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The green synthesis and characterisation of silver nanoparticles from *Serratia* spp

Síntesis verde y caracterización de nanopartículas de plata de *Serratia* spp

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Abstract

The biological means of silver nanoparticles synthesis is an evolutionary step in the process of synthesizing nanoparticles to produce particles within the nano range. With the side effects of the use of chemical and physical synthesis methods yielding toxic and unstable, researches have turned to green synthesis to produce nanoparticles using the mechanism of the bacteria specifically the nitrate reductase enzyme present in most bacteria to break down silver nitrate to silver nanoparticles which are stable and reduces the cost of production. Marine bacteria play it role by being a reservoir that can intake heavy metals and breaking down with no difficulty as a result of their harsh habitat which is rich in heavy metals. This study was done to prove that marine bacteria can synthesize silver nanoparticles and the nanoparticles are stable and has a range of size 1 nm to 100 nm and shape through preliminary studies. Marine bacteria were isolated from marine soil samples and later identified to belong to the genus Serratia. Synthesis of the nanoparticles were done using extracellular synthesis where equal amounts of bacterial supernatant and 0.5 M silver nitrate was added and incubated under dark condition for 72 hours. Screening showed twenty isolates showed positive results for synthesis which were further examined using UV-Visible Spectra analysis showing all 20 samples having a range of size and shape of nanoparticles with peaks observed between the ranges of 340 nm to 440 nm, with the sample strain AQ5-NT6 being the most potent. This study will provide fundamental data and add knowledge to the field of green synthesis of silver nanoparticles.

Keywords: Green synthesis, silver nanoparticles, *Serratia* spp.

Resumen

El significado biológico de la síntesis de nanopartículas de plata es un paso evolutivo en el proceso de la síntesis de éstas para producir partículas dentro del rango " nano-". Los efectos colaterales de los métodos de síntesis química y física consisten en la generación de productos tóxicos e inestables. Por ello, las investigaciones se han centrado en la síntesis verde para producir nanopartículas utilizando los mecanismos de bacterias, específicamente la enzima nitrato reductasa presente en la mayoría de las bacterias que a partir de nitrato de plata producen nanopartículas de plata, las cuales son estables y reducen los costos de producción. Las bacterias marinas actúan como un reservorio que puede captar metales pesados y descomponerlos sin dificultad como resultado de su hábitat hostil, el que es rico en metales pesados. Este trabajo se realizó para probar que bacterias marinas pueden sintetizar nanopartículas de plata, las cuales son estables y tienen una forma y rango de tamano de 1 nm a 100 nm según estudios preliminares. Bacterias marinas fueron aisladas a partir de muestras de suelo marino y, posteriormente, identificadas como bacterias pertenecientes al género Serratia. La síntesis de nanopartículas se llevó a cabo mediante síntesis extracelular en donde se anadieron cantidades iguales de sobrenadante bacteriano y nitrato de plata 0,5 M y a continuación se incubó en oscuridad durante 72 horas. En la búsqueda se encontraron 20 aislados que resultaron positivo para la síntesis de nanopartículas de plata, las que luego se analizaron mediante espectroscopía UV-visible. Estos resultados mostraron que las 20 muestras tenían forma y rango de tamano de nanopartículas con picos en el rango de 340-440 nm, siendo la cepa AQ5-NT6 la más potente. Este estudio proporcionará datos fundamentales y aportará conocimiento en el campo de la síntesis verde de nanopartículas de plata.

Keywords: Síntesis verde, nanopartículas de plata, *Serratia* spp.

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1 Introduction

Nanomaterial have become crucial in many aspects of live today. The use of nanomaterials have improved the quality and performance of many products in the medical, cosmetics, agriculture, water treatment and food industry. A wide range of nanomaterial have been synthetized through chemical, physical and biological processes such as chitosan, calcium chloride, gold, silver and combinations of nanoparticles such as silver-chitosan complex. Silver has shown success in its nanoparticle form giving rise to various research related to silver nanoparticles and its potential abilities (Velasco-Rodriguez *et al*., 2012; Sotelo-boyas et al., 2015; Cervantes-Avilés *et al*., 2017; Flores-Hernández *et al*., 2020).

Silver nanoparticles are generally particles of silver from 1 nm to 100 nm (Graf *et al*., 2003). Although often defined as silver, some are made up of a large proportion of silver oxide due to their large surface to volume ratio of silver atoms. The process of synthesizing silver nanoparticles from marine microorganisms such as bacteria is a steppingstone in producing stable and uniform sized nanoparticles. Primarily being that marine bacteria are theoretically capable of the uptake of heavy metals thus allowing the bacterial cell components to break down the bulk silver into nano sized particles via various mechanisms such as the role of the nitrate reductase enzyme present in bacterial cells due to the harsh habitat of marine microorganism which consists of various earth metals and metals as a result of pollution (Iyer *et al*., 2005). Nanomaterials are sourced from one of three general methods which are the chemical, physical and biological methods respectively, where the non-biological methods yield nano scaled particles that are unstable, of unpredictable shape and size as well as being costly and the production of hazardous by products, whereas biological synthesis methods yield nanoparticles of stable, uniform, cost effective and little to no environmental effects due to production of by products (Mock *et al*., 2002).

Silver based nanomaterials has sparked the interest of researches in the nanomaterials field in the last few decades due to their wide range of benefits in fields such as cosmetics, food, medicine and agriculture due to its antibacterial activity (Bretado-Aragón *et al*., 2016). The role of silver nanoparticles due to one of two mechanisms being either the slow oxidation into Ag^+ or the bonding of the nanoparticles to the surface protein thiol groups allowing for the penetration into bacterial cells thus attacking important cellular components leading to cell death (Majdalawieh *et al*., 2014). The exact means of action of the nanomaterials activity were not fully understood. Studies have shown that nanoparticles are able to interrupt with membrane proteins causing morphological changes which in turn disrupts membrane permeability inhibiting exchange processes across the plasma membrane (Majdalawieh et al., 2014). While other research reports show that silver binds to surface proteins with sulphur and phosphorus the primary component of DNA resulting in chain reactions leading to apoptosis (Majdalawieh et al., 2014).

