ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES BIOSYNTHESIZED USING THE SULFATED POLYSACCHARIDES FROM *ARTHROSPIRA PLATENSIS* BIOMASS

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Abstract

Arthrospira platensis (spirulina) is known for its high biological activity, including antioxidant, anti-inflammatory, and antiviral properties. In recent years, spirulina polysaccharides have been actively researched in fields such as tissue engineering, targeted drug delivery, and wound healing. These substances also possess a high reducing capacity, making them suitable matrices for the biosynthesis of metallic nanoparticles from metal ions. For instance, silver, known for its antimicrobial properties, can be biosynthesized as AgNPs on polysaccharide matrices derived from Spirulina biomass, potentially combining the effects of the nanoparticles and the matrix for developing wound coverings. In this study, sulfated polysaccharides were extracted from Spirulina biomass grown under controlled conditions to stimulate carbohydrate accumulation. AgNPs biosynthesis was carried out using polysaccharides solubilized in deionized water at 10 mg/ml and $AgNO_3$, under different process parameters. The nanoparticles obtained were tested for antibacterial properties against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, and antifungal properties against *Candida albicans*. The AgNPs demonstrated significant antimicrobial activity, with MIC and MBC values varying based on the species. These results indicate that AgNPs biosynthesized on spirulina sulfated polysaccharide matrices possess antimicrobial properties, making them promising for developing wound dressing materials.

Keywords: Silver nanoparticles, sulfated polysaccharides, antimicrobial activity

1. INTRODUCTION

It is now well-established that metallic nanoparticles, including silver nanoparticles, can be synthesized in an environmentally friendly manner through biosynthetic procedures using various microorganisms, cell cultures, and biological extracts. The resulting nanoparticles' shape, size, and biological properties vary depending on the matrix used for synthesis [1-3]. Cyanobacterial extracts are being studied due to their high reducing power and the abundance of components that enable the synthesis of zero-valent particles from target ions [4-6]. Most research focuses on using complex cyanobacterial extracts as bionanosynthesis matrices, while specific extracts, such as phycobiliprotein and protein extracts, are applied less frequently [5, 7]. Cyanobacterial polysaccharides can also be utilized in the biosynthesis of silver nanoparticles, resulting in polydisperse nanoparticles coated with a thin layer of polysaccharides [8].

Arthrospira platensis, commonly known as Spirulina, is one of the most intensively studied cyanobacteria due to its unique properties, including its diverse content of compounds with potent therapeutic effects [9]. In addition to its well-documented antioxidant, anti-inflammatory, and immunomodulatory activities, the antimicrobial effects of certain components of Spirulina biomass have also gained recognition in recent years

[10]. In this context, the sulfated polysaccharides of Spirulina, which exhibit inhibitory and biocidal effects against various viruses, bacteria, and fungi, have been particularly studied [11]. Moreover, whole biomass and different Spirulina biomass fractions are considered suitable matrices for the biosynthesis of silver nanoparticles [7, 8, 12].

The potential of biosynthesized silver nanoparticles using sulfated polysaccharide extracts from Spirulina biomass as a biosynthesis matrix for treating various wounds, including infected ones, is significant. A successful combination of Spirulina's sulfated polysaccharides and silver nanoparticles could provide a promising solution for joint use in treating wounds. Therefore, as a first step toward developing active therapeutic agents, we aimed to synthesize silver nanoparticles using sulfated polysaccharide extracts from Spirulina biomass and to test their activity against microorganisms with the potential to infect various skin lesions.

