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Biosynthesis of Silver Nanoparticles by *Serratia marcescens* ssp *sakuensis* and its Antibacterial Application against some Pathogenic Bacteria

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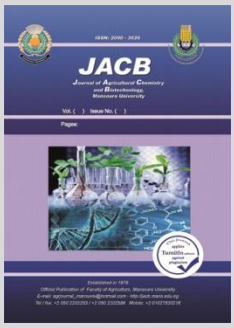

ABSTRACT

The utilize of microorganisms in the biosynthesis of nanoparticles from metals ions appear as an eco-friendly approach and alternative to the harmful traditional approaches in the environment. In this study, we synthesized silver nanoparticles (AgNPs) using *Serratia marcescens* subsp. *sakuensis* supernatant. The optimization factors of the production silver nanoparticles were carried out by seven parameters (medium growth, incubation time, the ratio of mixing culture supernatant to silver nitrate, temperature degree, pH level, silver nitrate concentration and agitation speed). The optimum silver nanoparticles production was achieved at pH 7, $AgNO_3$ concentration 2mM, temperature 30ºC, mixing ratio of silver nitrate to volume of culture supernatant 20:50 ml, incubation time 48h and agitation speed 140 rpm for AgNPs production. Also, the synthesized silver nanoparticles were characterized by UV-vis spectroscopy, transmission electron microscopy (TEM), powder X-ray diffraction (XRD), Fourier transform infra-red spectroscopy (FTIR) and Energy-dispersive X-ray spectroscopy (EDX). The surface plasmon absorbance spectra of AgNPs was observed at 430 nm, and transmission electron microscopy images showed that the diameter of well-dispersed AgNP (10–20 nm). In addition, the antibacterial activity was studied and the obtained results of the synthesized AgNPs by *Serratia marcescens* subsp. *sakuensis* showed a good antibacterial activity against the studied pathogenic bacteria.

Keywords: Biosynthesis, Silver nanoparticles, Optimization, Characterization, *Serratia marcescens*, Pathogenic bacteria

INTRODUCTION

In fact, silver is considered as one of the most inorganic metals ions applications especially in the form of nanoparticles and used as antimicrobial agents against broad spectrum of pathogenic bacteria and fungi as well (Dhand *et al.*, 2016). Their property led to their use in different industrial products such as plastics, soaps, pastes, food, and textiles and other applications (Zhang *et al.*, 2016). Similarly, the increasing concerns on antibiotic resistance by health organizations around the world are pushing researchers and pharmaceuticals to find other ways to conflict microorganisms, either through development of new antibiotics or other substances that can inhibit them. This then, ignited interest in AgNPs and their applications as antimicrobial particles (Sharma *et al.*, 2009). Also, it is commercialized as an important nanomaterial, with high tons of production every year, and is expected to increase year after year. Therefore, an ideal way for the synthesis of silver nanoparticles that provides a simple, eco-friendly method is deeply required (Larue *et al.*, 2014). Currently, there are several physical, chemical and biological methods available to biosynthesis of silver nanoparticles (NPs). In general, nanoparticles which are synthesized by physicochemical methods which highly effect and may due to a highly threat to the human, animals and plants environment (Kalaivani *et al.*, 2018).

Nowadays, nanotechnology is appearing as rapidly growing field with its applications in applied sciences. It is used as a good mechanism to explore the darkest avenues of applied sciences to combat disease by antibiotic resistant bacteria (Karthika *et al.*, 2015 and Zhang *et al.*, 2016).

In the biological approach, microorganisms have many enzymes in the extra or intracellular forms which can make nanoparticles. Among these microorganisms, bacteria are the most extensively exploited natural resources for the biosynthesis of metallic nanoparticles (Musarrat *et al.*, 2011).

Serratia marcescens is a species of rod-shaped Gram-negative bacteria, belongs to the Family Enterobacteriaceae. *Serratia marcescens* often produce a bright red colored pigment called prodigiosin. This bacterium can also reduce nitrate to nitrite which make it suitable to synthesis silver nanoparticle (Akilandeswari *et al.*, 2014 and Krithika *et al.*, 2014). This research was carried out to examine the ability of *Serratia marcescens* subsp. *sakuensis* strain which was isolated from Egyptian soil to synthesize silver nanoparticles, and used them as eco-friendly tool against some pathogenic bacteria.

MATERIALS AND METHODS

Isolation and Screening

Rhizosphere soil samples from El-Gabal El-Asfar region, which had been exposed to heavy metals pollution for more than 50 years in Egypt, were collected for the isolation of the target bacterial isolates. The collected samples were plated onto modified nitrate agar plates using silver nitrate (AgNO3/Sigma Aldrich-USA) at 2 mM (Adan *et al.*, 2018). After 48h of incubation period at 30°C, a clear zone around best colonies indicated the reduction of nitrate (Lakshmipathy

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and Nanda, 2013). The colonies which given a clear zone were selected and purified on nutrient agar plates. The isolates that formed the best zone were selected, and among them, only one isolate, which showed a good growth appearance and activity, was chosen and used for this study.

