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Antibacterial Potency of Extracellular Silver Nanoparticles Synthesized By *Bacillus* Species on Enteric Bacteria Isolated From Some Water Samples

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Abstract
Aim and Objectives: *Human diseases are usually treated with antibiotics but most microorganisms have developed resistance to chemically synthesized antibiotics necessitating the use of alternatives such as biologically synthesized silver nanoparticles (AgNPs). This study examined antibacterial potency of extracellular AgNPs synthesized by Bacillus species on enteric bacteria isolated from some water samples from Onyearugbulem market, Akure.*
Methodology: *Isolation of enteric bacteria and Bacillus species was carried out according to standard microbiological procedures. Isolates were identified using biochemical tests and molecular analysis. Silver nanoparticles were synthesized from Bacillus spp broth. Antibacterial assay was carried out on some enteric bacteria and antibiotic sensitivity discs containing 10 antibiotics was used as positive control.*
Results: *Escherichia coli, Shigella flexneri, and Salmonella enterica were isolated from waste water sample and Escherichia coli, Shigella flexneri, Sh. sonnei and Salmonella enterica from well water sample. Six Bacillus species were obtained from the rhizosphere soils of Amaranthus hybridus L, Musa paradisiaca L., Manihot esculenta Crantz and Zea mays L. Aqueous silver ions were reduced to extracellular AgNPs when added to all the Bacillus species separately. The UV-visible spectroscopy results showed that the absorption bands were observed for all the biosynthesized nanoparticles near UV region. Bacillus species from Zea mays 1 (BSZM1) had the highest wavelength. The Fourier Transmission Infra-Red (FT-IR) spectra of nanoparticle from BSAH2 was observed at 1651/cm and 3300/cm. Nanoparticles showed antibacterial activities against all isolated enteric bacteria with zones of inhibition from 1.33 ±2.05^{e}mm to 36.33±1.26^{a}mm. Antibacterial assay of nanoparticle from BSZM2 was significantly higher than others at $P \leq 0.05$.*
Conclusion: *The test bacteria showed varied significant susceptibility to different antibiotics with zones of inhibition ranging from 12.00 ±2.00^{e} to 35.33±3.21^{a} mm. Synthesized nanoparticles can be used as antibiotics since they compared favorably with the commercial antibiotics used in this investigation.*

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I. Introduction

Human diseases are usually treated with antibiotics. However, most microorganisms have developed resistance to chemically synthesized antibiotics so there is the need to develop alternatives. Nanoparticles have good antibacterial properties due to their large surface area to volume ratio since multiple resistance of microorganisms to various antibiotics are on the increase[1]. Among the several promising nanomaterials, silver nano particles (AgNPs) are good potential antibacterial agents due to their large surface-to-volume ratios and crystallographic surface structure. They can be used as therapeutic agents against many pathogenic microorganisms since they possess antibacterial, antifungal, anti-inflammatory, anti-viral, anti-angiogenic and anti-cancer properties [2] and therefore, used in the development of new pharmaceutical products [3]. They have become one of the most commonly used nanomaterials in consumer products having enhanced chemical and physical properties as compared with normal silver metal [4] demonstrating better antibacterial, antifungal and antiviral [5] properties in comparison with metallic silver and various silver compounds. There is the need to develop alternatives for antibiotics as some pathogenic microorganisms have become resistant to it. *Bacillus* species have a unique ability to replicate rapidly, resistant to adverse environmental conditions as well as have broad spectrum of biocontrol ability [6]. This study aims to use cell free supernatant of *Bacillus* species obtained from the rhizosphere of some plants for the synthesis of silver nanoparticles (AgNPs) and use them as antibacterial agents on enteric bacteria.

II. Materials and Methods

Collection of samples: Well water and waste water samples for the isolation of enteric bacteria were collected in labeled sterile bottles from Onyearugbulem market, Akure, Ondo State in January, 2018. *Bacillus* species were isolated from rhizosphere soil samples of some plants; *Zea mays* L., *Amaranthus hybridus* L., *Musa paradisiaca* L. and *Manihot esculenta* Crantz, collected from Afe Babalola University, Ado-Ekiti (ABUAD) farm in October, 2017 and used for the synthesis of nanoparticles. The samples were collected by manually uprooting the selected plants. *Escherichia coli* and *Proteus vulgaris* were collected from Microbiology Unit, Department of Biological Sciences, ABUAD. The whole experiments were conducted between October 2017 and April 2018.

