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Biogenic Synthesis of Silver Nanoparticles with Antimicrobial Properties

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Abstract

The transmission of a wide range of diseases, related to the infection by pathogenic microorganisms is a major public health problem that daily endangers the safety of human population. Silver has been thoroughly studied and used against bacteria due to its antimicrobial properties. Nanostructured silver gathers all the advantages of the silver itself, as well as the advanced performance of the nanomaterials. Thus, currently, silver nanoparticles constitute the most widely used kind of nanoparticles in biomedicine, due to their attractive antimicrobial properties. A variety of physical and chemical methods are employed for the AgNPs synthesis. However, many of them include the use of toxic reagents or require large amounts of energy, during the synthesis process. For this reason, many eco-friendly methods are proposed in order to synthesize AgNPs. Hence, biogenic synthesis of AgNPs, utilizing biological resources opens a novel route for the development of alternative production processes. These methods seem to have significant advantages, as the extracts contribute positively to the formation and enhancement of the antimicrobial activity of AgNPs, also acting as protective agents of the produced particles. In this review an integrated approach of AgNPs bio-synthetic methods using microorganisms, such as bacteria and fungi, plants and plant extracts, as well as several templates, like DNA and viruses is discussed, shedding light on the comparative advantages of them.

Keywords: Silver Nanoparticles; Antibacterial Properties; Biosynthesis; Biogenic Synthesis; Green Synthesis; Microorganisms; Plant Extracts; Templates

Introduction

Nowadays, the uncontrolled spread of diseases and infections caused by various pathogenic agents is a major public health problem [1]. Particularly, the microbial resistance opposed to a wide range of microbes, is one of the most crucial issues in the field of medicine [2]. Thus, in the light of the aforementioned issues and thanks to the recent advances in science and technology worldwide, which have led to the growth of nanotechnology, these problems may be controlled [3]. Nanotechnology is emerged as a promising interdisciplinary section dealing with research and development in various fields [4] and is currently considered to be a powerful medical tool, consisting a separate branch, called nanomedicine [5].

Within that framework, during the last decade, a new concept has been introduced, that of biogenic nanotechnology [6]. Biogenic nanotechnology constitutes an area of interest with important objective of facilitating the production of nanomaterial, such as metal nanoparticles [7]. Typically, MNPs can be synthesized using various approaches, such as biological, physical, or chemical methods with the conditions to control the size/shape and stability of nanoparticles [8]. The biogenic synthesis development of MNPs involves the use of non-toxic chemical substances, such as solvents, reducing and stabilizing agents [9,10]. Thus, apart from the non-toxic nature of the process, the simple development design and the cost effectiveness are the distinctive advantages of biogenic synthesis of MNPs, over the physical and chemical synthesis methods [11]. Biogenic methods of synthesis are performed through intracellular or extracellular approaches, generally utilizing plants and microorganisms [12]. The MNPs which are synthesized through a biogenic method, display multiple applications in fields such as catalysis, agriculture and electronics, with eminent that of biomedicine[13,14], with reported studies focusing on cancer treatment targeted drug delivery, DNA analysis, gene therapy, antibacterial agents, magnetic resonance imaging , enhancing reaction rates, and biosensors [15,16].

Silver has been thoroughly studied and used to treat bacterial infections due to its antimicrobial potential [17]. Silver nanoparticles gather all the advantage of the silver itself, as well as the advanced performance of the nanomaterials [18]. Thus, among the other noble MNPs, AgNPs possess potent antibacterial, antiviral and anticancer properties [19-21]. Additionally, AgNPs are utilized for bio-sensing, coating surgical instruments, water treatment, etc [22-26].

The promising antimicrobial properties of AgNPs against both gram-positive and gram-negative bacterial cells and their stability have attracted the interest of a great part of the scientific society [27]. It seems that the use of AgNPs, leads the bacterial cells to be less prone to develop antibacterial resistance [28].

Numerous strategies for the synthesis of AgNPs have been developed, including a variety of physical and chemical methods [29]. However, as mentioned above, the majority of them either employs toxic compounds or requires large amounts of energy during the synthesis process [30]. Under such circumstances, the elaboration of biogenic methods based on plants, fungi and bacteria has proven to be extremely promising for the AgNPs production, without negative impact on human health and the environment [31]. These methods seem to have significant advantages, as the extracts contribute positively to the formation and enhancement of the antimicrobial activity of AgNPs, also acting as protective agents of the produced particles [32].