Identification of the isolated bacteria

The primary identification of the selected isolate was identified firstly according to the testes outlined in Bergey's Manual of Systematic Bacteriology (Grimont and Grimont, 2005). To confirm the primary identification, the isolate was subjected again to the identification using Matrix-assisted laser desorption ionization time of flight (MALDI TOF) mass spectrometry (Bille *et al.*, 2012; Krasny *et al.*, 2013; Kocaman and Aras, 2019).

Biosynthesis of silver nanoparticles

For biosynthesis of the silver nanoparticle studies, *S. marcescens* isolate was grown in 100 ml sterilized nutrient broth and incubated for 48h at 30°C in shaking incubator at 150 rpm. The growth was harvested and centrifuged at 10000 rpm for 10 min. The supernatant sterilized through filter membrane (0.45 μm), and 10 ml of the supernatant were transferred to test tubes and the solution of AgNO3 was added to make a final concentration of 1mM. The mixture was incubated for 24h at 30°C. Color was visually monitored from a clear to brown color confirmed the biosynthesis of AgNPs (Manivasagan *et al.*, 2013).

Optimization of the biosynthesis of AgNPs

Seven parameters were studied to optimize the production of AgNPs during the biosynthesis process, including the growth medium (Nutrient broth (NB), Mueller-Hinton broth (MHB) and Luria-Bertani broth (LBB)), incubation times (2, 12, 24, 36, 48 and 60 h), culture supernatant to silver nitrate ratio (20:50 ml, 30:40 ml, 35:35 ml, 40:30 ml or 50:20 ml), temperature degrees (20, 25, 30, 35 or 40°C), pH levels (6.0, 6.5, 7.0, 7.5 or 8.0), silver nitrate concentration (1.0, 2.0, 3.0,4.0 or 5.0 mM) and agitation speed (100, 120, 140, 160 or 180 rpm). Controlled treatment was done using culture supernatant without silver nitrate was kept as control. (Safekordi *et al.*, 2011). Lastly, for reach to the best yield of the AgNPs, the tested strain was grown under the optimized growth conditions recorded and the mixture reaction containing AgNPs were separated and concentrated by repeating washing and centrifugation at 17000 rpm for 20 min. at 4°C, and the final suspension was redispersed in sterile deionized water, and the obtained solution were then dried using hot air oven at 60°C for overnight (Nagarajan and Kuppusamy, 2013).

Characterization of the biosynthesized AgNPs

The change of color from slight yellow to dark brown after the incubation of *Serratia* supernatant with AgNO3 is considered as a primary characteristic of the formation of AgNPs (Fang *et al.*, 2005).

UV–visible spectral analysis

UV–visible spectral analysis was performed to measure the absorbance of the reaction mixture by a dual-beam UV-Vis spectroscopy on Laxco™ (model Laxco™, Alpha-1502 Alpha Series Spectrophotometer, 200 - 1000 nm) according to El-Batal *et al.* (2013).

Fourier transform infra-red spectroscopy (FTIR) analysis

The AgNPs produced by *Serratia marcescens* isolate are subjected to Fourier transform infra-red spectroscopy (PerkinElmer Frontier, U.S.A) according to Aguilar *et al.* (2011).

Powder X-ray diffraction (XRD) analysis

The crystal shape of AgNPs were analyzed by XRD (6000 - shimadzu - Japan) using the radiation of CuKα which provides information regarding arrangement of atoms through the measurement of diffraction of these rays, according to Kalabegishvili *et al.* (2012).

Transmission electron microscopy (TEM) analysis

The image of silver nanoparticles filmed by TEM (JEOL JEM-1010, Japan) to visualize the morphological shape and the size of AgNPs. The procedure was performed according to Kalaivani *et al.* (2018).

Energy-dispersive X-ray spectroscopy (EDX) analysis

This technique was determined to confirm the existence of elemental Ag in the particles in addition to detect the particle primary elements (Najitha and Balasubramanian, 2015).

Antimicrobial activity assay

Four pathogenic bacteria including Gram-positive bacteria (*Listeria monocytogenes*, and *Bacillus cereus*) and Gram-negative bacteria (*Escherichia coli*, and *Pseudomonas aeruginosa*) were kindly provided by the Dept. of Microbiology, Faculty of Medicine, Zagazig University. They were used to determine the antibacterial activities of the synthesized silver nanoparticles by the agar disc diffusion method. One hundred microliter of fresh culture of each pathogenic bacterium was swabbed individually on Mueller-Hinton agar in Petri dishes. Different concentrations of AgNPs (25, 50, 75, 100 and 125μl/disc) was transferred to sterile discs placed on agar medium and incubated at 37° C for 48 h. A diameter of inhibition zone was measured in millimeters (Kalaivani *et al.*, 2018).

