Characterization of Silver Nanoparticles Produced by Wildtype and Mutants *Serratia marcescens* Bizio

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ABSTRACT

Silver nanoparticles (AgNPs) are silver species characterized by their nanoscale size (< 100 nm) and antimicrobial properties with a broad spectrum of activity. Bacterial mediation demonstrates a rapid method of synthesis of AgNPs from silver nitrate precursors. In this study, the ability of wild-type and five mutant strains of *Serratia marcescens* to mediate the biosynthesis of AgNPs, and their antimicrobial activity against medically important bacteria and fungi were investigated. Cell-free culture supernatants of *S. marcescens* were used to produce AgNPs. Visual inspection of crude cell-free supernatants showed a color change from light yellow to light brown. UV-Vis absorption spectroscopy showed a peak absorbance at 360 – 380 nm, indicative of $AgNO_3$ reduction. Similarly, the AgNP suspensions fluoresced at 380 – 400 nm under a fluorescence microscope. Scanning electron microscopy revealed the presence of spherical, monodispersed AgNPs with a size range of 11.71 – 23.20 nm (mean size = 17.05 nm; n = 20). The nanoparticles were identified as AgNPs based on energy dispersive X-ray analysis, where the mean silver content (% w/w) of the sample was 73.63%. The antimicrobial activity of biosynthesized AgNPs was tested against *E. coli* wildtype and mutant, *Salmonella* sp, *Staphylococcus aureus*, *Bacillus megaterium*, *B. subtilis*, *Candida glabrata*, *C. albicans*, *C. parasilopsis*, and *Fusarium* sp. via agar-well diffusion assay. Strong inhibitory activity was elicited by AgNPs against Gram-negative and Gram-positive bacteria, and yeast, and even stronger activity among drug-resistant strains.

KEYWORDS

Serratia marcescens, mutants, silver nanoparticle, antimicrobial resistance

INTRODUCTION

Antimicrobial resistance and the rapid emergence of multi-drug resistant microorganisms are a growing public health concern as it threatens the efficacy of traditional antimicrobial compounds against its target pathogens (Karthika et al. 2015; Bakkeren 2020; WHO 2020). Antimicrobial resistance occurs when microorganisms adapt in response to the presence of antimicrobial drugs, thus developing resistance. Antifungal resistance, although relatively uncommon at present, also poses a great threat to public health (CDC 2014). The misuse and overuse of antibiotics are often cited as the most common cause of antimicrobial resistance. It is therefore critical to find new

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alternatives to the existing array of antimicrobials to halt the rapid decline of antibiotics' efficacy and control the emerging threat of multidrug resistance.

Nanotechnology is a complex and highly interdisciplinary field that focuses on the properties, application, and manipulation of matter at an atomic, molecular, and supramolecular scale for the creation of nanoscale products (Tarafdar 2016). Advancement in nanotechnology allows a deeper understanding of nanoparticles and their relevance to several areas of study including organic chemistry, molecular biology, and molecular engineering, among others, and the implications of nanotechnology extend from its medical, ethical, and environmental applications to fields such as biology and chemistry. The need for novel antibiotics drove the investigation of the potential use of natural antibacterial materials such as zinc and silver when reduced to the nanoscale (Seil and Webster 2012). The physical structure of a nanoparticle allows greater interaction and penetration into the bacterial membrane besides providing unique bactericidal mechanisms, making nanoparticles a suitable alternative in antimicrobial control.

Silver, usually in the form of silver nitrate, silver sulfadiazine, and silver zeolite (Rai et al. 2009), is known to have antimicrobial effects. However, the nanoparticle counterpart of silver has been demonstrated to have more potent activity against a broad spectrum of Gram-negative and Gram-positive bacteria, fungi, and drug-resistant microorganisms (Maliszewska and Sadowski 2008; Nanda and Saravan 2009; Abkhoo and Panjehkeh 2016). Silver nanoparticles (AgNPs), a variant of silver with unique optical, electrical, and thermal properties, were observed to have more effective inhibitory activity against pathogenic microorganisms due to their larger surface area to volume ratio which leads to increased antimicrobial activity compared to its bulk counterpart (Rai et al. 2009; Durán et al. 2016).

Biogenic production of nanoparticles covers a variety of methodologies including the use of plant or cell extracts to reduce dissolved metals into nanoparticles. Microorganisms were also explored in the production of nanoparticles (Shahverdi et al. 2007; Lakshmipathy and Nanda 2013; Karthika et al. 2015). The ease of growth and genetic manipulation of the microbial genome makes bacteria and other microorganisms a promising candidate for the study of biogenic nanoparticle production and as an alternative for chemical processes. Furthermore, the use of extracellular factors secreted by microbes in the growth medium offers advantages in the commercial-scale production of these nanoparticles. *Serratia marcescens* was previously reported to biosynthesize silver nanoparticles from silver nitrate, and the production of prodigiosin, the pigment responsible for the red coloration of the bacteria was hypothesized to aid in the production of AgNPs by acting as a metabolic sink, thereby reducing the effects of toxic waste accumulation (Williams 1973; Saifuddin et al. 2008; Karthika et al. 2015). Further, Adan et al. (2018) reported the biosynthesis of AgNPs exhibiting antimicrobial activity by wildtype *Serratia* sp. NBL1001 using cell-free culture supernatant. In this study, wildtype and mutant strains of *Serratia marcescens*, shown to exhibit varying pigmentation from red to white, were investigated for their ability to mediate the reduction of silver nitrate into silver nanoparticles. Moreover, the antimicrobial properties of the extracellularly synthesized AgNPs were tested against medically important Gram-negative and Gram-positive bacteria and yeasts.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

