors or other chambers. Other silver tolerant fungi like *Fusarium oxysporum*, *F. solani* USM 3799, *Aspergillus niger*, *Coriolus versicolor*, etc are capable of synthesizing silver nanoparticles of different sizes but with spherical shapes (Saha *et al.*, 2011). A novel biological method for synthesis of silver nanoparticles using *Vericillium* was proposed in two-step mechanism. The first step involves trapping of  $\text{Ag}^+$  ions at the surface of the fungal cells.

Recently has reported the synthesis of silver nanoparticles using white rot fungus *C. versicolor* (Nithya *et al.*, 2009). Various fungi such as *Fusarium oxysporum*, *Trichoderma reesei* and *Trichoderma viride* which contain the hydrogenase enzyme which was demonstrated with washed cell suspensions that had been grown aerobically or anaerobically in a medium with glucose and salts amended with nitrate. The nitrate reductase was apparently essential for ferric iron reduction. Many fungi that exhibit these



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characteristic properties, in general, are capable of reducing Au (III) or Ag (I). Besides these extracellular enzymes, several naphthoquinones and anthraquinones with excellent redox properties were reported in *Fusarium oxysporum* that could act as electron shuttle in metal reductions (Vahabi *et al.*, 2011). Comparison with bacteria, fungi can produce larger amounts of nanoparticles due to secretion larger amounts of proteins which directly translate to higher productivity of nanoparticles. Considering the example of *F. oxysporum*, it is believed that the NADPH-dependent nitrate reductase and a shuttle quinone extracellular process are responsible for nanoparticle formation (Prabhu *et al.*, 2012).

### Silver Synthesizing Plant

Plants are easily available, safe, and nontoxic in most cases, have a broad variety of metabolites that can aid in the reduction of silver ions, and are quicker than microbes in the synthesis of AgNPs, that is the major advantage of using plant extracts for silver nanoparticle synthesis. The main mechanism considered for the process is plant-assisted reduction due to phytochemicals. The main phytochemicals involved are terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. Flavones, organic acids, and quinones are water-soluble phytochemicals that are responsible for the immediate reduction of the ions (Prabhu *et al.*, 2012). The Neem (*Azadirachta indica*) leaf broth and aqueous solution of silver nitrate or chloroauric acid were used for the extracellular synthesis of pure metallic silver and gold particles (Sadowski *et al.*, 2008). Compared with chemical and physical method of synthesis green synthesis method provides a low cost, environment friendly, easily scale up for large scale synthesis. Green synthesis method there is no need to use high pressure, energy, temperature and toxic chemicals (Sivakumar *et al.*, 2011).

### Antimicrobial Activity

Nanotechnology refers broadly to a field of applied science and technology whose unifying theme is the control of matter on the atomic and molecular scale. The metal microbe interactions have an important role in several biotechnological applications including the fields of bioremediation, biomineralization, bioleaching, and microbial corrosion (Oza *et al.*, 2012). Recent studies have demonstrated that specially formulated metal oxide nanoparticles have good antimicrobial activity (Nithya *et al.*, 2009). Silver and its compounds have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities for bacteria, fungi, and virus since ancient times (Nasrollahi *et al.*, 2011). In recent years, due to the development of resistant strains resistance of bacteria to bactericides and antibiotics has increased. Some antimicrobial agents are extremely irritant and toxic and there is vital need and much interest in finding ways to formulate new types of safe and cost-effective biocidal materials (Sondi *et al.*, 2004). By agar disk diffusion method the antimicrobial activity of Ag nanoparticles against microorganisms is evaluated (Jain *et al.*, 2012). The antimicrobial nature of silver nanoparticles is the most exploited nature of silver nanoparticles in the medical field. The anti-inflammatory nature of AgNPs is also considered immensely useful in the medical field (Prabhu *et al.*, 2012). The silver nanoparticles have unique physical and chemical properties which make them excellent candidates for the antimicrobial and anti-inflammatory activity. These properties make them excellent candidates for many purposes in the medical field and pharmaceuticals (Saha *et al.*, 2011). Thus, silver ions, as an antibacterial component; have been used in the formulation of dental resin composites and ion exchange fibers and in coatings of medical devices. Tiller and co-workers recently showed that hybrids of silver nanoparticles with amphiphilic hyperbranched macromolecules exhibit effective antimicrobial surface coatings (Sondi *et al.*, 2004). Initial studies have suggested that the acceleration of wound healing in the presence of nanoparticles is due to the reduction of local matrix metalloproteinase (MMP) activity and the increase in neutrophil apoptosis within the wound. A reduction in the levels of pro-inflammatory cytokines was also demonstrated in a mouse model with burn injury when silver nanoparticles were introduced. Dr. Robert Burrell is said to have developed the world's first nanosilver-based wound dressing in 1995. He developed Acticoat that speeds up the healing process and removes scars if any. The antimicrobial property of silver nanoparticles is reported and it has immense potential to be used in disinfectants. There is also believed that most medical treatments such as intravenous catheters, endotracheal tubes, wound dressings, bone