The use of biological systems as tools in synthesizing nanoparticles has gained the interest of researches over the years, primarily being that stable and uniform nano scaled products can be obtained and the methods of synthesis are simple and cost effective. Termed as 'green synthesis' this means of nanoparticle synthesis uses plants, bacteria, algae, fungi and their components to synthesise nanoparticles. Generally, plants have shown prominent results in this field and is preferred than bacteria, algae and fungi. Plants such as Azadirachta indica (Shankar *et al*., 2004), Aloe vera (Mukunthan *et al*., 2011), Acalypha indica (Mohanpuria *et al*., 2008) and Plectranthus amboinicus (Zheng *et al*., 2017) have showed success.

While plants have been successful nano factories in synthesizing nanoparticles, factors such as temperature, pH, time and preparation costs are the challenges that must be taken into account. The use of bacteria as a tool of synthesis is more appropriate because bacteria cells can be cultivated under controlled conditions in the laboratory while the temperature and pH can be maintained as required whereas in plants these factors are affected by the cellular functions of the particular plants. As compared to plants, bacteria can be culture easily and requires lesser time and cost. An ongoing issue is that the synthesis of nanomaterials using plant nanofactories has adverse effects on the plant biological system due to an influx of toxicity as the plant itself has its own toxicity mechanism to fight against predators. The influx of both the plants toxicity and the silver nanoparticles toxicity can results in damage to the cellular components of the plants thus eventually leading to death (Jha *et al*., 2009).

Research into the use of marine bacteria as a potential nano tool could eliminate the above problems. Bacterial cells can be easily controlled by influencing necessary factors and their microscopic

structure makes them perfect for research purposes. Marine bacteria will be the most suitable of all bacterial strains as they are theoretically tolerant to heavy metals due to the sea being a source of heavy metals as a result of various environmental and man-made issues. The stability, size and shape of the nanoparticles is affected by the incubation period of the reaction medium and the presence of the nitrate reductase enzyme (Darroudi *et al*., 2011). Similarly, the synthesized nanoparticles properties are also changed over time and are influenced by the method of processing, light exposure and storage conditions. Time variations can occur in various means, such as particle conglomeration due to long storage, reduction or growth of particles during long storage, or shelf life, which affects their potential (Baer, 2011). In bacterial synthesis, variability is less likely because same bacterial cells in a colony have identical genome (McCarthy, 2013). This research aims at synthesizing silver nanoparticles from marine bacteria and validating the presence of nanoparticles using basic UV-Visible Spectra analysis. Additional and new data will be added to existing research on silver nanoparticles synthesized from marine bacteria.

2 Materials and method

2.1 Sample collection

Seawater and marine soil sediments were collected from Port Dickson (Pantai Dickson: 2.424234, 101.886885; Pantai Cermin: 2.4145012, 101.85877149999999 and Pantai Purnama: 2.4436069, 101.85590430000002), a coastal area located in the state of Negeri Sembilan, Malaysia. About 20 marine soil samples and seawater were collected from the location. The 20 soil sediment samples were labelled based on the location of collection.

2.2 Isolation and identification of marine bacteria

2.2.1 Serial dilution

The seawater was filtered and autoclaved. Serial dilution up to 10^{-3} was carried out for the samples from Port Dickson. Serial dilution method following the method reported by Rajeshkumar *et al*. (2013) was used in this study. Four boiling tubes were prepared and labelled 10^{0}, 10^{-1}, 10^{-2} and 10^{-3} for each of the marine soil sediment samples with 9 mL of filtered and autoclaved seawater was added to each of the tubes.

Approximately 1 g of the marine soil sediment sample was weighed and added to 9 mL of filtered and autoclaved seawater denoted as 10^{0} dilutions. The mixture was then mixed using a vortex shaker for a 10 to 15 seconds. Then, 1 mL of the mixture from the 10^{0}dilution was taken to prepare 10^{-1}, $10^{-2}$and 10^{-3} dilution. After that, 100 μL of the diluted sample from each dilution was taken and spread plated on marine agar. Replicates of each dilution samples were made. The plates were left at room temperature for 5 days after which the morphological characteristics of each colony present were noted and selected based on morphological differences.

2.2.2 Subculture for pure colony

The selected single colonies were streaked on marine agar under aseptic conditions. The plates were incubated at room temperature for 5 days. Once a single strain was obtained, the bacteria samples were sub-cultured every 2 to 3 weeks to maintain a pure strain.

2.3 Biosynthesis of silver nanoparticles

An amount of 10 mL of marine broth was inoculated with the bacteria samples in a 50 mL falcon tube and placed onto a rotary shaker at 120 rpm for 24 hours. After 24 hours of incubation, the bacterial culture was centrifuged at 7000 rpm and 4°C for 20 min using high speed centrifuge. The culture supernatant was collected in a falcon tube.

Extracellular synthesis using culture supernatant was carried out as reported by Shivakrishna *et al.* (2013) with slight modifications. The bacterial supernatant was mixed with equal volume of 0.5 M silver nitrate solution in a falcon tube. The colour change from mixture of silver nitrate and bacterial supernatant was observed and recorded. The mixture was left in a dark condition for 3 days. The control containing only silver nitrate solution was done alongside the samples. The colour of the control was recorded before and after incubation. Colour change dark brown indicated the presence of silver nanoparticles.