In the current manuscript, we provide an overview of the preparation of AgNPs with antimicrobial properties, using biogenic methods, highlighting the great impact of the incorporation of green chemistry in innovative research protocols.

Physical	Chemical	Biological
Ball-milling Arc-discharge Vapor condensation Laser ablation	Chemical reduction Electrochemical Pyrolysis Irradiation-assisted	Microorganisms Plants / plant extracts Templates

Table 1: Methods for the synthesis of AgNPs.

Biogenic Synthesis of Silver Nanoparticles

Since the conventional methods for the synthesis of AgNPs utilize toxic and non-environmentally friendly reagents [33,34], researchers worldwide were triggered to develop and optimize the biogenic synthesis as a new route for the production of AgNPs [35,36]. Three main categories of biogenic synthesis of AgNPs would be determined, the synthesis based on the utilization of microorganisms, such as fungi and bacteria [37], the green synthesis, based on the use of plants and their extracts [38] and the approach which applies templates, such as viruses, membranes and DNA [39,40] .

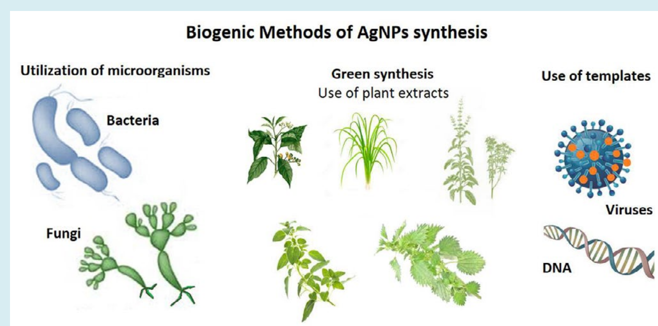

Figure 1: The main biogenic methods of AgNPs synthesis.

Synthesis of AgNps Using Microorganisms

The potential of microorganisms, like fungi and bacteria, acting as human, eco-friendly precursors for the production of AgNPs with antimicrobial properties, has gained a lot of interest [41-45]. The use of these microorganisms as bio-factories gathers several advantages, such as the production of nanoparticles with various sizes and chemical compositions in a mono-disperse way [46,47].

Researchers have focused on prokaryotes as a mean for producing AgNPs, since they are characterized by some significant virtues, such as abundance and adaptation to extreme conditions, low cost for cultivation, fast growth and growth conditions like temperature, oxygenation and incubation time can be easily controlled [48-52]. In addition, bacteria constitute efficient bio-factories for the synthesis of AgNPs, as they produce many extra- and intracellular inorganic materials [53]. Generally, AgNPs can be synthesized by bacteria through bioreduction, in which the reductase enzymes reduce Ag+ ions, commonly obtained by the addition of $AgNO_3$, resulting to AgNPs. This reaction can be held either in the intracellular or in the extracellular environment, depending on the location of the reduction of the Ag cations [54].

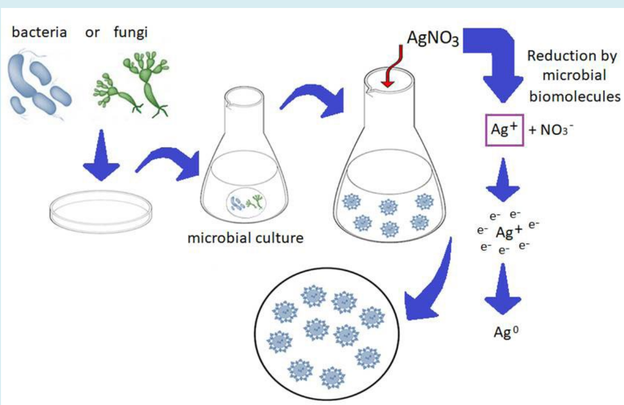

Figure 2: AgNPs synthesis through bacteria or fungi bioreduction.