RESULTS AND DISCUSSION

Among the purified bacterial isolates, the best isolate giving a good clear zone on the nitrate agar medium and have the ability of synthesizing AgNPs as observed by the change of the color in the reaction was chosen for this study. From the obtained results of morphological, physiological and biochemical tested carried out in the selected bacterial isolate and comparison them with those of known taxa in Bergey's Manual of Systematic Bacteriology (Grimont and Grimont, 2005), the chosen bacterial isolate was identified to genus and species level as *Serratia marcescens*.

The selected isolate was identified again by MALDI-TOF mass spectrometry, the results showed its maximum identity of 98% to various *Serratia* sp. mainly *Serratia marcescens* subsp. *sakuensis* CIP 107489T HAM (Krasny *et al.*, 2013; Kocaman and Aras, 2019). Thus, the local bacterial isolate, *Serratia marcescens* subsp. *sakuensis* is similar *Serratia marcescens* subsp. *sakuensis* CIP 107489T HAM.

As shown in Fig. 1, the difference in color of the reaction mixture from slight yellow to dark brown within a time of incubation indicated the synthesis of AgNPs by the supernatant of the bacterial strain culture. In this respect, Krithika *et al.* (2014) and El-Batal *et al.* (2016) reported the same observation since they found that the production of silver nanoparticles by *Serratia marcescens* was identified by the formation of a brown color after an incubation time. Also, Akilandeswari *et al.* (2014) stated that the primary

confirmation of biosynthesis of AgNPs from *Serratia marcescens* was noticed visually by the color change of a medium from light yellow to dark brown.

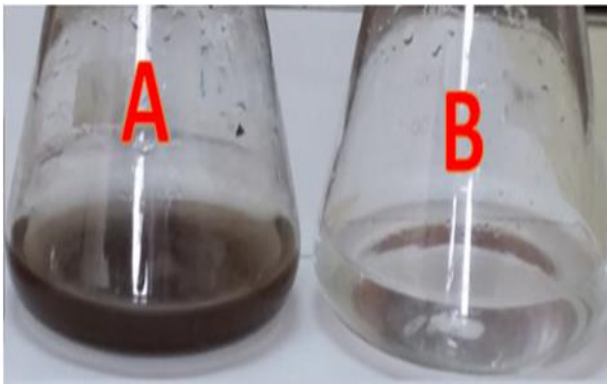

Fig.1. Color change of the reaction mixture indicating the formation of AgNPs by the tested bacteria A) $AgNO_3$ solution with bacterial supernatant. B) $AgNO_3$ solution without bacterial supernatant, after 24h of incubation period

Optimization Factors

The biosynthesis of AgNPs by *Serratia marcescens* subsp. *sakuensis* strain was optimized through different factors like mixing ratio of the supernatant and AgNO3, media type, temperature degree, pH level, incubation time, AgNO3 concentration and agitation speed.

Effect of mixing ratio

After mixing different volumes of AgNO3 (1mM) with different volumes of supernatant of *Serratia marcescens* subsp. *sakuensis* for 24h, UV- Visible spectra of silver nanoparticles were recorded as shown in Fig. 2. The obtained results showed that culture supernatant to silver nitrate ratio (20:50ml) gave maximum absorbance at 420 nm, thus it was considered as the optimal ratio of mixing supernatant with AgNO3 solution (1 mM). It seems that by increasing the filtrate, the amount of nanoparticles become smaller as reported by Safekordi *et al.* (2011). On increasing the amount of cell extract in the culture mixture, the plasmon absorbance maximum was recorded to be changed slightly towards longer wavelengths, which is an indication of increase in the particle size (Kajani *et al.*, 2014).

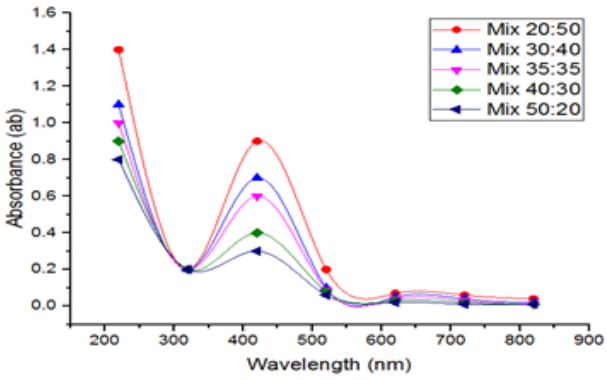

Fig. 2. UV-Vis spectra of the AgNPs biosynthesis by the tested strain as affected by the mixing ratio of culture supernatant and $AgNO_3$ solution