2.4 Identification of positive marine bacteria using 16 rRNA gene amplification

Bacterial DNA was extracted using NucleoSpinR Microbial DNA (Macherey Nagel) based on the manufacturer's instruction. After extracting the bacterial DNA, agarose gel was prepared at a concentration of 0.1% for agarose gel electrophoresis. Followed by, 16S rRNA PCR amplification was carried out using Vivantis master mix, forward and reverse primers. PCR cycling parameters were as followed. The initial denaturation was 94°C for 3 min. Denaturation step was at 94 °C for 1 min, annealing temperature was lowered to the ranges 53°C- 54°C for 1 min, and elongation step at a temperature of 72°C for 2 min. After PCR, the PCR product was stored at -20°C overnight. Then, the PCR product underwent 0.1% gel electrophoresis and once the bands were obtained, the gel was viewed on a GelDoc. The PCR product on the gel was sent for sequencing to NextGene. The sequencing results obtained were compared to known sequences in Basic Local Alignment Search Tool (BLAST) at National Centre of Biotechnology Information (NCBI) to search for sequence homology and determine the family of positive marine bacteria for silver nanoparticles biosynthesis.

2.5 Characterization of silver nanoparticles by UV Visible Spectroscopy

Change in colour was observed in the silver nitrate solution with the bacterial samples. The UV-visible spectra of this solution were recorded. The absorbance of each positive isolate was taken from the wavelength of 340 nm to 500 nm (range increased at 20 nm gradually).

3 Results and discussion

3.1 Isolation and screening for the synthesis of silver nanoparticles

About 19 strains of bacteria with different morphology were isolated from twenty soil samples collected. Among 19 samples, twenty indicated positive synthesis of silver nanoparticles through preliminary indication of colour change. The colour change from milky white to brown of the aqueous solution was caused by the elation of surface plasmon resonance from the nanoparticles (Krishnaraj *et al.*, 2010). This indicates the reduction of silver ion in silver nitrate solution by marine bacteria (Vanaja *et al*., 2013). Table 1 illustrates 20 positive bacterial samples based on the location of collection, which varies from Pantai Dickson to Pantai Purnama, Port Dickson.

Marine bacteria have shown success in synthesizing nanoparticles in research in past years. *Oscillatoria wille* NTDM01 has been successfully used to synthesize silver nanoparticles, the marine cyanobacterium was incubated with $AgNO_3$ and after 72 hours a colour change to yellow indicated the presence of silver nanoparticles. The same was reported when $AgNO_3$ was added to *Spirulina platensis* but in this case it took 120 hours of incubation to produce silver nanoparticles. In both conditions spherical silver nanoparticles were produced that maybe the result of proteins present in the bacteria (Govindaraju *et al.*, 2008). Shivakrisna *et al.* (2013) reported that marine bacteria *Pseudomonas aerogenosa* was able to synthesize Ag NP's within 24 hours of incubation however half the incubation period was done in the dark. Dhandapani and Supraja (2012) reported that marine thermophilic *Bacillus* sp. produced silver nanoparticles in 48 hours. In all the conditions above the pH was maintained in the range of 7-8, temperature between 25°C and 30°C and different bacteria required different culture medium such as Luria broth and Zobell marine broth.

3.2 Biosynthesis of silver nanoparticles

The synthesis of silver nanoparticles was initiated with the addition of silver nitrate causing a change in colour from milky white and dark brown after incubation at room temperature for 72 hours as shown in Fig. 1. Throughout the incubation period, two layers were observed; the top layer being the clear brown layer intensifies over time while the bottom layer with precipitate was suspected to be of organic material from the bacteria and other forms of silver apart from silver nanoparticles. The conversion of silver nanoparticles from silver nitrate to silver nanoparticles started from the reduction of Ag^+ to Ag^0 and to silver nanoparticles. The bacterial metabolic pathway was able to use nitrate as a source of nitrogen where nitrate was converted to nitrite via the enzyme nitrate reductase, causing the reduction from Ag^+ to silver nanoparticles (Lee and Jun, 2019). The silver nanoparticles were maintained in colloid form to maintain the stability of the nanoparticles.

Table 1. Isolates with the positive indication of silver nanoparticles synthesis.

No	Sample	Location	Sediment
1.	AQ5-NT1	Pantai Dickson, Port Dickson 2.424234, 101.886885	Soil
2.	AQ5-NT2	Pantai Dickson, Port Dickson 2.424234, 101.886885	Soil
3.	AQ5-NT3	Pantai Dickson, Port Dickson 2.424234, 101.886885	Soil
4.	AQ5-NT4	Pantai Dickson, Port Dickson 2.424234, 101.886885	Soil
5.	AQ5-NT5	Pantai Dickson, Port Dickson 2.424234, 101.886885	Soil
6.	AQ5-NT6	Pantai Dickson, Port Dickson 2.424234, 101.886885	Soil
7.	AQ5-NT7	Pantai Dickson, Port Dickson 2.424234, 101.886885	Soil
8.	AQ5-NT8	Pantai Dickson, Port Dickson 2.424234, 101.886885	Soil
9.	AQ5-NT9	Pantai Dickson, Port Dickson 2.424234, 101.886885	Soil
10.	AQ5-NT10	Pantai Cermin, Port Dickson 2.4145012, 101.85877149999999	Soil
11.	AQ5-NT11	Pantai Cermin, Port Dickson 2.4145012, 101.85877149999999	Soil
12.	AQ5-NT12	Pantai Cermin, Port Dickson 2.4145012, 101.85877149999999	Soil
13.	AQ5-NT13	Pantai Cermin, Port Dickson 2.4145012, 101.85877149999999	Soil
14.	AQ5-NT14	Pantai Cermin, Port Dickson 2.4145012, 101.85877149999999	Soil
15.	AQ5-NT15	Pantai Cermin, Port Dickson 2.4145012, 101.85877149999999	Soil
16.	AQ5-NT16	Pantai Cermin, Port Dickson 2.4145012, 101.85877149999999	Soil
17.	AQ5-NT17	Pantai Purnama, Port Dickson 2.4436069, 101.85590430000002	Soil
18.	AQ5-NT18	Pantai Purnama, Port Dickson 2.4436069, 101.85590430000002	Soil
19.	AQ5-NT19	Pantai Purnama, Port Dickson 2.4436069, 101.85590430000002	Soil
20.	AQ5-NT20	Pantai Purnama, Port Dickson 2.4436069, 101.85590430000002	Soil

Colloid form ensures stability and monodispersity of the nanoparticles and prevents the nanoparticles from agglomerating (Sánchez-Juárez *et al*, 2019).