The *Serratia marcescens* NBL1001 wildtype strain used in this study was previously described by Adan et al. (2018) and Mendoza et al. (2019). Mutant strains (designated as M01, M10, M17, M72, and M85) were obtained via transposon mutagenesis of *S. marcescens* NBL1001 following the protocol of Ault et al. (2011) using *E. coli* BW20767 strain as donor. Pure cultures were routinely cultivated in Luria-Bertani (LB) medium (Merck). Mutant strains were grown in the same medium supplemented with 100 ppm Kanamycin (Sigma-Aldrich) throughout the study unless otherwise specified. The strains were characterized based on colony morphology, Gram-stain reaction, cell shape, and arrangement.

Nitrate Reductase Assay

The ability of the wildtype and five mutant strains of *Serratia marcescens* to elaborate the enzyme nitrate reductase was determined via the nitrate reductase assay following the methods of Kumar et al. (2007). Briefly, the strains were grown in Nutrient Broth (NB, TM Media) supplemented with 0.1% KNO_3, and gas formation was monitored with the Durham tubes. A confirmatory test was done with the addition of nitrate reagents A and B to elicit a color change from yellow to red or red-brown color, indicative of the reduction of NO_3 to NO_2 (Durán et al. 2005). An uninoculated broth subjected to the same conditions served as the negative control.

Biosynthesis of Silver Nanoparticles

Silver nanoparticles were synthesized from silver nitrate precursor, following the methods of Malarkodi et al. (2013) with modifications. Briefly, the *S. marcescens* cultures were grown in 30 ml Nutrient Broth at room temperature with shaking (150 rpm) for 24 h. For the mutant strains, 100 ppm Kanamycin was supplemented. About 15 mL of each of the cultures was centrifuged at 3,200 ×g for 15 minutes at room temperature. The supernatant was collected and filtered through a 0.22 µm membrane filter. Aqueous silver nitrate ($AgNO_3$, Sigma-Aldrich) was added to a final concentration of 2 mM to the cell-free supernatants. The tubes were wrapped with aluminum foil to prevent exposure to light. Positive and negative controls were prepared by subjecting uninoculated nutrient broth in the same incubation conditions with and without light exposure, respectively. Visual inspection by color change relative to the controls was observed.

About 15 mL of the supernatants in tubes with positive reactions were centrifuged at 9,000 ×g for 30 min at room temperature. The pellet was collected and resuspended in 5 mL sterile HPLC-grade water and washed by centrifugation. The resulting pellet was resuspended in 1 mL sterile HPLC-grade water in aluminum covered vials and were stored at 4ºC.

Characterization of Silver Nanoparticles

The UV-visible spectra of the $AgNO_3$-supernatant mixtures were measured using GENESYS 10S UV-Vis spectrophotometer (v4.003, Thermo Scientific, USA). Silver nanoparticles harvested from the wildtype *S. marcescens* were sent to the Advanced Device and Materials Testing Laboratory, Department of Science and Technology (ADMATEL, DOST, Taguig) for Field Emission Scanning Electron Microscopy with Energy Dispersive X-Ray Spectroscopy (FESEM-EDX) analysis using the xT software ver. 5.2.2 and Aztec software, respectively. Briefly, 100 µL of AgNP suspension was drop-casted on an aluminum sheet and air-dried overnight under dark conditions. The sample was viewed under 10,000×, 25,000×, 100,000×, 160,000×, and 200,000× magnification. The diameter of 20 individual nanoparticles from two separate fields of vision was measured. Additionally, the elements present in the sample and their relative abundance were determined.

Further, the biosynthesized AgNPs were observed under a fluorescence microscope (Carl Zeiss AXIOPlan2 SN: 235430) and photomicrographs were captured using Olympus DP70 microscope camera, analyzed using the AxioVision software. Briefly, 10 µL concentrated samples were observed under the bright field and fluorescence lenses #1 (Excitation BP 365/12 nm, Emission LP 397 nm), #5 (Excitation BP 395 – 440 nm, Emission LP 470 nm), #9 (Excitation BP 450 – 490 nm, Emission LP 515 nm) and #15 (Excitation BP 546/12 nm, Emission LP 590 nm), under 20× objective magnification.

Antimicrobial Activity of Silver Nanoparticles

The antimicrobial activity of the biosynthesized AgNPs was determined against three Gram-positive species (*Staphylococcus aureus, Bacillus subtilis, B. megaterium*), three Gram-negative species (*Serratia marcescens*, *Escherichia coli, Salmonella enterica*), three species of yeast (*Candida albicans, C. glabrata, C. parasilopsis*), and one mold species (*Fusarium* sp.) via the agar well diffusion assay (Staub et al. 2005). Briefly, test organisms were grown in 10 mL Tryptic Soy Broth (TSB, Pronadisa) or LB overnight at 37 ºC. Cultures were diluted to 0.50 level McFarland standard and 100 µL was swabbed on Mueller-Hinton agar (Scharlau). For *Fusarium* sp., 50 µL of spore stock suspension was swabbed on Potato Dextrose Agar (PDA, Scharlau) plates to create a lawn. Nine 3-mm diameter wells were cut from the inoculated agar at equal distance and 20 µL of cell-free AgNP solutions were dispensed, with 200 ppm Streptomycin (Sigma-Aldrich) for bacteria, 200 ppm Nystatin (Sigma-Aldrich) for yeasts and 200 ppm Fluconazole (Sigma-Aldrich) for molds, and sterile distilled water as positive and negative controls, respectively. The plates were incubated for 24 h at ambient room temperature under restricted light conditions (7 to 14 days for *Fusarium* sp.). The presence of a clear zone around the inoculated well indicates antimicrobial activity and the diameter of the inhibition zones were measured.