Effect of medium type

For study this factor, three different media were selected (NB, MHB and LBB). NB medium was found to be the best medium for the manufacturing of AgNPs by *Serratia marcescens* subsp. *sakuensis*. UV Vis spectra of AgNPs by *Serratia marcescens* subsp. *sakuensis* in NB, LBB and/or MHB media are given in Fig. 3. Similar result was reported by Divya *et al.* (2016) who found that nutrient broth was the standard medium for the biosynthesis of silver nanoparticles by *E. coli.*

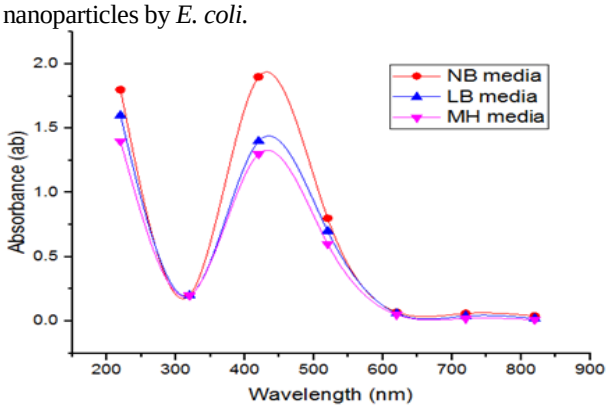

Fig.3. UV-Vis spectra of the AgNPs biosynthesis by the tested strain as affected by media type

Effect of pH

For the standardization of pH level for silver nanoparticles manufacture, five different pH levels were tested. pH 7 was considered to be the best pH for the formation of AgNPs by *Serratia marcescens* subsp. *sakuensis*. UV-Vis spectra of AgNPs formed in pH levels are presented in Fig. 4. Akilandeswari *et al.* (2014) stated that the maximum synthesis of AgNPs by *Serratia marcescens* was observed at pH 8, and the synthesis of nanoparticles increased gradually with the increasing of pH until pH 8, and then it starts decrease.

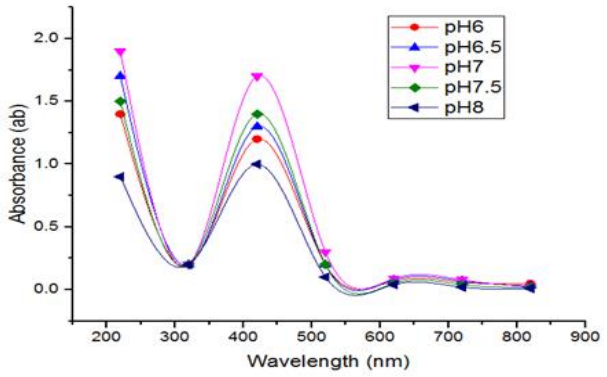

Fig.4. UV-Vis spectra of the AgNPs biosynthesis by the tested strain as affected by pH level

Effect of $AgNO_3$ concentration

Five different silver nitrate concentrations were selected to study this factor. 2mM of $AgNO_3$ was found to be the optimum silver nitrate concentration for the manufacturing of AgNPs by *Serratia marcescens* subsp. *sakuensis*. UV Vis spectra of AgNPs by *Serratia marcescens* subsp. *sakuensis* in silver nitrate concentration are shown in Fig. 5. These results were correlated with those obtained by Akilandeswari *et al.* (2014) who stated that the sample with low concentration of silver nitrate showed dark brown color when compared with the samples with higher concentration. This may due to a higher concentrations of silver nitrate solution may produce toxic or inhibitory effect on the *Serratia marcescens.* Also, Singh *et al.* (2014) and

Saxcena *et al.* (2016) reported that maximum AgNPs biosynthesis of 2mM $AgNO_3$ concentration using *Penicillium* sp. and *Sclerotinia sclerotirum* were recorded, respectively.

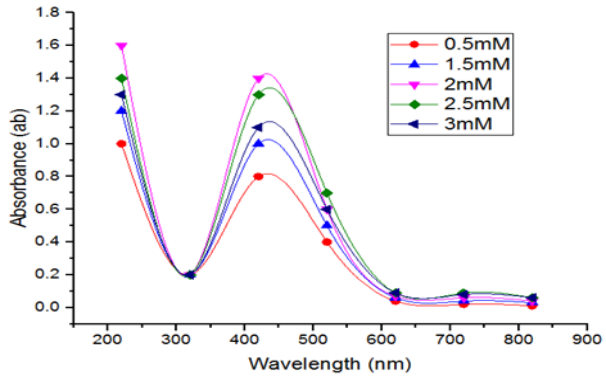

Fig.5. UV-Vis spectra of the AgNPs biosynthesis by the tested strain as affected by different silver nitrate concentrations

Effect of temperature degree

In this experiment, five different temperature degrees were performed. 30ºC was recorded to be the standard temperature degree for the manufacturing of AgNPs by *Serratia marcescens* subsp. *sakuensis*. UV Vis spectra of AgNPs by *Serratia marcescens* subsp. *sakuensis* in temperature degrees are shown in Fig. 6. Amit *et al.* (2012) stated that absorbance increased with the increasing of the temperature degrees from 25 to 45°C, thereafter decreased at the higher of the temperature degree.