3.3 Identification of positive bacterial isolates

Fig. 2 shows the phylogenetic tree generated using the neighbour-joining method where a species of *Bacillus* was used as an outgroup. The analysis of the phylogenetic trees showed a close relation to the genus

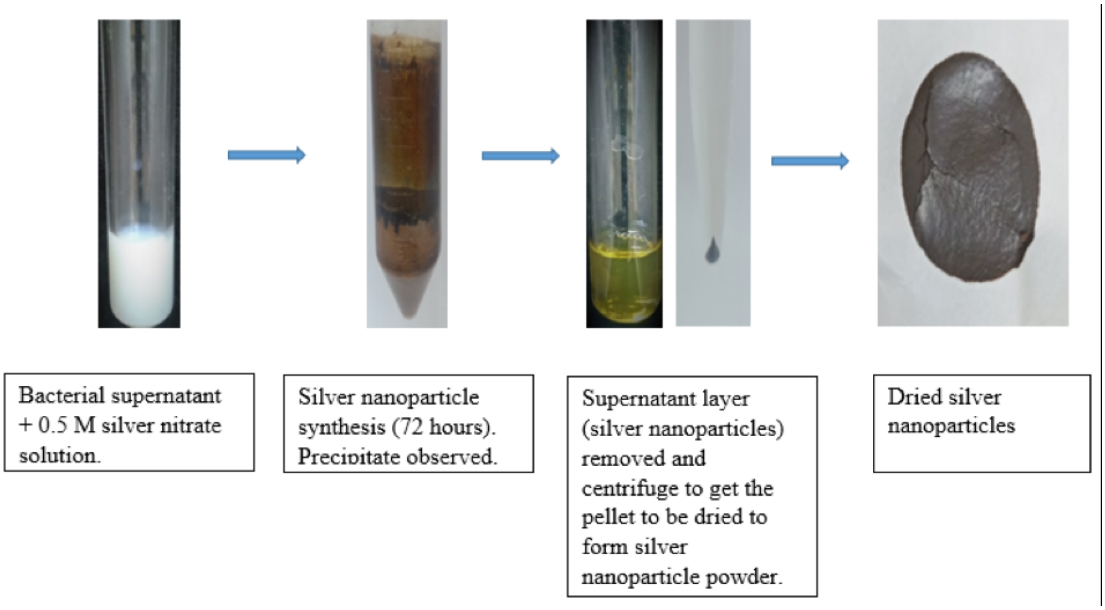
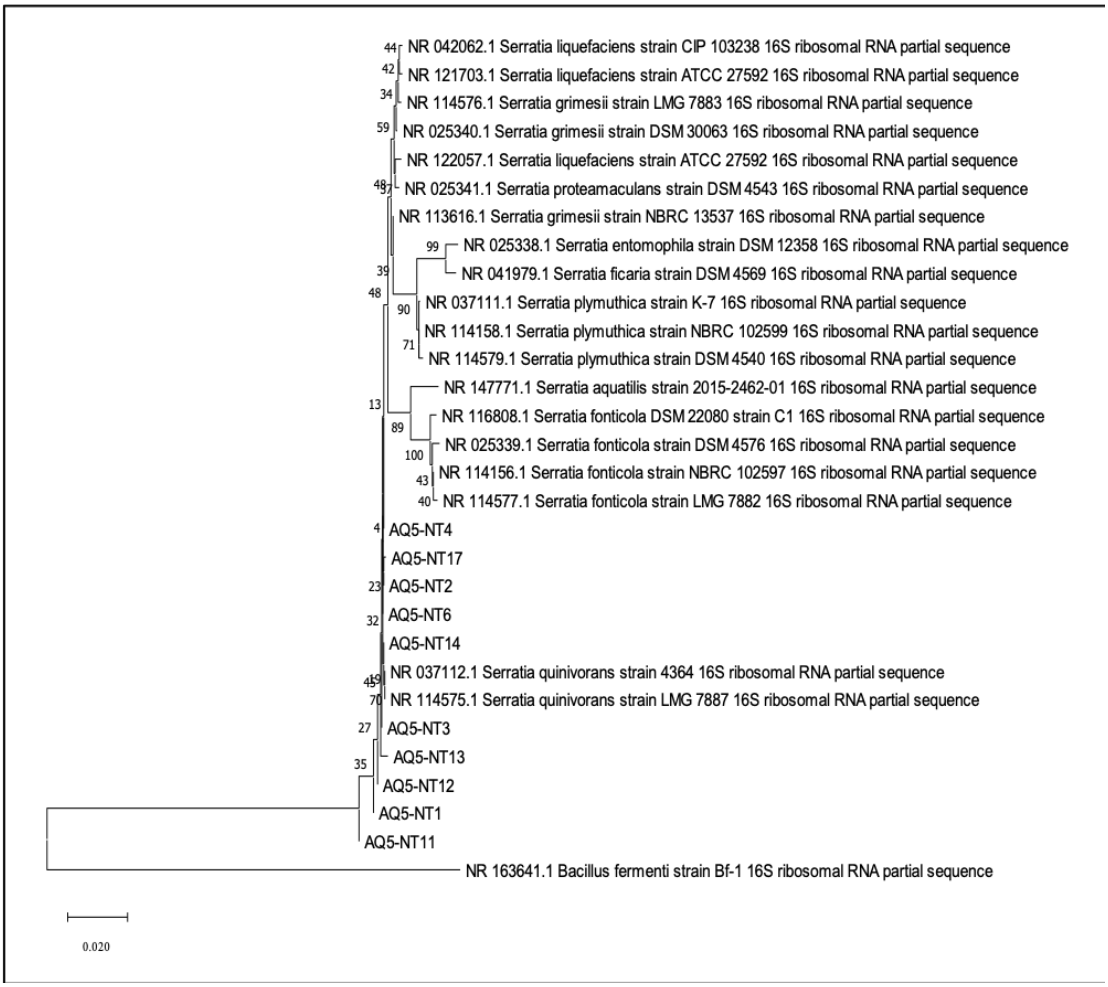