The first reported attempt to synthesize AgNPs by bacteria was made using *Pseudomonas stutzeri*, which was isolated from a silver mine [55-58]. In general, it is known that AgNPs are toxic to bacteria; thus, these bio-factories can become resistant to these nanoparticles through the incorporation of the "sil" gene using plasmids [59,60]. However, Pseudomonas stutzeri is mainly resistant to silver, owing this property to the intracellular buildup of silver crystals of about 200 nm in diameter and of a certain shape and composition [61-65].

Otari, et al. [66] investigated the synthesis of AgNPs using *Rhodococcus spp.* Specifically, they reported that the AgNPs synthesis was carried out after 10 h of incubation at room temperature, while the produced nanoparticles were spherical, having a mean diameter of about 10-12 nm. The synthesized AgNPs were also tested regarding their antimicrobial activity and they demonstrated remarkable both bactericidal and bacteriostatic activity against a wide range of bacteria, such as *Escherichia coli* , *Enterococcus faecalis* , *Staphylococcus aureus* , *Klebsiella pneumoniae* , *Pseudomonas aeruginosa* , *Acinetobacter baumannii* and *Proteus vulgaris* [67,68].

Moreover Wang, et al. [69] performed synthesis of AgNPs in the culture supernatant of *Bacillus methylotrophicus*. The aforesaid nanoparticles were produced at 28°C within 48 h and their antimicrobial activity was tested against *E. coli*, *Candida albicans*, *Salmonella enterica* and *Vibrio parahaemolyticus* [70]. These AgNPs presented a better inhibition growth compared to antibiotics. The main drawback related to the AgNPs synthesis using bacteria is the limited variety of sizes and shapes of the produced nanoparticles [71].

The biogenic approach using fungi for the synthesis of nanoparticles has been well recognized, because of the Eukaryota have various documented and remarkable characteristics [72]. Generally, fungi can be used as excellent resources of a wide variety of extracellular enzymes for the synthesis of different MNPs, including AgNPs [73,74]. The fungal materials used in the synthesis of MNPs, include polysaccharides, proteins, mycelia and fruiting bodies. Fungi not exposed to a large concentration of metals possess an inherent ability to produce higher concentrations of proteins, in comparison to bacteria, which contributes to metal reduction [75,76].

Conducted synthesis of AgNPs in the culture supernatants of *Aspergillus terreus* [77]. The NPs were poly-dispersed with a mean diameter ranging from 1 to 10 nm, while the synthesis was facilitated by an extracellular enzyme [78]. Li, et al. [79]. have also reported the extracellular synthesis of AgNPs at room temperature using *Aspergillus terreus*. In addition, these NPs presented high antimicrobial activity over a wide variety of pathogenic microorganisms. Recently, Seetharaman used *Phomopsis liquidambaris* for the synthesis of AgNPs [80]. Specifically, the produced NPs were characterized by a spherical shape and a mean size equal to 18.7 nm. These NPs were also act effectively both as antimicrobial and mosquitocidal agents. Recently, showed the reduction of silver ions by phenolic compound homogentisic acid, presented in the aqueous extract obtained from *Lactarius piperatus* mushroom [81].

Macrofungi is another category of fungi that includes edible and medicinal mushrooms growing on organic substrates in nature [82]. Nowadays, many studies focus on synthesizing MNPs, using different genera of edible and medicinal mushrooms, due to the innumerable bio-active compounds with diverse biological activities that are present within them [83]. A quite wide variety of amino acids, proteins and polysaccharides that are present in the mushrooms have been utilized in both the intracellular and extracellular synthesis of MNPs, including AgNPs [84]. The aforementioned compounds found in the mushrooms result to the synthesis of NPs with high stability and good dispersion [85]. According to Owaid, the mushroom-assisted synthesis method has led to the mycosynthesis of AgNPs [86]. The term mycosynthesis is used to describe the biosynthesis of MNPs by extracts of edible and medicinal mushrooms or extracts of fungi in general as bioreducers and stabilizers, instead of other biomaterials [87,88].

Oyster mushroom *Pleurotus sp.* has been used to mycosynthesize AgNPs. *Pleurotus sp.* constitutes one of the well-known edible and medicinal mushrooms that has antimicrobial, antibacterial, antifungal, anti-candidal, anti-viral, anti-cancer and anti-diabetic properties [89].