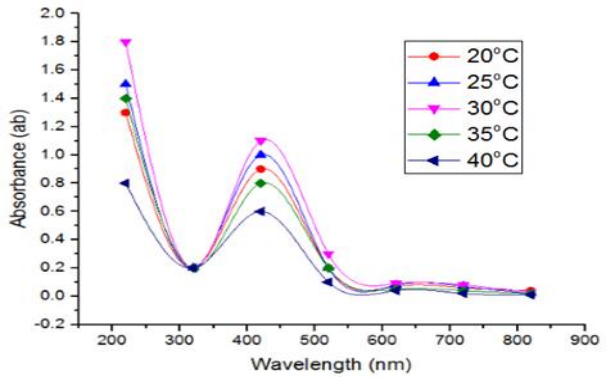

Fig.6. UV-Vis spectra of the AgNPs biosynthesis by the tested strain as affected by different temperature degree

Effect of agitation speed

In this factor, five different agitation speeds were selected to achieve this study. The best agitation speed for the biosynthesis of AgNPs by *Serratia marcescens* subsp. *sakuensis* was found to be 140 rpm. UV Vis spectra of AgNPs by *Serratia marcescens* subsp. *sakuensis* in agitation speeds are shown in Fig. 7. In the biosynthesis of AgNPs, the aeration is an important factor to maximize the reaction and provided a good homogenous suspension (Armenante and Nagamine, 1998). In this connection, Lakshmipathy and Nanda (2013) and El-Batal *et al.* (2016) used 150 rpm as agitation speed in their studies to biosynthesis of AgNPs by *Serratia marcescens.*

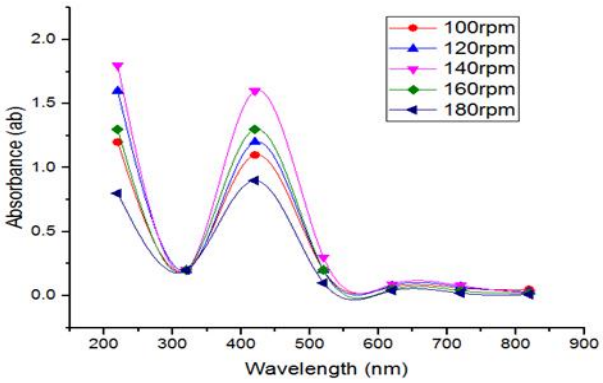

Fig. 7.UV-Vis spectra of the AgNPs biosynthesis by the tested strain as affected by agitation speed

Effect of incubation time

In this test, five different incubation times were studied. UV Vis spectra of AgNPs by *Serratia marcescens* subsp. *sakuensis* in incubation time are given in Fig. 8. From the results, 48h was found to be the optimum incubation time for the manufacturing of AgNPs by *Serratia marcescens* subsp. *sakuensis*. Lakshmipathy and Nanda (2013) studied the influence of incubation time by reductase assay using supernatant of *Serratia marcescens* and they found that, the maximum reduction was observed after 72 h.

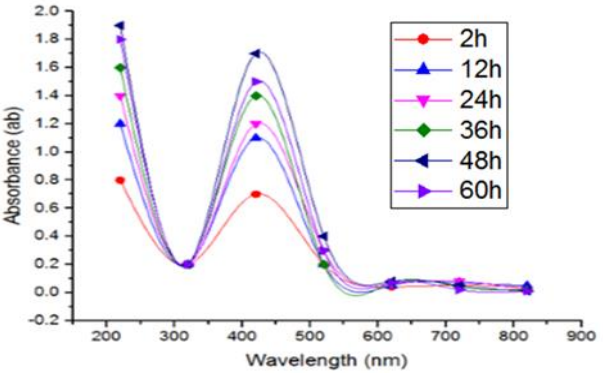

Fig. 8. UV-Vis spectra of the AgNPs biosynthesis by the tested strain as affected by incubation time

Characterization of AgNPs produced by *Serratia marcescens* subsp. *sakuensis*.

Characterization of silver nanoparticles is important to understand their intrinsic characters. The primary confirmation of biosynthesis AgNPs from *Serratia marcescens* was observed visually by the change of the color in medium growth from yellow to dark brown as shown in Fig. 1.Characterization was accomplished using different techniques such as UV-Vis spectra, FTIR, XRD, TEM, and EDX spectroscopy analyses.