Inhibitory Activity of AgNPs against Drug-Resistant Bacteria

The antibacterial activity of biosynthesized AgNPs was determined against drug-resistant strains of *Serratia marcescens* (n = 5) and *Escherichia coli* (n = 1) via the agar-well diffusion assay (Staub et al. 2005). The strains of *S. marcescens* were grown in 10 mL TSB with 100 ppm Kanamycin (LB + 100 ppm Rifampicin + 100 ppm Kanamycin for *E. coli*) overnight at 37ºC. Then, 100 µL of the culture (0.50 McFarland level) was swabbed on Mueller-Hinton agar. Nine 3 mm diameter wells were cut from the inoculated agar at equal distance and 20 µL of cell-free AgNP solutions were dispensed, with 200 ppm Streptomycin and sterile distilled water as positive and negative controls, respectively. The plates were incubated for 24 h at ambient room temperature under restricted light conditions. The presence of a clear zone around the well served as a positive result and the diameter of the inhibition zones were measured.

Statistical Analysis

All assays were performed in triplicates. The computed antimicrobial indices (AI) values of the treatments against each test organism were subjected to One-way ANOVA test to determine whether the AI means are significantly varied at p-value < 0.05. Pairwise comparison using Tukey's test was done to compare the mean AI of AgNPs with positive control. All statistical analyses were computed using Minitab 18 (Minitab 18).

RESULTS

Partial Characterization of *Serratia marcescens*

The wildtype *Serratia marcescens* in LB agar without antibiotic produced the characteristic red colonies, whereas mutant strains grown in LB agar supplemented with 100 ppm Kanamycin showed varying changes in pigmentation observed as white to light red colonies. Specifically, *S. marcescens* M01 produced light pink colonies, *S. marcescens* M07 produced light red colonies, *S. marcescens* M10 and *S. marcescens* M72 produced white colonies, and *S. marcescens* M85 produced the typical red colonies (Figure 1). The wild type strain did not grow in Kanamycin-supplemented medium. All strains showed Gram-negative, short-rod cells under the microscope.

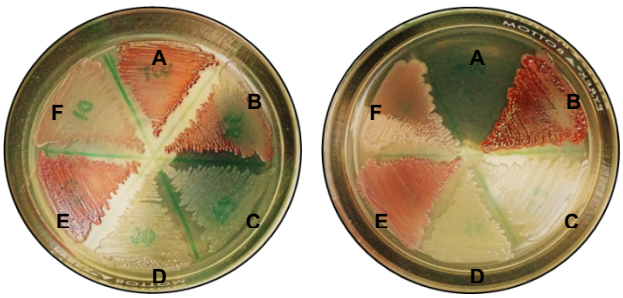

Figure 1: Colony pigmentation of *Serratia marcescens* on LB Agar. (I) without antibiotics; (II) with antibiotics – (A) *S. marcescens* wildtype, (B), *S. marcescens* mutant M01, (C) *S. marcescens* mutant M07, (D) *S. marcescens* mutant M10, (E), *S. marcescens* mutant M72, (F) *S. marcescens* mutant M85.

Nitrate Reduction Assay

Turbidity was observed after 24 h incubation at room temperature. All strains produced nitrate gas after 72 h incubation, observed as gas trapped in the upside-down Durham tubes. A confirmatory test was performed through the addition of nitrate reagents A and B. Except for *S. marcescens* M72, which produced a bright red-orange color change, all strains produced the characteristic brown-orange coloration. The negative control showed no cell growth, gas production, and color change following the confirmatory test.

Silver Nanoparticle Production

To determine the ability of *S. marcescens* strains to mediate the reduction of silver nitrate to silver nanoparticles, cell-free supernatants of overnight cultures were inoculated with 2 mM $AgNO_3$ and incubated under dark conditions. The light-restricted reduction of $AgNO_3$ was observed as a color change to light brown, whereas the positive control exposed to light showed an intense brown color after incubation (Figure 2). The absorption spectra of the reduced $AgNO_3$ showed a peak shift from 300-310 nm to 360-380 nm (Figure 3), indicating a change in the properties of the silver ions (Ag^{1+}) to elemental silver (Ag^0), whereas the photoreduced $AgNO_3$ showed a single broad peak at 420 nm, characteristic of large, spherical silver nanoparticles (Chhatre et al. 2012).