The first attempt to mycosynthesize AgNPs was made by the scientific group of Vigneshwaran [90]. The AgNPs synthesis included the use of spent mushroom substrate proteins, which contained fungal mycelia and fruiting bodies residues of oyster mushroom. After that, many studies were conducted using extracts of different oyster mushroom species [91]. The AgNPs biosynthesized using oyster mushroom species were applied against bacteria in vitro and showed excellent antibacterial efficacy [55].

Synthesis of Agnps Using Plants

Plant-based synthesis of MNPs, also known as green synthesis, constitutes one of the emerging fields of nanotechnology in the recent era [92]. It is widely acknowledged way of NPs development, because of the plentiful and diversified cellular metabolites present in plant extracts. These bioactive metabolites are capable to act both as reducing and capping agents, thus eliminating the need to add any further chemical agent to synthesize the nanoparticles [61-65].

Thus, AgNPs can be prepared with a biogenic composition, using aqueous extracts of natural raw materials, such as oregano, nettle, propolis and various others, as reducing agents [93]. Plant extracts are rich in different chemical compounds, such as polyphenols, flavonoids, fatty acids, that make them a potential candidate to be used as a green reducing and stabilizing agent for the synthesis of MNPs. The methods for the AgNPs synthesis based both on plants and their extracts are non-pathogenic, simple and characterized by a higher bio-reduction potential, compared to microbial culture filtrates. Several researchers have reported the AgNPs synthesis with antimicrobial properties using plants [42-49].

AgNPs can be synthesized using either the whole plant or its extract; thus the availability of the reducing and stabilizing agents is greater in their extract than in the plant itself, so as a result the majority of the studies have focused on the use of plant extracts for the AgNPs synthesis [72]. In general, the plant-based method for the production of AgNPs involves the stage of mixing each of plant extract, which is selected, with an aqueous solution of $AgNO_3$. The synthesis process takes place at room temperature and lasts from several minutes to a few hours to be completed [94]. The principal mechanism responsible for the production of silver nanoparticles is based on the reduction process that is controlled by phytochemicals, such as terpenoids, flavones, ketones, aldehydes, quinones, amines and carboxylic acids. In particular, flavones, quinones and organic acids are in charge of the immediate reduction of the Ag^+ ions.

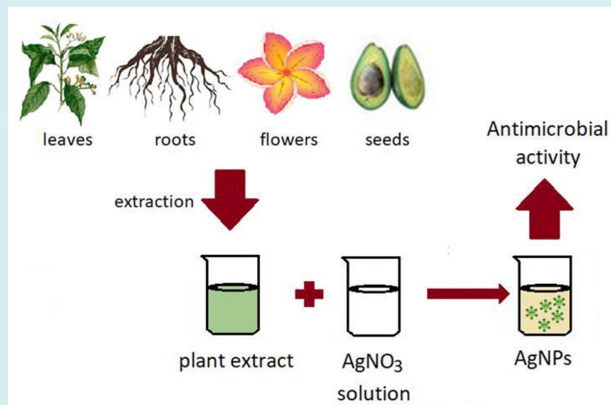

Figure 3: Green synthesis of AgNPs via plant extraction.

According to Veerasamy and his colleagues, the extract from the leaves of *Garcinia mangostana* led to the biosynthesis of AgNPs characterized by an adequate antimicrobial activity. Prabu and Johnson used the leaf extracts of *Solanum verbascifolium*, *Tylophora ovata*, *Cymbopogon citronella* and *Tragia involucrate*, in order to synthesize AgNPs via a green method. The formation of AgNPs was observed within 15 min, while the results of the several characterization techniques revealed that the produced NPs were of high stability and had no impurities.

Another study for the green synthesis of AgNPs using plant extracts was conducted by the group of Ahmed, who used *Azadirachta indica* aqueous leaf extracts. Only 15 min were required for the conversion of silver ions into AgNPs at room temperature, without the involvement of any hazardous chemical. The produced AgNPs showed antibacterial properties against both gram positive and gram negative microorganisms [95].