UV-Visible absorption

UV-Visible absorption spectroscopy is one of the most important tools which has been utilized in nanoparticles characterization. The biosynthesis of AgNPs in solution was recorded by the absorption spectra at a wavelength range of 200:1000 nm (Fig. 9). The solution of $AgNO_3$ turned to dark brown after the addition of *Serratia* supernatant; this result confirmed the formation of AgNPs, on contrast no color change was noticed with the absence of *Serratia* supernatant. In UV-Vis spectrum, a broad surface

plasmon resonance (SPR) peak was recorded at 420 nm which confirmed the biosynthesis of silver nanoparticles. Zaheer and Rafiuddin (2012) reported that a SPR peak was located between 410 and 450 nm for AgNPs. Color transitions arise during the reduction of metal salts to metals, thus leading to changes in the ability to absorb light in visible region at the electromagnetic field (Akilandeswari *et al.*, 2014). Also, Karthika *et al.* (2015) observed a sharp peak for biosynthesis of the silver nanoparticles was recorded at 400-425 nm by UV-Visible spectroscopy.

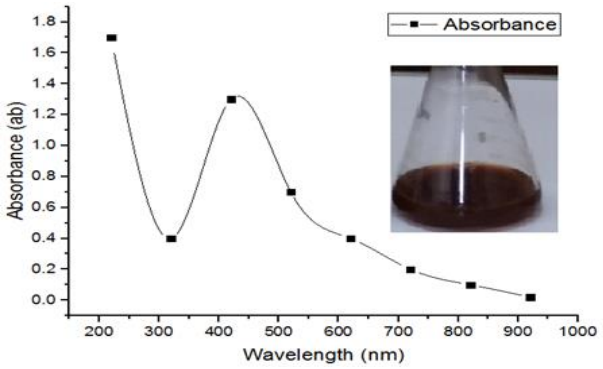

Fig. 9. UV-Vis Spectrum of AgNPs biosynthesized by *Serratia marcescens* subsp. *sakuensis*

FTIR spectroscopy

FTIR measurement was used to identify the presence of various functional groups in biomolecules responsible for the reduction of Ag^{+} to Ag^{o} and also capping/stabilization of AgNPs. The bands were compared with standard values to identify the functional groups. Absorption bands were recorded by FTIR spectrum at 3419.39, 2962.66, 2094.08, 1650.16, 1551.47, 1449.39, 1408.49, 1337.27, 1160.02, 920.66 and 619.39 cm^{-1} refer to the presence of capping agent with nanoparticles (Fig. 10). These bands represented different functional groups like primary, secondary amines, amides, alcohols, phenols (strong and broad peak with the bonds of O–H stretching and N–H stretching at 3419.39 cm^{-1} while at 2962.66, 2094.08 gave a strong and weak peak, having functional groups of aromatics and alkynes, respectively.

The bonds appearing at 1650.16, 1551.47, 1449.39, 1408.49 and 1337.27cm^{-1} were assigned for – C≡C – stretching and C–C stretching (in – ring) of alkenes and aromatics, respectively. Krithika *et al.* (2014) reported FTIR spectra of silver nanoparticle produced from *Serratia marcescens* supernatant and observed a strong peak value at 3761.47 cm^{-1} corresponding to N-H stretching vibration amide or primary and second amines linkages in the proteins. Also, Kalaivani *et al.* (2018) stated that the peak near 3431cm^{-1} corresponding to OH groups.

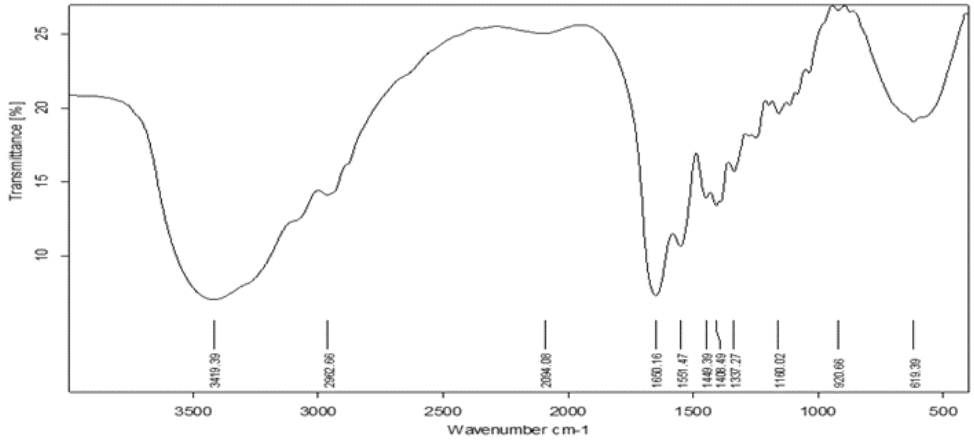

Fig. 10. FTIR analysis of biosynthesized AgNPs by *Serratia marcescens* subsp. *sakuensis*