2. EXPERIMENTAL DESIGN

2.1. Silver nanoparticles biosynthesis

The sulfated polysaccharide extract from Spirulina (SPES) was used as a reducing agent for the biosynthesis of AgNPs. The source of SPES was the cyanobacterium *Arthrospira platensis* CNMN-CB-02 (Spirulina) strain from the National Collection of Non-Pathogenic Microorganisms at the Institute of Microbiology and Biotechnology, Technical University of Moldova. The cyanobacterium was cultivated in SP1 medium under 12:12 (L) photoperiod conditions, which ensured the production of up to 26% carbohydrates from the dry Spirulina biomass [13]. SPES was extracted from the native Spirulina biomass using deionized water at a 1:45 (mg/mL) ratio at a temperature of 90°C for 120 minutes. After centrifugation for 10 minutes at 4500 rpm, a 1% cetyltrimethylammonium bromide solution was added to the supernatant to precipitate the polysaccharides. Following centrifugation under the same conditions, the residue was washed with a saturated solution of sodium acetate in 95% alcohol, then with 96% alcohol, solubilized in deionized water at a concentration of 10mg/ml [14]. SPES contains 89.2% carbohydrates and 10.8% peptides.

Silver nitrate ($AgNO_3$) (SIGMA-ALDRICH GmbH, Germany) was used as the precursor substance in the biosynthesis of AgNPs. $AgNO_3$, at a silver concentration of 200 mg, was resuspended in 250 ml Erlenmeyer flasks with 100 ml of a sulfated polysaccharide extract from Spirulina (SPES) at a concentration of 10 mg/ml. The mixture was placed on a WU-4 universal laboratory shaker at 38-40°C and stirred at an oscillation frequency of 200 rpm for 3 hours. As a result, the active AgNPs-SPES mixture was obtained.

2.2. Methods of investigation

The FTIR method (Perkin-Elmer FTIR spectrometer (PerkinElmer Inc., USA) equipped with an air-cooled DTGS (Deuterated Triglycine Sulfate) detector) was used to identify the active chemical groups involved in the biosynthesis of silver nanoparticles.

Scanning electron microscopy was used to visualize the obtained nanoparticles. Scanning Electron Microscopy (SEM) was performed using the Quanta 3D FEG (FEI Company, USA). The operational features of the microscope used in the experiment were as follows: magnification 100,000-250,000x; voltage 30 kV.

The antimicrobial activity of AgNPs-SPES was evaluated against three bacterial strains: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and fungal strain *Candida albicans* (ATCC 10231). Determination of the MIC (minimum inhibitory concentration, µg/ml) and MBC (minimum bactericidal concentration, µg/ml) was done using the serial dilutions in liquid broth method. As a pozitive control was used SPES.

3. RESULTS AND DISCUSSION

The formation of nanoparticles was visually monitored by observing changes in the color of the reaction mixture, and their presence was confirmed through scanning electron microscopy (**Figure 1)**.