Fig 1: Colour change as a result of the conversion of silver nanoparticles from silver nitrate + bacterial supernatant.



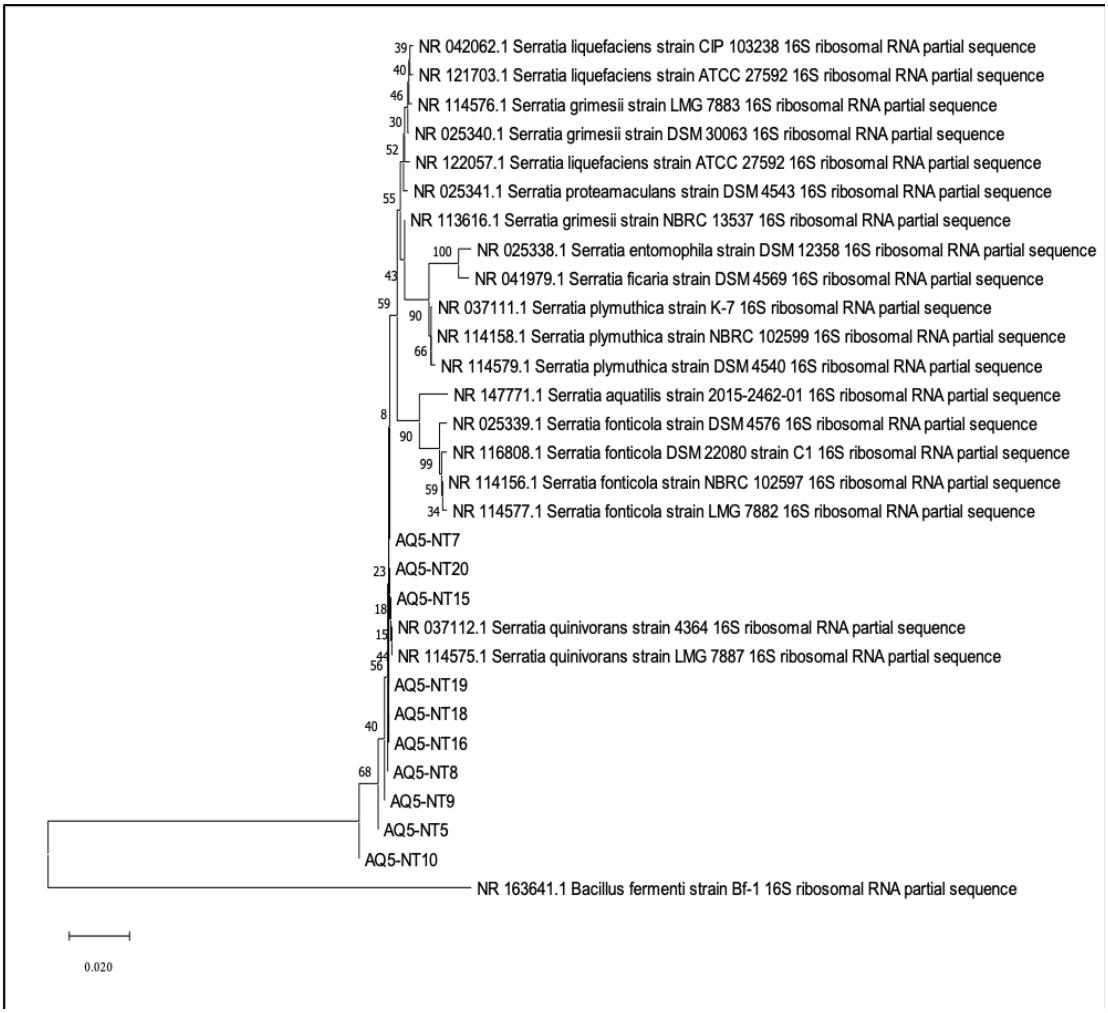


Fig 2: Phylogenetic tree. A) Shows the phylogenetic tree for samples AQ5-NT1, AQ5-NT2, AQ5-NT3, AQ5-NT4, AQ5-NT6, AQ5-NT11, AQ5-NT12, AQ5-NT13, AQ5-NT14 and AQ5-NT17 based on percent homology to with their nearest neighbours *Serratia quinivorans* and *Serratia grimesii*. B) Shows the phylogenetic tree for samples AQ5-NT5, AQ5-NT7, AQ5-NT8, AQ5-NT9, AQ5-NT10, AQ5-NT15, AQ5-NT16, AQ5-NT18, AQ5-NT19 and AQ5-NT20 based on percent homology to with their nearest neighbours *Serratia quinivorans* and *Serratia liquefaciens*.