X-ray diffraction

Crystalline nature of silver nanoparticles was carried out by X-ray diffraction. The XRD pattern of the biosynthesis of AgNPs is shown in Fig. 11. The diffracted intensities were recorded from 20 to 80. Four strong bragg reflections at 32.10º, 46.15º, 57.17º and 76.13º corresponding to the planes of (111), (200), (220) and (311), respectively. This result confirmed that AgNPs were spherical and crystalline. Lakshmipathy and Nanda (2013) stated that a typical XRD pattern of reduced silver nitrate produced by *Serratia marcescens* supernatant shows diffraction peaks at 38º and 45º that can be indexed to (111) and (200) plans of the silver, respectively. The biosynthesis of AgNPs synthesized by culture supernatant of *Serratia marcescens* was supported by XRD measurements. XRD of the formation of AgNPs showed distinct diffraction peaks at 31.75 and 45.50 indexed to the planes 111 and 211, respectively (Karthika *et al.*, 2015).

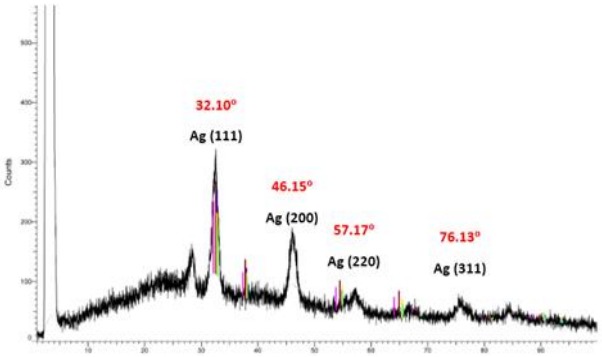

Fig. 11. XRD Spectrum of AgNPs synthesized by *Serratia marcescens* subsp. *sakuensis*

Transmission electron microscopy.

TEM micrograph of AgNPs produced by Serratia marcescens ssp sakuensis is shown in Fig. 12. This analysis is used to know the size and the shape of AgNPs

biosynthesized. It was observed that the nanoparticles were spherical and dispersed well without agglomeration. The particle size of AgNPs was sized less than 52 nm. In this respect, Karthika *et al.* (2015) used TEM to characterize the size distributions of silver nanoparticles obtained with a culture supernatant of Serratia marcescens, and found that the morphology of silver nanoparticles is nearly spherical having particle size less than 100 nm. Also, El-Batal *et al.* (2016) reported the synthesis of spherical shape of AgNPs with a diameter about 10.7 nm by Serratia marcescens. Saifuddin *et al.* (2009) and Durairasu *et al.* (2017) reported that TEM images revealed that the morphology of AgNPs produced by Bacillus sp. synthesized spherical NPs with the size of 5-50nm.

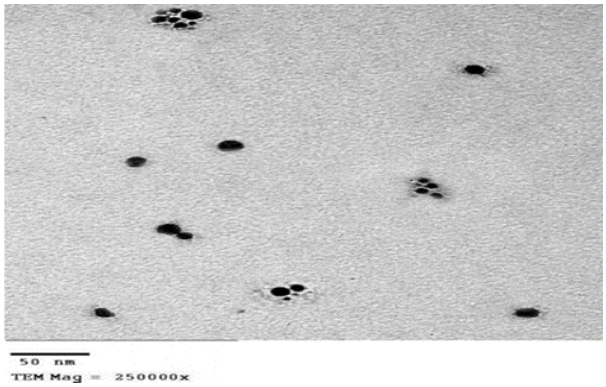

Fig. 12.TEM electron micrograph of silver nanoparticles produced by *Serratia marcescens* subsp. *sakuensis*

EDX spectrum analysis

EDX instrument was used in this experiment to confirm the existence of silver metal in the AgNPs. Also, to confirm the formation of the AgNPs from *Serratia marcescens* subsp. *sakuensis*. A strong peak of the Ag ions was obtained at 3 KeV (Fig. 13). A sharp signal was recorded at 3 keV of AgNPs along with other peaks in EDX spectrum of 0–4keV. Also, a sharp signal with the absorption band peaks in a range of 3 to 4 keV is typical confirmed the presence of silver metal by the absorption of metallic silver nanoparticles, which indicates the presence of elemental silver nanoparticles in the suspension. Karthika *et al.* (2015) reported a similar result with the biosynthesis of AgNPs from *Serratia marcescens*, and stated that analysis through EDX confirmed the presence of a strong signal of silver along with another element that are bound to surface of AgNPs.