Characterization of Silver Nanoparticles

Field Emission Scanning Electron Microscopy (FESEM) analysis of wildtype strain mediated AgNPs revealed spherical, monodispersed silver nanoparticles with a size range of 11.71 – 23.20 nm (average size = 17.05 nm; n = 20), viewed under 106,667× magnification (Figure 4). Additionally, the Energy Dispersive X-Ray analysis revealed a total of seven elements present in the sample (Aluminum, Silver, Carbon, Oxygen, Sodium, Sulfur, and Chloride). The relative abundance (% w/w) of the six elements (Figure 5), revealed that Silver comprised most of the sample (73.63%), followed by Oxygen (9.19%), and Carbon (8.56%). Aluminum was excluded from the analysis

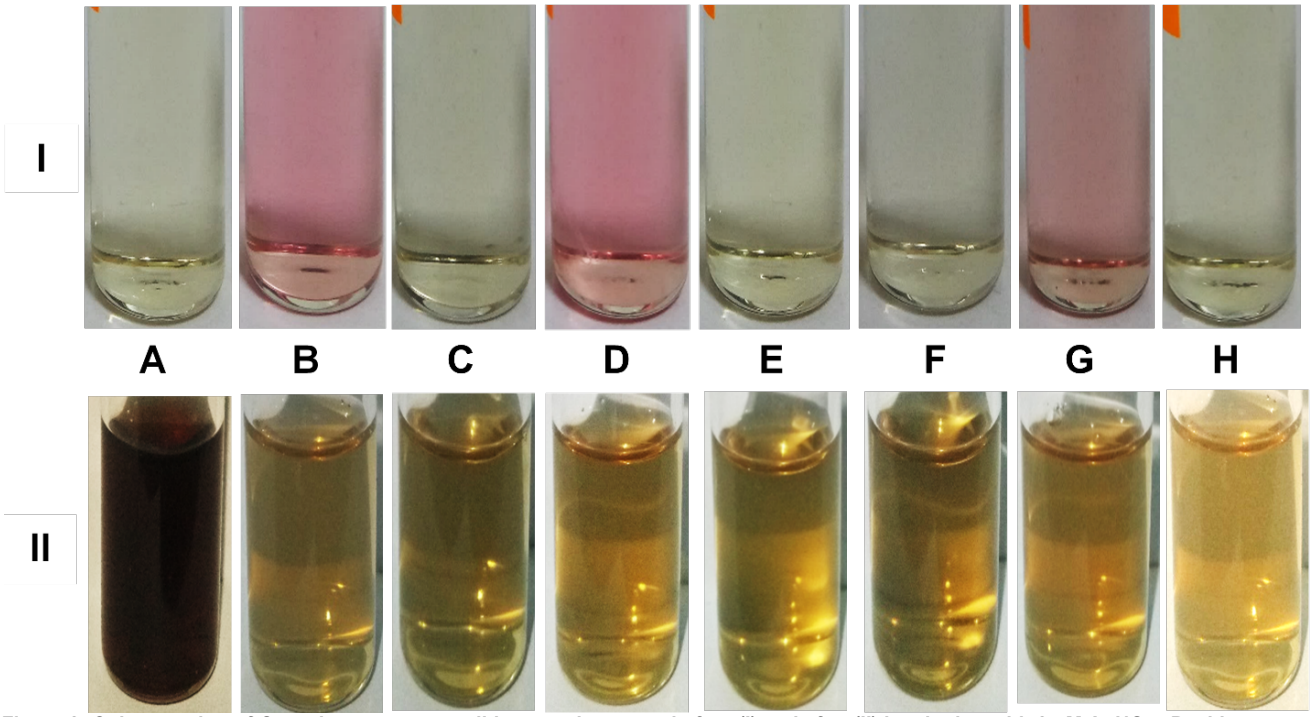

Figure 2: Color reaction of *Serratia marcescens* wildtype and mutants before (I) and after (II) incubation with 2mM $AgNO_3$ - Positive control (A), *S. marcescens* wildtype (B), *S. marcescens* mutant M01 (C), *S. marcescens* mutant M07 (D), *S. marcescens* mutant M10 (E), *S. marcescens* mutant M72 (F), *S. marcescens* mutant M85 (G), Negative control (H).