Investigated a green, efficient and sustainable route for the AgNPs synthesis using aqueous $AgNO_3$ solution and leaf extracts of three plants, *Musa balbisiana*, *Azadirachta indica* and *Ocimum tenuiflorum* , which are well known for their wide availability and medicinal applications. AgNPs obtained showed significantly higher antimicrobial properties against *E. coli* and *Bacillus sp.* in comparison to both $AgNO_3$ and raw plant extracts. Furthermore, de Aragão, et al. [96] biosynthesized AgNPs using as reducing and stabilizing agent a polysaccharide extracted from red algae *Gracilaria birdiae*, present in the coast of Pique [35-38]. The AgNPs were tested for antimicrobial activity using *E. coli* and *S. aureus* and all samples showed antimicrobial properties.

Good antibacterial performance against common pathogens presented also the AgNPs produced by Ahmad, et al. [42] using *Desmodium triflorum* extract. The AgNPs when combined with antibiotics presented synergic effect in suppressing the growth of bacteria [97]. Have successfully biosynthesized AgNPs using aqueous leaves extract of *Guiera senegalensis*. The prepared AgNPs showed excellent bactericidal effect against *E. coli* and *S. aureus* with about 99% colony reduction yield.

Synthesized AgNPs by *Crocus haussknechtii* extract, which showed strong inhibitory effect against both *S. aureus* and *P. aeruginosa*. Also, Hernández-Morales and colleagues reported for the first time the green synthesis of AgNPs using extracts of two varieties of *Salvia hispanica* seeds. The antibacterial study indicated that the prepared AgNPs have powerful antimicrobial activity against *E. coli* and *S. aureus*. Biosynthesized silver nanoparticles using babassu mesocarp starch. Starches have mainly been used in nanobiotechnology in the development of MNPs, due to their reduction and stabilization capacities [98]. The biosynthesized AgNPs showed an antimicrobial effect against *S. aureus* and *E. coli*, resulting in notable membrane damage. Recently, Sharma et al. reported *Coptidis rhizome* eco-friendly ex-situ biosynthesis of AgNPs with antimicrobial potential against both Gram-positive and Gram-negative microorganisms. *Coptidis rhizome* has been known for its medicinal properties, due to the existence of berberine, coptisine, jatrorrhizine and palmatine [99,100].

Finally, reported a green, facile and highly efficient process for the preparation of AgNPs using *Crataegus pentagyna* fruit extracts as reducing and capping agent. *Crataegus spp.* constitutes one of the oldest medicinal plants that are extensively employed throughout the world, mainly in Turkey and South Europe. *Crataegus spp.* is primarily employed for the treatment of cardiovascular deseases, while *Crataegus* extract contains phenolic and flavonoid compounds that exhibit antioxidant activity. The so-produced NPs presented efficient antimicrobial activity against *S. aureus*, *E. faecalis*, *P. aeruginosa*, *A. baumanii* and *E. coli*.

Synthesis of AgNPs Using Templates

During the last years, the use of viruses as templates for the synthesis of AgNPs have been studied, as they offer confined cages, high symmetry, functional protein capsids and unique architecture structure, except for the easy manipulation [101]. Furthermore, viral scaffolds can stimulate both the nucleation and assembly of inorganic materials. Some examples of viral components are tobacco mosaic virus and cowpea chlorotic mottle virus [102].

Biopolymers, such as DNA, proteins and polysaccharides, have the advantage of availability in a wide variety of sources and thus have great potential to be used as a template in synthesis of other materials. By means of inducing the biomineralization of inorganic NPs, biopolymers can synthesize nanomaterials that have certain structures, like gold NPs and Tin Oxide [99,100]. Peptides are utilized as a frame for the synthesis of AgNPs. Kashmery has described the synthesis of sugar-modified peptides to template the synthesis of AgNPs using Tollens' reagent. He correlated the quantity of sugar units and the size of AgNPs. Furthermore, he proposed a novel template-directed triazole ligand strategy for the synthesis of AgNPs.

DNA has been also used as template for the synthesis of AgNPs. Davis and co-researchers reported that a nucleoprotein filament from polymerizing RecA proteins on a single strand DNA probe was mixed with a long aldehyde-derived double strand DNA substrate [103]. Afterwards, the sample was exposed to $AgNO_3$ ions which binded to DNA, when RecA proteins were absent; so, the aldehyde groups reduced the Ag^+ ions to Ag^0 resulting to the formation of silver nanoparticles [99,100].