Serratia. Based on 16S rRNA sequences, isolates in Fig 2A: AQ5-NT1, AQ5-NT2, AQ5-NT3, AQ5-NT4, AQ5-NT6, AQ5-NT11, AQ5-NT12, AQ5-NT13, AQ5-NT14 and AQ5-NT17 showed a 97% homology with their nearest neighbours *Serratia quinivorans* and *Serratia grimesii*. Isolates in Fig 2B: AQ5-NT, AQ5-NT7, AQ5-NT8, AQ5-NT 9, AQ5-NT10, AQ5-NT15, AQ5-NT16, AQ5-NT18, AQ5-NT19 and AQ5-NT20 showed a 97% homology with their nearest neighbours *Serratia quinivorans* and *Serratia liquefaciens*. *S. quinivorans*, *S. grimesii* and *S. liquefaciens* were Gram negative and rod-shaped bacteria with red colour pigment prodigiosin when grown on McConkey agar, which were the characteristics of the *Serratia* genus. Based on Table 2, the isolates AQ5-NT1, AQ5-NT2, AQ5-NT3, AQ5-NT4, AQ5-NT5, AQ5-NT6, AQ5-NT7, AQ5-NT8, AQ5-NT9, AQ5-NT10, AQ5-NT11, AQ5-NT12, AQ5-NT13, AQ5-NT14, AQ5-NT15, AQ5-NT16, AQ5-NT17, AQ5-NT18, AQ5-NT19 and AQ5-NT20 were all Gram-negative rod-shaped bacteria, demonstrating a positive catalase and negative oxidase result. Furthermore, Ashelford *et al*. (2002) reported that *S. quinivorans*, *S. grimesii* and *S. liquefaciens* were some of the many *Serratia* sp.

Table 2. Identification of isolated bacteria.

No.	Sample	Gram Staining	16sRNA	Gene bank
1.	AQ5-NT1	Negative	*Serratia* sp.	MN726448
2.	AQ5-NT2	Negative	*Serratia* sp.	MN726449
3.	AQ5-NT3	Negative	*Serratia* sp.	MN726450
4.	AQ5-NT4	Negative	*Serratia* sp.	MN726451
5.	AQ5-NT5	Negative	*Serratia* sp.	MN726452
6.	AQ5-NT6	Negative	*Serratia* sp.	MN726453
7.	AQ5-NT7	Negative	*Serratia* sp.	MN726466
8.	AQ5-NT8	Negative	*Serratia* sp.	MN726467
9.	AQ5-NT9	Negative	*Serratia* sp.	MN726469
10.	AQ5-NT10	Negative	*Serratia* sp.	MN726493
11.	AQ5-NT11	Negative	*Serratia* sp.	MN726470
12.	AQ5-NT12	Negative	*Serratia* sp.	MN726474
13.	AQ5-NT13	Negative	*Serratia* sp.	MN726475
14.	AQ5-NT14	Negative	*Serratia* sp.	MN726477
15.	AQ5-NT15	Negative	*Serratia* sp.	MN726478
16.	AQ5-NT16	Negative	*Serratia* sp.	MN726480
17.	AQ5-NT17	Negative	*Serratia* sp.	MN726486
18.	AQ5-NT18	Negative	*Serratia* sp.	MN726488
19.	AQ5-NT19	Negative	*Serratia* sp.	MN726489
20.	AQ5-NT20	Negative	*Serratia* sp.	MN726490

bacteria which were isolated from soil, while and the isolates AQ5-NT1, AQ5-NT2, AQ5-NT3, AQ5-NT4, AQ5-NT5, AQ5-NT6, AQ5-NT7, AQ5-NT8, AQ5-NT9, AQ5-NT10, AQ5-NT11, AQ5-NT12, AQ5-NT13, AQ5-NT14, AQ5-NT15, AQ5-NT16, AQ5-NT17, AQ5-NT18, AQ5-NT19 and AQ5-NT20 were all isolated from marine soil sediments.

Silver nanoparticles have been synthesised using strains of *Serratia* including *Serratia marcescens* and *Serratia nematodiphila*. Lakshmipathy and Nanda (2013) were successfully synthesised silver nanoparticles using nitrate reductase screened and isolated from *S. marcescens* S01. According to El-Batal *et al.* (2016), using strains of *S. marcescens,* successfully synthesised silver nanoparticles with the average size of 11.5 nm. Rajeshkumar *et al.* (2013), using *S. nematodiphila,* synthesised silver nanoparticles between the ranges of 10 - 31 nm. Rajeshkumar and Malarkodi (2017) used response surface methodology to optimise *S. nematodiphila* for producing silver nanoparticles in large scale for wider applications. However, there were no papers found on the synthesis of silver nanoparticles using *S. quinivorans*, *S. grimesii* and *S. liquefaciens*.

Based on Fig. 3, the UV-Visible spectra analysis indicated the presence of a mixture of size and shape present in each of the 20 positive silver nanoparticle colloidal sample. Fig. 3A shows that for samples AQ5-NT1, AQ5-NT2 and AQ5-NT5, a peak of 400 nm was observed indicating the presence of small and fine shaped nanoparticles, whereas for sample AQ5-NT3, a peak at 440 nm was observed indicating the presence of large nanoparticles. For sample AQ5-NT4, a peak at the wavelength 380 nm was observed indicating small and fine nanoparticles present in the colloid. Fig. 3B shows peaks at the wavelength of 400 nm for sample AQ5-NT8 and AQ5-NT9 indicating small and fine nanoparticles and peaks at the wavelength of 420 nm for samples AQ5-NT6 and AQ5-NT10 indicating the presence of large nanoparticles. Sharp peaks at both blue and red wavelengths were observed for sample AQ5-NT7 at the wavelengths of 380 nm and 440 nm indicating the presence of small and fine nanoparticles as well as large nanoparticles. Fig. 3C shows peaks at the wavelength of 420 nm for sample AQ5-NT14 and 440 nm for samples AQ5-NT12 and AQ5-NT13 indicating the presence of large nanoparticles in the colloidal solution of the samples. Whereas peaks at blue and red wavelengths of 340 nm and 420 nm as well as 380 nm and 420 nm were observed for samples AQ5-NT11 and AQ5-NT15, respectively, indicating the presence of a mixture of size and shape in the colloid solutions. Fig. 3D shows peaks at wavelengths of 400 nm for sample AQ5-NT17, 420 nm for samples AQ5-NT 16 and AQ5-NT18 and 440 nm for samples AQ5-NT19 and AQ5-NT20 indicating the presence of