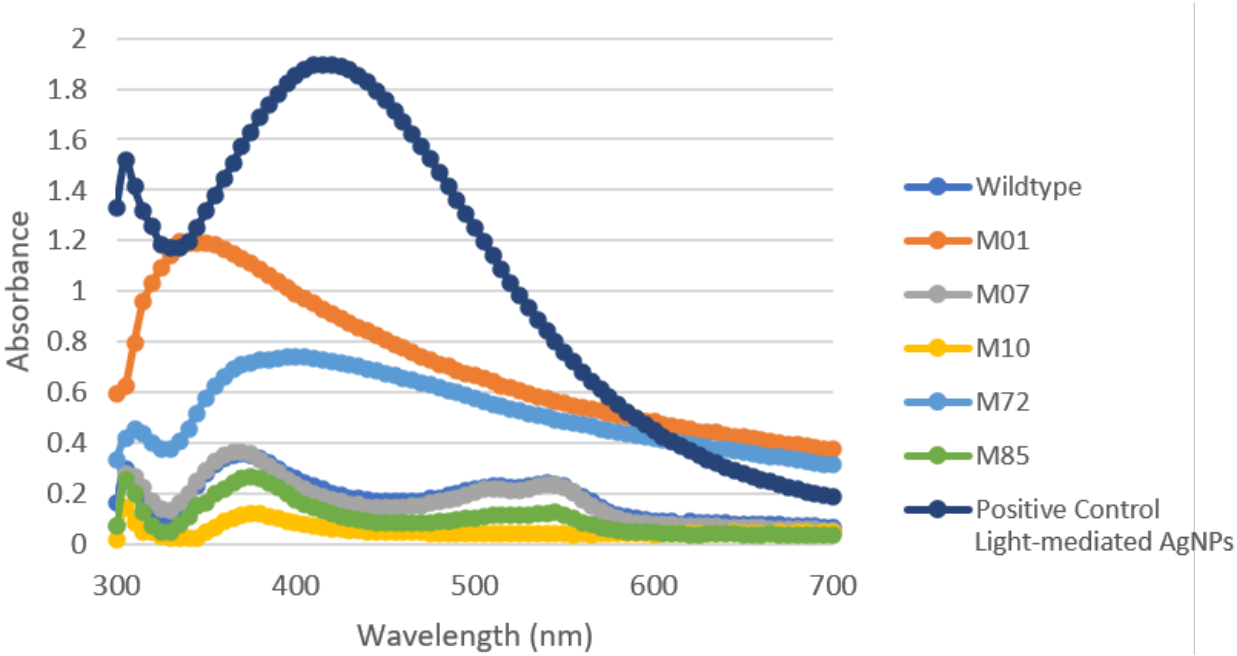

Figure 3: Absorption spectra of silver nanoparticles extracellularly produced by wildtype and mutant *Serratia marcescens*.

since the strong signal masks the other elements when Aluminum was only attributed to the material to which the AgNPs were casted upon. The presence of other elements may indicate impurities from the culture media which may aid the stability of the nanoparticles (Sperling and Parak 2010).

The AgNPs showed fluorescence under a fluorescence microscope (Filter #1, Excitation BP 365/12 nm, Emission LP 397 nm; Filter #5, Excitation BP 395 – 440 nm, Emission LP 470 nm). The photo-reduced and biosynthesized AgNPs fluoresced bright cyan to yellow-green under 380 – 400 nm wavelength and fluoresced bright green-yellow under 480 – 500 nm wavelength. The negative control, consisting of $AgNO_3$ solution, showed no fluorescence activity.

Antimicrobial activity of silver nanoparticles

Biosynthesized AgNPs showed inhibitory activity against both Gram-negative and Gram-positive bacteria, and yeasts (Table 1). Among the Gram-negative bacteria, *Salmonella enterica* subsp. *enterica* showed the largest inhibition zone (ZOI = 10.78 mm), followed by wild-type *Serratia marcescens* (ZOI = 9.45 mm). Wild-type *Escherichia coli* showed the smallest inhibition zone (ZOI = 8.00 mm). Inhibition zones observed against Gram-positive bacteria are larger than that of Gram-negative bacteria. *Staphylococcus aureus* showed the largest inhibition (ZOI = 16.72 mm) followed by *Bacillus subtilis* (ZOI = 10.39 mm), and *B. megaterium* with the smallest inhibition zone (ZOI = 9.61 mm). The inhibitory properties of biosynthesized AgNPs were also tested against three species of *Candida*, where *C. glabrata* showed the largest inhibition zone (ZOI = 10.72 mm), followed

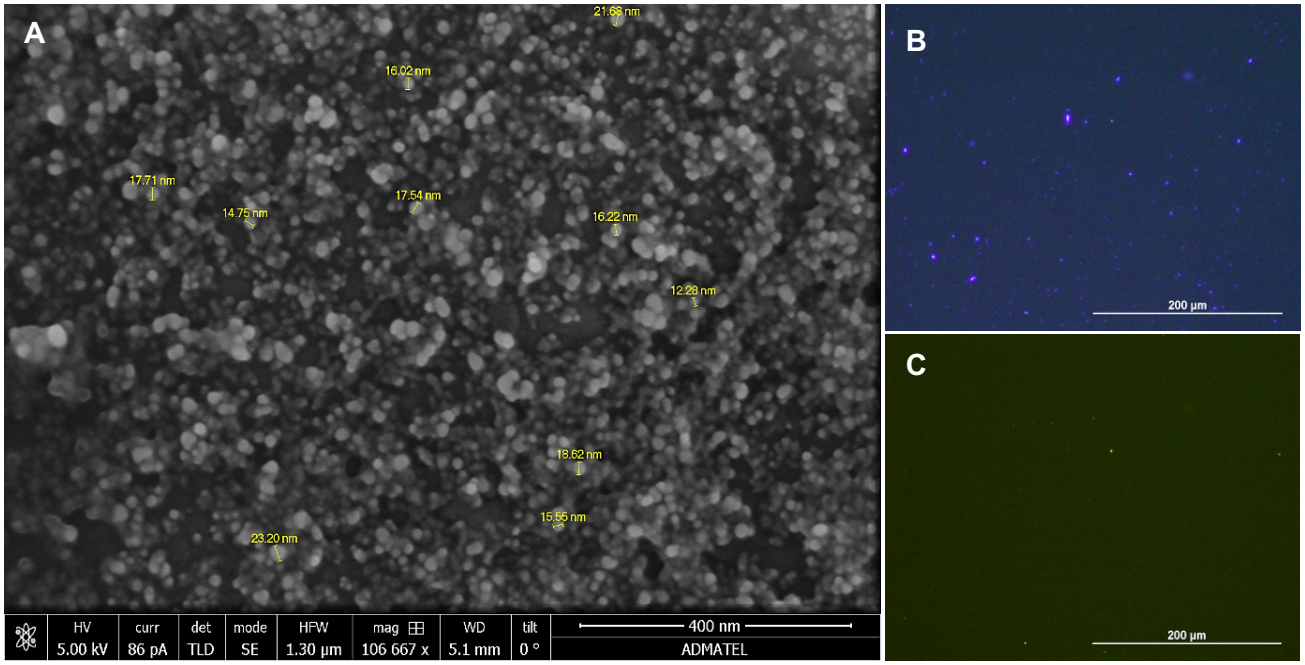

Figure 4: Photomicrograph of silver nanoparticles extracellularly produced by wildtype *Serratia marcescens*: (A) SEM image, (B) Filter #1, Excitation BP 365/12 nm, Emission LP 397 nm, (C) Filter #5, Excitation BP 395 – 440 nm, Emission LP 470 nm.