AgNPs in Chemical Doping

Since AgNPs are one of the most vital and fascinating nanomaterials among several MNPs that are involved in biomedical applications, it is reasonable that they are selected for the development of advanced composite materials. Studied the anticandidal properties of AgNPs embedded mesoporous silicon dioxide nanospheres, indicating very promising results [104]. Developed a novel green biomimetic approach for synthesis of zinc oxide/silver nanocomposites with antimicrobial activity against food-borne pathogens. Nguyen et al. also focused on the antibacterial activity of both titanium dioxide and ZnO decorated with AgNPs [105]. Various conventional methods of AgNPs synthesis include the use of toxic reagents or require large amounts of energy. For this reason, biogenic synthesis AgNPs in order to be used for chemical doping or binding to other nanomaterials should be selected. For instance, Su et al., developed AgNPs/N-Doped Carbon-Dots nanocomposites, derived from *Siraitia Grosvenorii*, focusing on their surface-enhanced characteristics. He proposed a green-synthesized system based on AgNPs encapsulated on nitrogen-doped graphene, for hydrogen peroxide detection. Ruíz-Baltazar exploited the extracts from *Mellissa officinalis* in order to apply a novel biosynthesis method of AgNPs embedded to hydroxyapatite for dental and orthopedic applications [106]. The increasing interest for eco-friendly techniques and processes indicates the impact of their advantages.

TiO_2 Doped with AgNPs Produced via Biogenic Methods

Recently, important scientific effort is observed in the field of chemical doping of TiO_2 in order to be utilized in environmental, antimicrobial, anticancer and other biomedical applications. He achieved the green synthesis of AgNPs and TiO_2NPs using *Euphorbia prostrata* extracts, showing shift from apoptosis to G_0/G_1 arrest, followed by necrotic cell death in *Leishmania donovani*. Synthesized Ag-TiO_2-G photocatalysts with an innovative and ecofriendly method by *pranus cerasus*, in order to investigate the effect in photodegradation of organic pollutants in water and showed that this composite material presented higher photocatalytic performance in the photodegradation [107]. Created by green process a system based on Ag-TiO_2 NPs bound on porous glass, presenting enhanced photocatalytic performance for oxidative desulfurization and removal of dyes under visible light [108]. Hariharan reported that green hydrothermal synthesis of Ag-TiO_2 NPs led to enhanced photocatalytic and anticancer properties [109]. They developed TiO_2-Ag nanocomposites, via green route, for dye degradation, possessing significant antimicrobial potential [110-116]. Recently, our group focused on the green synthesis of AgNPs, which are then bound to chemically doped TiO_2NPs with nitrogen, and embedded in a metallic matrix, employing pulsed electrodeposition. The preparation of AgNPs was achieved by the extractions of natural raw materials, such as origin and nettle, because these herbs contain bioactive phenolic compounds with antioxidant and antimicrobial properties, acting as reducing agents and as stabilizers, preventing from the formation of NPs aggregations [117]. The combination of $AgNP_S$ and N-TiO_2NPs leads to the synthesis of hybrid systems with improved photocatalytic and antimicrobial properties. These composites can be stabilized on metal surfaces, with strong mechanical properties in order to be used in multifunctional every-day-touched surfaces.

Conclusion and Future Perspectives

AgNPs constitute perhaps the mostly used kind of NPs in biomedical research, due to their antimicrobial properties. A variety of physical and chemical methods are employed for the AgNPs synthesis. However, many of them include the use of toxic reagents or require large amounts of energy. For this reason, biogenic methods are proposed instead of the conventional methods of AgNPs synthesis. Three main categories of biogenic synthesis of AgNPs would be determined, the synthesis based on the utilization of microorganisms, the green synthesis, based on the use of plants and their extracts and the approach which applies templates and scaffolds. These methods utilize biological resources, as reducing agents and seem to have significant advantages, since the extracts contribute positively to the formation and enhancement of the antimicrobial activity of AgNPs. Overall, in this review, an integrated approach of AgNPs bio-synthetic methods using microorganisms, such as bacteria and fungi, plants and plant extracts, as well as several templates, like DNA and viruses was discussed, in order to highlight the significance of the incorporation of green chemistry in basic and applied research.

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Conflicts of Interest

The authors declare no conflict of interest.

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