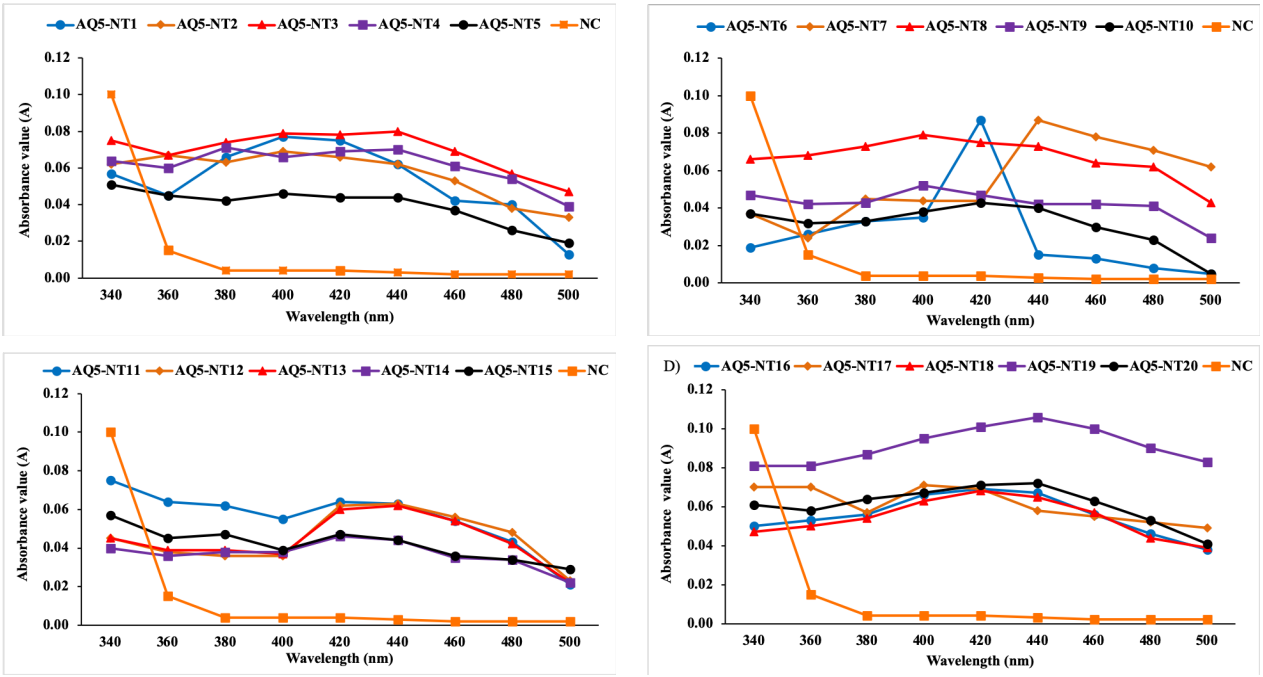


Fig. 3: UV-Visible spectra of silver nanoparticles (72 hours). A) AQ5-NT1, AQ5-NT2, AQ5-NT3, AQ5-NT4 and AQ5-NT5. B) AQ5-NT6, AQ5-NT7, AQ5-NT8, AQ5-NT9 and AQ5-NT10. C) AQ5-NT11, AQ5-NT12, AQ5-NT13, AQ5-NT14 and AQ5-NT15. D) AQ5-NT16, AQ5-NT17, AQ5-NT18, AQ5-NT19 and AQ5-NT20. (NC: Negative control).

large nanoparticles. Suber *et al.* (2005), reported that nanoparticles of spherical shape exhibit a maximum absorption spectrum between 420 nm to 440 nm with 420 nm being the optimum peak. Samples AQ5-NT3, AQ5-NT7, AQ5-NT12, AQ5-NT13, AQ5-NT19 and AQ5-NT20 indicated the presence of stable spherical nanoparticles at peaks of 440 nm and samples AQ5-NT6, AQ5-NT10, AQ5-14, AQ5-NT15, AQ5-NT16 and AQ5-NT18 indicating the presence of stable spherical nanoparticles at the optimum peak of 420 nm with the most potent sample being AQ5-NT6, thus proving that the *Serratia* strains used to synthesise the silver nanoparticles are good nano factories.

Fig. 4 shows a comparative analysis of peaks at 0 hours, 72 hours and 96 hours of synthesis for samples AQ5-NT3, AQ5-NT6, AQ5-NT8, AQ5-NT19 and AQ5-NT20 respectively, these sample showed the best peaks based on absorbance reading from Fig 3. Fig. 4 shows that at 0 hours a very low absorbance reading was recorded indicating the presence of silver nitrate and not silver nanoparticles. At 96 hours, there was a reduction in absorbance readings as compared to absorbance reading of Fig. 3 indicating the presence of larger sized nanoparticles. As time on incubation goes on the nanoparticles become larger due to large surface tension energy which cause a greater cohesion force causing interactions between nanoparticles thus forming clusters of nanoparticles (Badi'ah *et al.,* 2019).