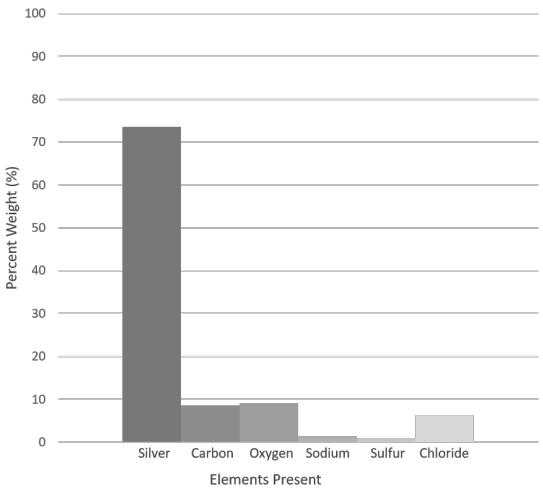

Figure 5: Average percent weight (w/w) of elements detected via EDX point analysis of AgNPs produced by wildtype *Serratia marcescens*.

by *C. parasilopsis* (ZOI = 9.00 mm), and *C. albicans* (ZOI = 8.28 mm). For *Fusarium* sp., the biosynthesized AgNPs failed to elicit inhibition. However, increasing the volume of AgNPs (wildtype and M72 mediated AgNPs) to 40 µL showed slight inhibition (data not shown). The ZOI values are the average of the six *S. marcescens* mediated AgNPs treatments.

Antimicrobial activity of silver nanoparticles against drug-resistant bacteria

Silver nanoparticles produced larger inhibition zones against drug-resistant bacteria compared to their wild-type counterpart (Table 1). Among the *Serratia marcescens* mutants, *S. marcescens* M10 was the most sensitive (ZOI = 18.05 mm), followed by *S. marcescens* M72 (ZOI = 15.78 mm), *S. marcescens* M17 (ZOI = 14.89 mm), *S. marcescens* M01 (ZOI = 14.00), and the least inhibited was *S. marcescens* M85 (ZOI = 12.50 mm). Rifampicin and Kanamycin resistant *Escherichia coli* showed a significantly larger inhibition (ZOI = 10.67 mm) compared to the wild type strain. The ZOI values are the average of the six *S. marcescens* mediated AgNPs treatments. The positive control, 200 ppm Streptomycin, did not inhibit the mutant strains of *S. marcescens.*

DISCUSSION

Wild-type and five mutant strains of *Serratia marcescens* were investigated for their ability to mediate the production of silver nanoparticles (AgNPs) extracellularly. *S. marcescens* is known for its red pigment, prodigiosin, which is a secondary metabolite that could act as a metabolic sink and aid in the production of AgNPs (Williams 1973; Saifuddin et al 2008). Mutant strains of *S. marcescens* are resistant to Kanamycin and exhibit varying phenotypes, particularly in the color of the colonies formed, suggesting that the expression of the prodigiosin (*pig*) cluster is affected and production of prodigiosin is altered. Nevertheless, all strains can produce nitrate reductase, an enzyme hypothesized to reduce silver nitrate to elemental silver and essentially AgNPs (Durán et al. 2005; Kumar et al. 2007; Krithika et al. 2014). It has been previously reported that prodigiosin is necessary for the production of gold nanoparticles but the results of the present work suggest otherwise (Dozie-Nwanchuku et al. 2017). It is possible that nanogold and nanosilver biosyntheses operate with different pathways.

Extracellular biosynthesis of AgNPs was observed using the cell-free culture supernatants of the six strains of *S. marcescens*. Similarly, extracellular biosynthesis of AgNPs was also reported using different bacterial species including *Klebsiella penumoniae, Escherichia coli, Enterobacter cloacae*, and *Staphylococcus aureus* (Shahverdi et al. 2007; Nanda and Saravan 2009). The reduction of silver ions (Ag^{1+}) to elemental silver (Ag^{0}) was accompanied by a color change of the culture supernatant to light brown. The optical properties of AgNPs are dependent on its stability and physical properties, observable as changes in the optical density of the nanoparticles. Smaller silver nanospheres have peaks near 400 nm wavelength whereas larger AgNPs tend to have a broader peak and a shift towards longer wavelengths (Paramelle et al. 2014). Moreover, aggregation of nanoparticles results in broad peaks or a secondary peak at longer wavelengths. Optical properties are also affected by the

Table 1: Test organisms, growth media, and sensitivity to biosynthesized AgNPs.