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cements, and dental fillings can all make use of nanosilver to prevent microbial infections (Saha *et al.*, 2011). Viruses represent one of the leading causes of disease and death worldwide. For some of there is still no vaccine available for most pressing viral pathogens. Viruses are also able to cause considerable distress and sometimes persistent infections that may lead to cancer or to acquire immunodeficiencies, such as hepatitis viruses (mainly HBV and HCV) or human immunodeficiency virus (HIV). There have been improvements in antiviral therapy, but with a wide margin of ineffectiveness, therefore new antiviral agents are urgently needed to continue the battle between invading viruses and host responses. The advantage of using silver nanoparticles for impregnation is that there is continuous release of silver ions enhancing its antimicrobial efficacy. Antimicrobial effectiveness was shown for both Gram-positive and Gram-negative bacteria. The antibacterial activity of silver nanoparticles was mainly demonstrated by *in vitro* experiments. Activity against methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio cholera* and *Bacillus subtilis* has been documented. Low concentrations of silver nanoparticles were able to consistently inhibit *E. coli* while the growth-inhibitory effect on *S. aureus* was minor. Synergistic antimicrobial activity of silver or zinc nanoparticles with ampicillin, penicillin G, amoxicillin, kanamycin, erythromycin, clindamycin, chloramphenicol and vancomycin against *S. aureus*, *E. coli*, *Salmonella typhi* and *Micrococcus luteus* was observed. Theoretically, any metal could be analysed for antiviral activity, however, little effort has been done to determine the interactions of metal nanoparticles with viruses. Some recent studies have emerged showing that metal nanoparticles can be effective antiviral agents against HIV-1, hepatitis B virus, respiratory syncytial virus, herpes simplex virus type 1, monkeypox virus, influenza virus and Tacaribe virus. Elechiguerra *et al.* were the first to describe the antiviral activity of metal nanoparticles. They also found that silver nanoparticles undergo size-dependent interactions with HIV-1 (Kaler *et al.*, 2010). Shirley *et al.*, observed the antibacterial activity of silver nanoparticles against various pathogenic bacteria of Gram positive (*S. aureus*, *S. epidermidis*) and Gram negative strains (*E. coli*, *S. typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus vulgaris*) using well diffusion technique. Nalenthiran *et al.* observed *Bacillus* sp. using TEM after exposure to a 3.5 mM aqueous AgNO<sub>3</sub> solution to confirm silver precipitation. Many researchers used *Escherichia coli* as a model for gram negative bacteria and proved that AgNPs may be used as an antimicrobial agent. Other workers also opined that the AgNPs have an antimicrobial effect on *S. aureus* and *E. coli* (Jain *et al.*, 2012).

## CONCLUSION

Nanoparticles of noble metals are of great interest today because of their possible applications in microelectronics. Reducing the size of a particle has a greater impact on the property of the molecule and the properties also completely differ from the bulk material. But the synthesis of nanomaterials at nanoscale involves tedious phenomena (reducing agents) when synthesized chemically. Moreover, the chemically synthesized nanoparticles require another step for the prevention of aggregation of the particles. But, biological synthesis of silver nanoparticles uses harmless, eco-friendly reducing agents and the nanoparticle structure is stabilized by the proteins present in the environment eliminating the extra step in preventing the aggregation of chemically synthesized nanoparticles by stabilizers. The current chemical methods for synthesizing nanoparticles are energy intensive, employ toxic chemicals which are becoming outdated, expensive and inefficient. Silver has always been an excellent antimicrobial and has been used for the purpose for ages. Biologically synthesized silver nanoparticles could have many applications, such as spectrally selective coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors, catalysts in chemical reactions, biolabelling, etc. Hence, this current review concludes that the unique properties of these silver nanoparticles can be put to great use for human betterment without any controversies.

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