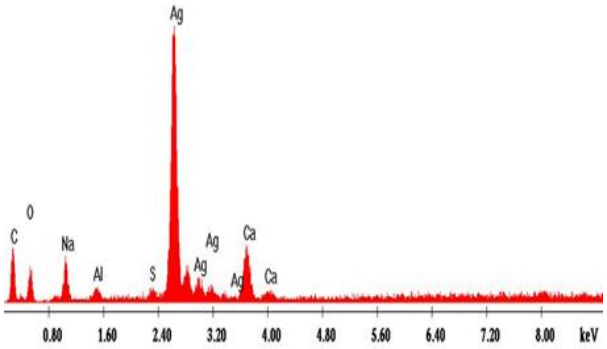

Fig. 13. EDX spectrum analysis of biosynthesized AgNPs by *Serratia marcescens* subsp. *sakuensis*

Antimicrobial activity against pathogens

The disc diffusion assay for antibacterial susceptibility testing was carried out to determine the antimicrobial activities of the silver nanoparticle produced from *Serratia marcescens* subsp. *sakuensis* against four pathogenic bacteria, namely, *Bacillus cereus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Escherichia coli*. Disc inhibition assay for silver nanoparticle supernatant showed a best potential antimicrobial activity against all tested microbes as shown in Table 1. The particles showed the highest activity against the pathogenic *Pseudomonas aeruginosa* (23 mm). The higher activity was followed by *Listeria monocytogensis* (21 mm), *Bacillus cereus* (19.5 mm) and *E. coli* (17 mm). The formation of a clear zone increased by increasing the concentrations of AgNPs. Silver nanoparticle exhibited antibacterial effect against Gram-positive bacteria as well as Gram-negative bacteria (Lakshmipathy and Nanda, 2013; Paul and Sinha, 2014 and Adan *et al.*, 2018). From this study, we revealed that the nanoparticles from *Serratia marcescens* subsp. *sakuensis* showed a good activity against pathogenic bacteria.

Table 1. Antibacterial activity of AgNPs at different concentrations against some pathogenic bacteria using agar well diffusion assay.

Concentration	**Zone of inhibition (mm)**				
Bacteria	**25 µl/disc**	**50 µl/disc**	**75 µl/disc**	**100 µl/disc**	**125 µl/disc**
Listeria monocytogenes	8.5	11.0	14.5	18.0	21.0
Bacillus cereus	8.0	10.5	13.0	15.0	19.5
Escherichia coli	7.5	10.0	13.5	14.0	17.0
Pseudomonas aeruginosa	8.5	12.5	15.5	20.0	23.0

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التخليق الحيوى لجسيمات الفضة متناهية الصغر بواسطة *Serratia marcescens* subsp. *sakuensis* وتطبيقه ضد بعض البكتريا المسببة للأمراض

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إن استخدام الكائنات الحية الدقيقة في التخليق الحيوي للجسيمات متناهية الصغر من أيونات المعادن يظهر كنهج صديق للبيئة وبديل للطرق التقليدية الضاره. في هذه الدراسة ، تم تخليق جسيمات الفضة متناهية الصغر باستخدام مستخلص بكتريا *Serratia marcescens* subsp. *sakuensis*. تم معظمة عوامل النمو المثلى لإنتاج جزيئات الفضة متناهية الصغر (AgNPs) بدراسة سبعة عوامل (بيئة النمو ، وقت التحضين، نسبة خلط مستخلص البكتريا إلى نترات الفضة ، درجة الحرارة، مستوى الـ pH، تركيز نترات الفضة وسرعة الرج). كانت الظروف المثلى لإنتاج جسيمات الفضة متناهية الصغر التى تحققت هى درجة pH 7، وتركيز نترات الفضه 2 ملليمول، ودرجة الحرارة 30 درجة مئوية ، ونسبة خلط نترات الفضة إلى حجم مستخلص المزرعة البكتيرية هو 20:50 مل، وكان وقت التحضين 48 ساعة وسرعة الرج 140 لفة في الدقيقة وذلك للإنتاج الأمثل من AgNPs. علاوة على ذلك، تم توصيف جسيمات الفضة متناهية الصغر بواسطة التحليل الطيفي للأشعة فوق البنفسجية، المجهر الإلكتروني النافذ (TEM)، حيود أشعة إكس (XRD)، الأشعة تحت الحمراء (FTIR)، التحليل الطيفي للأشعة السينية المشتتة للطاقة (EDX). وقد لوحظ أقصى امتصاص لـ AgNPs عند 430 نانومتر ، وأظهرت صور المجهر الإلكتروني النافذ أن قطر AgNP هو (10-20 نانومتر). بالإضافة إلى ذلك، تمت دراسة النشاط المضاد للبكتيريا وأظهرت النتائج التي تم الحصول عليها أن جزيئات الفضة متناهية الصغر المخلقة بواسطة بكتريا *Serratia marcescens* subsp. *sakuensis* لها نشاط جيد مضاد للبكتريا المسببة للأمراض التى تم دراستها.