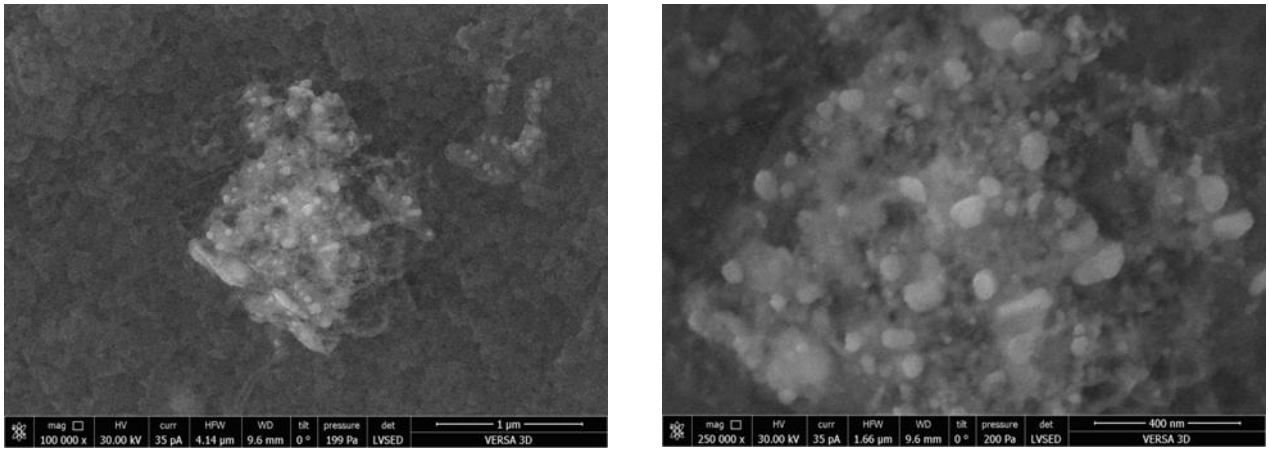

Figure 1 SEM of AgNPs-SPES

Due to their metallic nature, silver nanoparticles have a distinctive shine and are easily visualized against the amorphous polysaccharide matrix. They are spherical or nearly spherical, with sizes ranging from a few nanometers to several tens of nanometers, and are predominantly grouped in clusters.

Table 1 presents the FTIR absorption peaks for the sulfated polysaccharide extract from Spirulina (SPES) and the silver nanoparticles in the extract (AgNPs-SPES).

Table 1 FTIR Absorption peaks for sulfated polysaccharide extract from Spirulina (SPES) and silver nanoparticles synthesized in SPES (AgNPs-SPES)

Material	Regions			
	4000-2500, -OH; -NO	**2000-1500, C=O; N-H**	**1500-1000, C-O; C-N; S=O**	**<1000**
SPES	3283, 2971, 2930	1632, 1557	1450, 1406,1395, 1241, 1231,1065, 1037, 1027	919, 901, 893, 798, 696
AgNPs-SPES	3347, 2968, 2934	1634, 1557	1393, 1387, 1352, 1231, 1120, 1066, 1028	923, 828, 776, 715, 699

For the sulfated polysaccharide extract from Spirulina (SPES), the absorption peak at 3283 cm^{-1} suggests the presence of -OH or -NH groups, indicative of hydrogen bonds, reflecting a structure rich in hydrophilic functionalities. The values at 2930 cm^{-1} and 2971 cm^{-1} correspond to C-H stretching vibrations associated with hydrocarbon chains, suggesting the presence of methyl or methylene fragments. The peaks around 1632 cm^{-1} are attributed to C=O stretching vibrations, indicating carbonyl or amide groups. In the 1400-1600 cm^{-1} region, multiple peaks indicate the presence of carboxylate (COO^-) groups or N-H vibrations. In the 1500-1000 cm^{-1} region, the peaks at 1037 cm^{-1}, 1027 cm^{-1}, 1085 cm^{-1}, and 1025 cm^{-1} are typical of C-O stretching vibrations, characteristic of carbohydrates or polysaccharides, which are major components of the extract. A peak at 1120 cm^{-1} indicates S=O groups (from sulfated polysaccharides). The values below 1000 cm^{-1} correspond to molecular backbone vibration modes of organic compounds, indicating deformations characteristic of complex structures such as polysaccharides. For the active AgNPs-SPES mixture, the absorption peak at 3347 cm^{-1} indicates the presence of -OH and -NH groups that remain active. The peak at

1634 cm^{-1} suggests that carbonyl groups (C=O) play a role in the stabilization and potential functionalization of the formed nanoparticles. The 1400-1500 cm^{-1} region changes reflect possible interactions between carboxylate groups from Spirulina and silver ions (Ag^{+}). These interactions are essential for the ions' reduction process and the nanoparticles' stabilization. Peaks below 1000 cm^{-1} present signals associated with the vibrations of organic compounds and preliminary interactions between silver ions and the biomolecules from Spirulina. Thus, the FTIR spectrum for AgNPs-SPES indicates that in the mixture of silver nitrate and Spirulina extract, the biomolecules act as reducing and stabilizing agents for the silver ions. The spectrum reflects interactions involved in the reduction of Ag^{+} and the synthesis of AgNPs, highlighting the structural and chemical changes that occur.

The antimicrobial activity of SPES and AgNPs-SPES is presented in **Table 2**.