Indicator Organism	Growth Media	Antibiotic Supplement [a]	Inhibition Zone [b]
Gram-positive			
Staphylococcus aureus	TSB	-	+++
Bacillus subtilis	TSB	-	++
Bacillus megaterium	TSB	-	+
Gram-negative			
Salmonella enterica	LB	-	++
Serratia marcescens	LB	-	+
Escherichia coli	LB	-	+
Gram-negative mutants			
E. coli $Rif^{R}Kan^{R}$	LB	Rifampicin, Kanamycin	++
S. marcescens M01	LB	Kanamycin	++
S. marcescens M10	LB	Kanamycin	+++
S. marcescens M17	LB	Kanamycin	++
S. marcescens M72	LB	Kanamycin	+++
S. marcescens M85	LB	Kanamycin	++
Fungi			
Candida albicans	TSB	-	+
Candida glabrata	TSB	-	++
Candida parasilopsis	TSB	-	+
Fusarium sp.	PDA	-	-

[a] Antibiotics are supplemented up to a final concentration of 100 ppm (60 ppm for *S. marcescens* M10).
[b] Activity is expressed based on the diameter of inhibition zone around the well (average of the six AgNPs treatments from wildtype and mutant strains): -, no inhibition; +, up to 10.0 mm; ++, up to 15.00 mm; +++, up to 20 mm.

local refractive index near the surface of AgNPs (Lee and Jun 2019). Generally, the extinction peaks of AgNPs will shift to longer wavelengths if the local refractive index is increased and will shift to shorter wavelengths if the local refractive index is decreased.

Additionally, the biosynthesized AgNPs were observed to fluoresce under filter #1 (Excitation BP 365/12 nm, Emission LP 397 nm), #5 (Excitation BP 395 – 440 nm, Emission LP 470 nm). The fluorescence observed under filter #1 indicates that the excitation wavelength of AgNPs is within the UV range (380-400 nm), and fluorescence under filter #5 suggests that the emission spectra is within the range of 480-500 nm or the green to yellow spectra (Parang et al. 2012). Scanning electron microscopy images of concentrated AgNPs produced using the cell-free culture supernatant of wild-type *S. marcescens* showed spherical, monodispersed nanoparticles. The AgNPs ranges from 11.71 nm to 23.20 nm, with a mean diameter size of 17.05 nm (n = 20).

Results showed that biosynthesized AgNPs have antimicrobial activity against both Gram-positive and Gram-negative bacteria as well as yeasts. Smaller nanoparticles have better inhibitory activity due to the larger surface area to volume ratio (Castañon et al. 2008). The biosynthesis of AgNPs using the culture supernatant of *K. pneumoniae*, and other *Enterobacteriaceae* showed the formation of silver nanoparticles (28.2-122 nm diameter) through the reduction of silver ions (Shahverdi et al. 2007). In another study, the production of AgNPs (10-100 nm diameter) were mediated by *Penicillium* sp. and showed antibacterial activity against bacteria including *S. aureus, B. cereus, E. coli,* and *P. aeruginosaI* (Maliszewska and Sadowski 2008). *S. nematodophila* was observed to synthesize variably shaped AgNPs (10-31 nm diameter) with antimicrobial activity against *B. subtilis, P. aeruginosa,* and *Klebsiella planticola* (Malarkodi et al. 2013).

The small size of AgNPs allows it to cross membrane barriers and reach the nuclear region of target organisms and interact with the sulfur- and phosphorus-containing cellular constituents such as proteins and DNA. Interaction with thiol groups in enzymes and phosphorus-containing bases may inhibit DNA replication and cell division (Buszewski et al. 2018) Moreover, AgNPs disrupt the integrity of the cell membrane and plasma membrane which then leads to cell lysis (Lee et al. 2010). Table 2 shows the antimicrobial indices of biosynthesized AgNPs in comparison with antibiotics and light-mediated AgNPs. The Tukey's pairwise grouping show how significantly different the AI produced by the biosynthesized AgNPs with the positive controls. Generally, the AI of biosynthesized AgNPs is not significantly different among the group and against light-mediated AgNPs. However, AgNPs showed significantly

Table 2: Antimicrobial indices of silver nanoparticles produced by *Serratia marcescens* wildtype and mutants against test organisms with Pairwise comparison of means using Tukey's Test [a].

Test Organism	Antibiotic [b]	Silver Nanoparticle Treatments						
		Light-Mediated	Wild-type	M01	M07	M10	M72	M85
Staphylococcus aureus	1.56	5.00	5.89	4.78	4.67	4.11	3.78	4.22
	C	A/B	A	A/B	A/B	B	B	A/B
Bacillus subtilis	5.89	2.22	2.11	2.11	2.33	2.11	2.22	2.33
	A	B	B	B	B	B	B	B
B. megaterium	5.22	2.56	2.33	2.44	2.55	2.22	2.44	2.78
	A	B	B	B	B	B	B	B
Salmonella enterica subsp. *Enterica*	1.44	2.56	2.56	2.78	2.78	2.44	2.33	2.67
	B	A	A	A	A	A	A	A
Serratia marcescens	2.11	2.78	2.33	2.22	2.11	2.00	1.89	2.33
	A	A	A	A	A	A	A	A
Escherichia coli	2.33	2.33	1.78	2.00	1.78	1.44	1.45	1.56
	A	A	A	A	A	A	A	A
E. coli $Rif^{R}Kan^{R}$	2.78	2.11	1.89	1.78	2.56	3.00	3.33	2.78
	A/B	B/C	C	C	A/B/C	A	A	A/B
S. marcescens M01	0.00	3.89	3.78	3.67	3.78	3.56	3.22	4.00
	B	A	A	A	A	A	A	A
S. marcescens M10	0.00	4.89	5.11	4.78	5.11	4.67	5.11	5.33
	B	A	A	A	A	A	A	A
S. marcescens M17	0.00	4.33	4.22	4.00	4.00	3.67	3.33	4.56
	B	A	A	A	A	A	A	A
S. marcescens M72	0.00	4.22	4.22	4.56	4.33	4.22	4.00	4.22
	B	A	A	A	A	A	A	A
S. marcescens M85	0.00	4.00	3.89	3.78	2.89	2.55	2.89	3.00
	B	A	A	A	A	A/B	A	A
Candida albicans	4.11	2.11	2.00	1.78	1.78	1.67	1.89	1.44
	A	B	B	B	B	B	B	B
C. glabrata	2.89	3.11	2.89	2.56	2.56	2.33	2.33	2.78
	A	A	A	A	A	A	A	A
C. parasilopsis	4.00	1.89	2.22	2.11	2.11	1.78	1.89	1.89
	A	B	B	B	B	B	B	B
Fusarium sp. [c]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	-	-	-	-	-	-	-	-