Isolation of enteric bacteria from water samples: Each water sample (10 mL) was serially diluted with sterile water (90 mL). The serially diluted samples were inoculated separately on Nutrient agar, Eosin Methylene Blue agar, Salmonella Shigella agar and MacConkey agar plates for 24 hours at 37^{o}C using pour plate technique [7]. The colonies obtained were further subcultured on nutrient agar plates until pure cultures were obtained. They were then stored at 4^{o}C in the refrigerator until use.

Isolation of *Bacillus* species from the rhizosphere of selected plants: Each soil sample (1g) collected from the rhizosphere of *Zea mays* L, *Amaranthus hybridus* L, *Musa paradisiaca* L, and *Manihot esculenta* Crantz was weighed and serially diluted with 9 mL sterile water until the seventh fold. An aliquot (1 mL) of each serially diluted sample was inoculated on plate count agar and nutrient agar using pour plate technique. The plates were incubated at 37^{o}C for 24 to 48 hours. Colonies were subcultured on nutrient agar until pure cultures were obtained and stored at 4^{o}C in the refrigerator until use according to the method adopted by[8] . Gram staining was carried out using the method of [9] and some biochemical tests were carried out using the methods of [10], [7].

Molecular identification of the bacterial isolates

Extraction of genomic DNA: The genomic DNA was isolated by transferring 1.5 mLof each bacterial culture from well water, waste water and soil samples to a microcentrifuge tube and centrifuged for 2 min. Then the supernatant was discarded and the pellet was re-suspended in 467 µL TE buffer by repeated pipetting, to it were added 30 µL of 10% SDS and 3µL of 20 mg/mL proteinase K and incubated for 1 hr at 37°C. After incubation, an equal volume of chloroform was added and mixed well by inverting the tube until the phases are completely mixed. The DNA/phenol mixture was carefully transferred into a fresh tube and centrifuged for 2 min. The upper aqueous phase was transferred into a new tube. An equal volume of chloroform was added again mixed well and transferred to a new tube and centrifuged for 2 min. The upper aqueous phase was transferred to a new tube. Thereafter, 0.1mLof sodium acetate and 0.6 mL of isopropanol was added and mixed gently until the DNA precipitates. The DNA was spooled onto a glass rod and washed by dipping the end of the rod into 1 mLof 70% ethanol for 30 sec after which it was resuspended in 100-200 µL TE buffer [11].

PCR amplification of 16S rRNA: The PCR amplification of 16S rRNA gene from the purified genomic DNA was carried out using the universal bacterial primer. The reaction conditions were as follows: initial denaturation at 94°C for 4 min, 40 amplification cycles of denaturation at 94°C for 1 min, annealing at 48°C for 1 min and primer extension at 72°C for 3 min; followed by a final extension at 72°C for 10 min. Aliquots of the PCR products (5 µL) were analyzed in 1% (w/v) agarose gels by horizontal gel electrophoresis. The DNAs were visualized by UV excitation after staining with 0.5 mg/L ethidium bromide [11].

Purification of PCR product: The unpurified DNA (15µL) of each sample was dissolved in 50µL of PCR cleanup solution and incubated at 55ºC for 15-20 minutes. The mixture was centrifuged at 12, 000 rpm for 15 minutes, during which time the contaminants were released into the supernatant and the supernatant discarded at the end of the centrifugation. Furthermore, the DNA was precipitated by the addition of 600 µL of 80% ethanol and centrifuged at the same conditions as before. The residual cleanup solution and the contaminants were removed along with ethanol by discarding the supernatant. Finally, the DNA pellet was dried and dissolved in 10- 15µLof Milli Q water [11].

Sequencing of target genes from the bacterial isolates: The sequencing of the target gene was done using ABI-Big Dye Terminator v3.1 Cycle Sequencing Kit. The tubes were placed in the thermo cycler which was programmed as follows: 25 cycles of 96^{o}C for 10 sec, 50^{o}C for 5-10 sec, 60^{o}C for 4 min and then ramped to 4^{o}C [11].