Table 2 Antimicrobial activity of sulfated polysaccharide extract from Spirulina (SPES) and silver nanoparticles synthesized in SPES (AgNPs-SPES)

Strain	Product	MIC (µg/mL)	MBC (µg/mL)
Staphylococcus aureus (ATCC 25923)	AgNPs-SPES	0.48	0.48
	SPES	5.00	n/a
Escherichia coli (ATCC 25922),	AgNPs-SPES	0.11	0.22
	SPES	5.00	5.00
Pseudomonas aeruginosa (ATCC 27853)	AgNPs-SPES	0.11	0.22
	SPES	5.00	5.00
Candida albicans (ATCC 10231).	AgNPs-SPES	1.95	3.90
	SPES	5.00	n/a

The sulfated polysaccharide extract from Spirulina exhibits inhibitory action against all studied strains and bactericidal action against *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). The minimum inhibitory concentrations determined for the extract are the same, constituting 5.0 µg/mL. The silver nanoparticles obtained based on the sulfated polysaccharide extract from Spirulina show high antimicrobial activity against all the studied strains of microorganisms. For instance, the minimum inhibitory concentration for *Staphylococcus aureus* ATCC 25923 was 0.48 µg/mL, the minimum bactericidal concentration for this microbial strain. The AgNPs-SPES exhibited a more pronounced antibacterial activity against the two Gram-negative bacterial strains studied. The minimum inhibitory concentration for *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 was 0.11 µg/mL, while the minimum bactericidal concentration was 0.22 µg/mL. Importantly, the biosynthesized nanoparticles were also active against the yeast-like fungus *Candida albicans* ATCC 10231, with a minimum inhibitory concentration of 1.95 µg/mL and a minimum bactericidal concentration of 3.90 µg/mL.

In previous studies, polysaccharide extracts from Spirulina have demonstrated moderate antimicrobial activity against Gram-negative and Gram-positive strains. For example, Spirulina polysaccharides have been reported to have MIC values ranging between 2 and 10 µg/mL for *E. coli* and other Gram-negative bacteria. More recent studies highlight that Spirulina and its components may have bacteriostatic activity, while bactericidal effects are observed at higher concentrations. The MIC values for *E. coli* and *P. aeruginosa* obtained in our study are consistent with those reported in the literature, while the bactericidal activity is significantly higher. We believe this is likely due to applying a specific procedure for growing Spirulina under moderate stress conditions, which resulted in biomass with enhanced properties [13].

Silver nanoparticles are well-documented for their remarkable antimicrobial properties, particularly against Gram-negative bacteria, due to their thinner cell walls and higher nanoparticle permeability. In many studies, the MIC for *S. aureus* ranges between 0.5 and 1 µg/mL, consistent with our results. *E. coli* and *P. aeruginosa*

tend to have even lower MICs against AgNPs, ranging from 0.1 to 0.2 µg/mL, aligning with our study's 0.11 µg/mL value. The antifungal activity of silver nanoparticles against *C. albicans* is also well-documented in the literature, with MIC values ranging from 1 to 10 µg/mL, depending on the synthesis method of the nanoparticles. The values obtained in this study, 1.95 µg/mL, fall well within these limits [15-17].

4. CONCLUSION

It has been demonstrated that the sulfated polysaccharide extract from Spirulina biomass is a suitable matrix for the biosynthesis of silver nanoparticles from the $AgNO_3$ precursor. It provides conditions for reducing and stabilizing silver ions formed in solution. The formed nanoparticles aggregate into small clusters on the available polysaccharide matrix.

The antimicrobial test results demonstrate excellent antimicrobial activity for both SPES and AgNPs-SPES, particularly against Gram-negative bacteria and fungi. Compared to the literature, the values obtained are consistent with previously reported results, and AgNPs-SPES shows a substantial improvement in antimicrobial activity compared to SPES. In this context, the results obtained can serve as a basis for the use of the biosynthesized nanoparticles from this study in impregnating wound dressing textiles of various etiologies, allowing the combination of antibacterial effects with the known therapeutic properties of the polysaccharide matrix.

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