[a] Values are grouped as A, B, or C, depending on the closeness of their values, such that individuals belonging to group A have significantly higher AI values than those of group B and C, and individuals belonging to group B have significantly higher AI values than those of group C at p-value < 0.05.
[b] The antibiotics used as the controls for bacterial strains were 200 ppm Streptomycin, 200 ppm Nystatin for yeasts, and 200 ppm for molds.
[c] Tukey's test was not applied for *Fusarium* sp. since all responses have the same value.

different AI as compared with antibiotic control. When tested against Gram-positive test organisms, AgNPs have lower AI than Streptomycin, except for *Staphylococcus aureus*. The AI values were not significantly different against gram-negative bacteria. However, AgNPs showed significantly higher AI than antibiotics against the drug-resistant groups of test organisms. In particular, mutant strains of *S. marcescens* that produce white colonies are more sensitive to AgNPs than the pigmented strains which suggest that the production of the secondary metabolite, prodigiosin, may aid in reducing the effects of toxic wastes and delay the death phase (Williams 1973) and thus providing some resistance against the activity of AgNPs. In the case of the fungi, AgNPs have AI significantly lower than Nystatin against *C. albicans* and *C. parasilopsis*. In contrast, *Fusarium* sp. was not inhibited by AgNPs. However, increasing the volume of biosynthesized AgNPs inoculated onto the wells resulted in slight inhibition in the growth of the test organism. This suggests that a higher concentration of AgNPs is needed to significantly inhibit *Fusarium* sp.

Table 3 shows multiple comparisons of mean AI exhibited by biosynthesized AgNPs against fifteen test organisms and their respective groupings based on Tukey's test. It can be deduced that among the four groups of microorganisms tested, the drug-resistant bacteria are more sensitive to AgNPs activity, along with *Staphylococcus aureus*. On the other hand, AgNPs activity against Gram-positive and Gram-negative bacteria, as well as yeasts, are not significantly different, supporting the idea that silver nanoparticles have broad-spectrum anti-microbial properties. Although *Fusarium* sp. showed some resistance against AgNPs treatment, increasing the concentration of AgNPs might result in significant inhibition.

This study demonstrated the ability of wild-type and drug-resistant *S. marcescens* to mediate the production of silver nanoparticles with a broad spectrum of activity against certain microorganisms. Antimicrobial analyses demonstrated the potent activity of biosynthesized AgNPs – inhibiting bacteria, yeasts, and drug-resistant bacteria. Therefore, microbial driven synthesis of nanoparticles proves to be a strong alternative for

Table 3: Multiple comparison of average antimicrobial indices (AI) exhibited by biosynthesized AgNPs against fifteen test organisms.

	Test Organism	Mean AI of AgNPs	Tukey's Grouping [a]								
Gram-positive	*Staphylococcus aureus*	4.57	A	B							
	Bacillus subtilis	2.20							F	G	
	B. megaterium	2.46							F		
Gram-negative	*Salmonella enterica* subsp. *Enterica*	2.59						E	F		
	Serratia marcescens	2.15							F	G	
	Escherichia coli	1.67								G	
Drug-resistant strains	*E. coli* $Rif^{R}Kan^{R}$	2.56						E	F		
	S. marcescens M01	3.67			C	D					
	S. marcescens M10	5.02	A								
	S. marcescens M17	3.96		B	C						
	S. marcescens M72	4.26		B	C						
	S. marcescens M85	3.17				D	E				
Fungi	*Candida albicans*	1.76								G	
	C. glabrata	2.57						E	F		
	C. parasilopsis	2.00							F	G	
	Fusarium sp.	0.00									H

[a] Mean AI values grouped under the same letter are not significantly different, with A having significantly higher AI than the rest, and H having significantly lower AI than the rest (p-value < 0.05).

chemical and physical methods of synthesis due to the ease of production and relatively lower cost.

CONCLUSION

Serratia marcescens wildtype and mutants were shown to mediate the production of silver nanoparticles extracellularly. Characterization analyses revealed that the AgNPs produced by wild-type *S. marcescens* were spherical and monodispersed, with an average diameter of 17.05 nm. The biosynthesized AgNPs showed antimicrobial activity against both Gram-negative and Gram-positive bacteria and yeasts. Furthermore, it was observed that AgNPs exhibit more potent activity against drug-resistant Gram-negative bacteria (*E. coli* $Rif^{R}Kan^{R}$ and *S. marcescens* Kan^{R}) which suggests its potential role as an antimicrobial agent. Biosynthesis of AgNPs, particularly by microorganisms, may prove to be a better alternative for chemical and physical methods due to its cost-effectivity and environmentally friendly methodology.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

CONTRIBUTION OF INDIVIDUAL AUTHORS

All authors contributed to the form and content of this paper through data gathering, data analysis, and writing.

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