Purification of sequencing extension product: The content of the tube was spinned and transferred by pipetting entire sequencing reactions into 1.5 mL micro centrifuge tube. 40 mL of 75% ethanol was added and

mixed by vortexing and left at room temperature for 15 mins to precipitate products. The tube was centrifuged for a minimum of 20 min at maximum speed in a micro centrifuge. The supernatant was aspirated completely with a separate pipette tip for each sample, being careful not to disturb the DNA pellet, and then it was discarded. About 125 mL of 75 % ethanol was added to the tube and vortexed briefly and centrifuged for 5 min at maximum speed, and the supernatant was aspirated as in above step. The sample was dried for 10 - 15 minutes and stored at -20^{o}C until ready for electrophoresis. The purified extension products were separated in the ABI 3730xl DNA Analyzer by capillary electrophoresis. The resulting nucleotide sequences were analysed using BLAST (Basic Local Alignment Search Tool) at NCBI (National Center for Biotechnology Information) site. The multiple alignment of the related reference sequences from BLAST was performed using ClustalW after which the phylogenetic tree was constructed on molecular evolutionary genetic analysis (MEGA 6) software package [11].

Synthesis of silver nanoparticles from *Bacillus* species: *Bacillus* species obtained from the rhizosphere of *Zea mays* L, *Amaranthus hybridus* L, *Musa paradisiaca* L. and *Manihot esculenta* Crantz was inoculated into different 250 mL Erlenmeyer flask containing 100 mL sterile nutrient broth. The cultured flasks were incubated in a rotating shaker (KL2) at 200 rpm for 48 h at 28^{o}C. Thereafter, each of the cultures was centrifuged at 12,000 rpm for 10 min. The biomass and supernatant were separated and the supernatant used for extracellular production of silver nanoparticles by mixing it with filter-sterilized 1 mM $AgNO_3$ solution. All the reaction mixtures were incubated on rotating shaker KL2 (200 rpm) at 28 ±2^{o}C for a period of 72 h. Visual observation was conducted periodically to check for the nanoparticle formation. Primary confirmation of the synthesized nanoparticles was carried out by UV-visible (Model 1800) spectroscopy in the range of 200-800nm. Also, the chemical composition of the nanoparticles formed were characterized using Fourier Transform Infra- Red (FT-IR) (4100 Exo Scan) spectroscopy [12].

Standardization of inoculum: A loopful of each test organisms (enteric bacteria) was aseptically inoculated onto sterile nutrient broth in separate flasks and incubated for 24 hours. 0.2 mL from the 24 hour culture was dispensed into 20 mL sterile nutrient broth and incubated for 3-5 hours to standardize the culture to 0.5 McFarland standard (10^6cfu/mL) before use according to the method of [13].

Antibacterial activities of the synthesized silver nanoparticles and commercial antibiotics: The antibacterial activities of the AgNPs were examined according to the Kirby Bauer disk diffusion method against enteric bacteria obtained from water samples as well as the isolates obtained from ABUAD [14]. The standardized broth culture of each test bacterium was swabbed evenly onto sterile Mueller-Hinton agar plates. Four wells were bored on agar plate using a sterile 10 mm cork borer and different concentrations (0.2, 0.4 and 0.8mL) of the synthesized AgNPs solution was pipetted onto each well while silver nitrate was (0.8 mL) put in the 4th hole which served as negative control. The plates were incubated at 37ºC for 24 hours after the inhibition zone was measured with a metre rule by subtracting the diameter of the cork borer from the actual clear zones of inhibition.

For positive control, commercial antibiotic discs were impregnated on Mueller Hinton agar plates containing each test bacterium. The plates were incubated at 37°C for 24 h. The inhibition zone around each disc was observed and measured with a metre rule in mm. The zones of inhibition were classified into susceptible (17.00 mm and above), intermediate (13.00 -17.00 mm) and resistant (0-12 mm) following the protocol of [14].

Data analysis of results: The data collected were subjected to one - way Analysis of variance (ANOVA) and the means were separated by Duncan's New Multiple Range Multiple Test at $P \leq 0.05$ level of significance using SPSS version 20.0.

III. Results

Table no 1 shows bacteria obtained from both well water and waste water samples from Onyearugbulem market following the routine biochemical and morphological examinations. Three Gram negative bacilli were obtained from waste water sample and were identified as *Escherichia coli*, *Shigella flexneri* and *Salmonella enterica*. *Escherichia coli* and *Shigella flexneri* were methyl red, indole, and catalase positive but Vogue Proskauer and citrate negative. Also, the results of the sugar fermentation for *Escherichia coli* showed that it was positive for galactose, sucrose and glucose while *Shigella flexneri* was positive for all the sugars used. *Salmonella enterica* was catalase, indole, methyl red and citrate positive and VP negative.