The UV-Visible Spectra analysis indicates the presence of silver nanoparticles. Aslan *et al.* (2005) reported that the presence of silver nanoparticles can be indicated with the absorption spectrum ranging from 350 nm to 500 nm. The peaks or plasmon absorption maximum can be utilised to indicate size and shape of the nanoparticles ranging between 1 nm to 100 nm in size and shapes such as circular, triangular and rods. Generally, smaller nanoparticles peak at wavelengths of 350 nm to 400 nm, whereas larger nanoparticles peak at 410 nm to 500 nm. An increase in size of nanoparticles can cause a red shift, whereas a decrease in size causes a blue shift (Pillai and Kamat, 2004; Peng *et al.,* 2010). This plasmon absorption maximum gives a preliminary indication on the presence of either a mixture of size and shape of nanoparticles or a specific size and shape of nanoparticles in a particular solution.

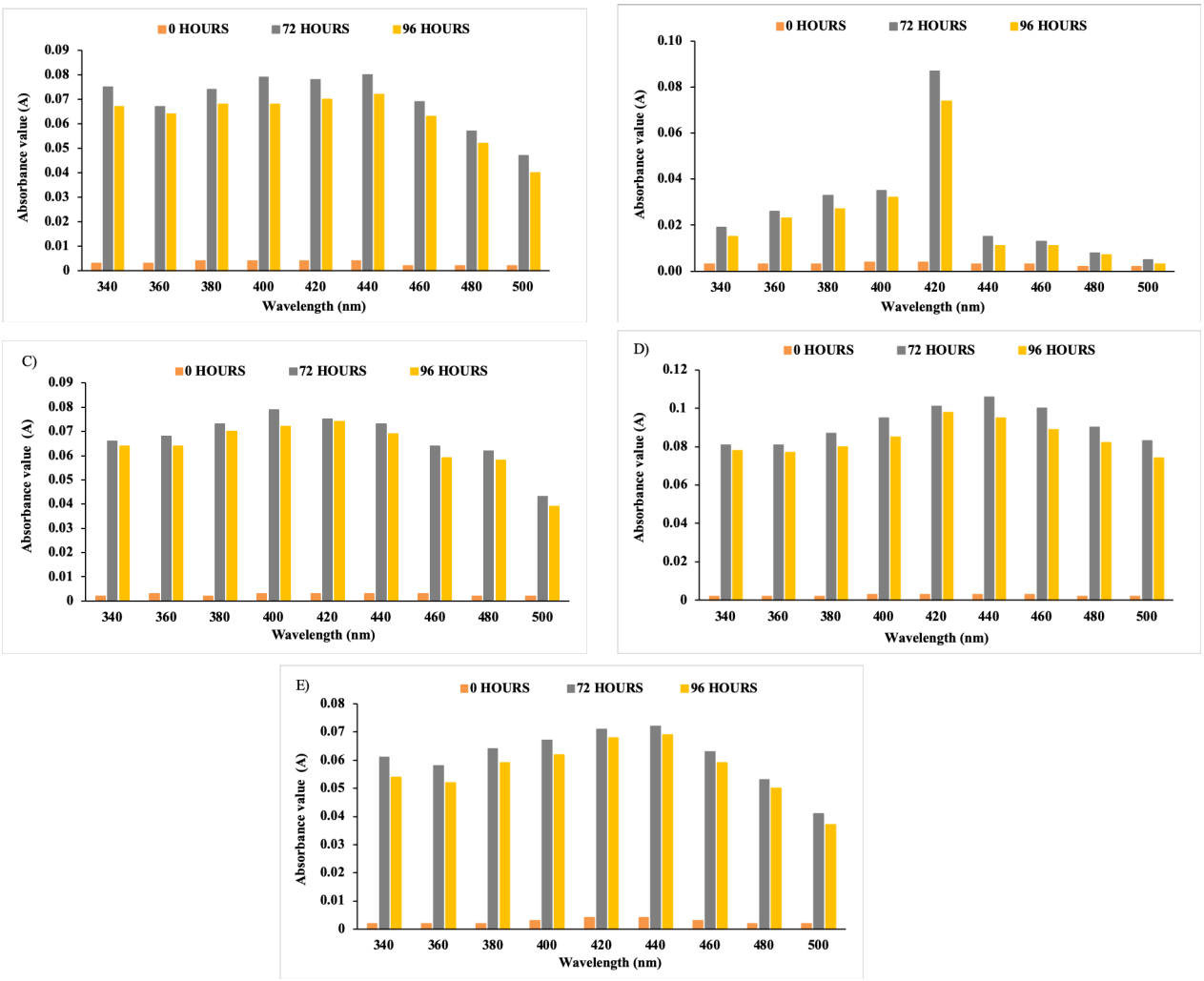


Fig. 4: Comparison of UV-Visible spectra of silver nanoparticles for samples AQ5-NT3, AQ5-NT6, AQ5-NT8, AQ5-NT19 and AQ5-NT20 at 0 hours, 72 hours and 96 hours respectively. A) AQ5-NT3, B) AQ5-NT6, C) AQ5-NT8, D) AQ5-NT19 and E) AQ5-NT20.

Conclusions

Twenty positive marine isolates have been screened and positively able to synthesise silver nanoparticles. 16s rRNA sequencing has been carried out and the 20 isolates were identified to belong to the genus Serratia. UV-Visible spectra analysis showed that all 20 isolates consist of silver nanoparticles of varied size and shape with the most potent being AQ5-NT6, which has a blue shift and the sharpest plasmon absorption peak at 420 nm indicating the presence of stable spherical nanoparticles at an optimum size. However, further analysis using microscopic analysis such as transmission electron microscopy (TEM) and scanning electron microscopy (SEM), dynamic light scattering (DLS) and Fourier-transform infrared spectroscopy (FTIR) need to be carried out to know the exact size and shape of the nanoparticles and components of the colloid solution as UV-Visible Spectra analysis only provided preliminary indications.

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