Four Gram negative bacilli were isolated from the well water sample and were identified as *Escherichia coli*, *Shigella sonnei*, *Shigella flexneri* and *Salmonella enterica*. *Escherichia coli*, and *Shigella sonnei* were methyl red, indole, and catalase positive but Vogue Proskauer and citrate negative. *Escherichia*

coli, was positive for fermentation of lactose, sucrose and glucose while *Shigella sonnei* fermented fructose, lactose and glucose. *Shigella flexneri* was indole and methyl red positive but VP, citrate and catalase negative.

***Bacillus* species obtained from the rhizosphere of selected plants**

Table no 2 Shows six Gram positive bacilli were obtained from the rhizosphere of *Amaranthus hybridus*, *Musa paradisiaca*, *Manihot esculenta* and *Zea mays.* Two were obtained from the rhizosphere of *Amaranthus hybridus*. Both isolates were positive to starch hydrolysis, catalase, motility and spore staining tests. They were indole negative, oxidase negative methyl red and VP negative. *Bacillus* species AH1 produced only acid from glucose, galactose and maltose while both acid and gas were produced from fructose. However, it did not produce neither acid nor gas from lactose, mannitol, sucrose and sorbitol. For *Bacillus* species AH2, acid and gas were produced from galactose and fructose, only acid was produced by the organism from glucose, maltose and sucrose while neither acid not gas was produced from lactose, mannitol and sorbitol. Only one Gram positive rod was isolated from the rhizosphere of *Musa paradisiaca.* The bacterium was positive to starch hydrolysis, catalase, motility and spore staining tests while negative to indole oxidase, methyl red and VP.

Table no 1: Morphological and Biochemical Characteristics of Enteric Bacteria obtained from water and waste water samples in Akure

Isolate code	Gram/Shape	Catalase	Sugar fermentation					Indole	Methyl red	Voges Proskauer	Citrate	Probable isolate
			F	Ga	La	S	G					
WA	- rod	+	-	+	-	+	+	+	+	-	-	*Escherichia coli*
WA	- rod	+	+	+	+	+	+	+	+	-	-	*Shigella flexneri*
WA	- rod	+	+	+	-	+	-	+	+	-	+	*Shigella enterica*
WW	- rod	+	+	-	+	-	+	+	+	-	-	*Shigella sonnei*
WW	- rod	+	+	+	-	-	+	-	+	-	+	*Shigella enterica*
WW	- rod	-	+	-	+	+	+	+	+	-	-	*Shigella flexneri*
WW	- rod	+	+	-	+	+	+	+	+	-	-	*Escherichia coli*

Key:
WA: Waste water from Onyearugbulem market, Akure;
WW: Well water from Onyearugbulem market, Akure
+: Positive; -: Negative; F: Fructose, Ga: Galactose, L: Lactose; S: Sucrose; G: Glucose

Table no 2: *Bacillus* species obtained from the rhizosphere of some plants obtained from Commercial farm of Afe Babalola University

Isolate code	Colour	Surface	Edge	Elevation	Shape	Grama reaction	Catalase	Starch hydrolysis	Motility	pore stain	Indole	Methyl red	VP	Oxidase	Sugar Fermentation								Probable isolates
															Glucose	Lactose	Mannitol	Galactose	Fructose	Maltose	Sucrose	Sorbitol	
AH1	White	Rough	Rhizoid	Flat	Rod	+	+	+	+	+	-	-	-	-	A	-	-	A	AG	A	-	-	*Bacillus* sp
AH2	White	Rough	Rhizoid	Flat	Rod	+	+	+	+	+	-	-	-	-	A	-	-	AG	AG	A	A	-	*Bacillus* sp
MP	White	Rough	Rhizoid	Flat	Rod	+	+	+	+	+	-	-	-	-	A	-	-	AG	AG	A	A	-	*Bacillus* sp
ME	White	Rough	Rhizoid	Flat	Rod	+	+	+	+	+	-	-	-	-	A	-	-	A	AG	A	-	-	*Bacillus* sp
ZM1	White	Rough	Rhizoid	Flat	Rod	+	+	+	+	+	-	-	-	-	A	-	-	A	AG	A	-	-	*Bacillus* sp
ZM2	White	Rough	Rhizoid	Flat	Rod	+	+	+	+	+	-	-	-	-	A	-	-	AG	AG	A	A	-	*Bacillus* sp

Keys: -: negative, +: positive, A: Acid production only, AG: Acid and gas production
AH: *Amaranthus hybridus* L, MP: *Musa paradisiaca* L; ME: *Manihot esculenta* Crantz; ZM: *Zea mays* L

Table no 3 shows the quality of the purified PCR products. The highest nucleic acid concentration of 134.40µg/L was obtained from *Bacillus* species from *Amaranthus hybridus* 1(BSAH1) while the least nucleic acid concentration of 21.30µg/L was obtained from BSAH2.

Table no 4 shows the length of the nucleotide sequence and percentage similarities of all the isolates subjected to molecular characterization in order of arrangement on the thermo cycler. Among the enteric bacteria, the highest length of the nucleotide sequence was obtained from *Shigella flexneri* from well water (SFWW) and it had 92% similarity with *Shigella flexneri* on NCBI site after blasting. The length of nucleotide sequence of *Bacillus* species from *Amaranthus hybridus* 1(BSAH1) was 969 bp (base pair) with 90% similarity with different species of *Bacillus* on NCBI site. Also, the lengths of nucleotide sequence of species from *Amaranthus hybridus* 2 (BSAH2) and *Bacillus* species obtained from *Musa parasidiaca* (BSMP) were 957 and 884 with percentage similarities of 86% and 81% respectively. *Bacillus* species from *Manihot esculenta* (BSME) and *Bacillus* species from *Zea mays* 1 (BSZM1) showed lengths of 911 and 972 bp with percentage similarities of 97% and 86%.

Table no 3: Quality of purified PCR products of bacterial isolates obtained from water and soil samples

Isolate code	Nucleic acid concentration (µg/L)	A260/280
***Bacillus* species**		
BSAH1	134.40	1.43
BSAH2	21.30	1.65
BSMP	33.30	1.47
BSME	50.80	1.73
BSZM	44.30	1.51
Enteric bacteria from ABUAD		
ECMB	29.70	1.66
PVMB	29.70	1.51
Enteric bacteria from waste water		
SEWA	37.10	1.50
ECWA	56.80	1.53
SFWA	40.60	1.54
Enteric bacteria from well water		
SEWW	42.50	1.73
SFWW	36.00	1.72
SSWW	45.50	1.56

Key: BS:*Bacillus* species from rhizosphere of: AH: *Amaranthus hybridus* L, MB: *Musa paradisiaca* L; ME: *Manihot esculenta* Crantz; ZM: *Zea mays* L ECMB: *Escherichia coli* from Microbiology laboratory, ABUADPVMB: *Proteus vulgaris* from Microbiology laboratory, ABUAD SEWA: *Salmonella enterica*; ECWA: *E. coli* SFWA: *Shigella flexneri*. SEWW: *S. enterica*; SFWW: *Sh. sonnei* SFWW: *Sh. flexneri*; ECWW: *E. coli*

Table no 4: Comparison of isolated samples from rhizosphere of some plants and water samples with isolates from NCBI Gene Bank

Sample identity	Nucleotide sequence length	Highest Percentage similarity
BSAH1	969	90
BSAH2	957	86
BSMP	884	81
BSME	911	97
BSZM	972	86
ECMB	907	96
PVMB	890	83
SEWA	887	95
ECWA	931	86
SFWA	936	93
SEWW	909	96
SFWW	949	92
SSWW	919	95

Key: BS: *Bacillus* species from rhizosphere of: AH: *Amaranthus hybridus* L, MB: *Musa paradisiaca* L; ME: *Manihot esculenta* Crantz; ZM: *Zea mays* L ECMB: *Escherichia coli* from Microbiology laboratory, ABUAD PVMB: *Proteus vulgaris* from Microbiology laboratory, ABUAD SEWA: *Salmonella enterica*; ECWA: *E. coli* SFWA: *Shigella flexneri*. SEWW: *S. enterica*; SFWW: *Sh. sonnei* SFWW: *Sh. flexneri*; ECWW: *E. coli* Figure 1 showed the phylogenetic tree having 11 nucleotide sequences.

Figure nos 1-3 show the molecular characterization showed that BSAH1 resembled *Bacillus nakamurai* strain NRRL B-401091. BSMP was identified as *Bacillus wiedmannii* using based on the comparison with

sequences submitted in the GenBank Database by using neighbor joining method (Figure 1). The phylogenetic tree involves 4 nucleotide sequences. SSWW was identified as different strains of *Shigella sonnei* (Figure 2) and the phylogenetic tree involves 9 nucleotide sequences. SEWW was identified as *S. enterica* strain (Figure 3). The phylogenetic tree involves 11 nucleotide sequences

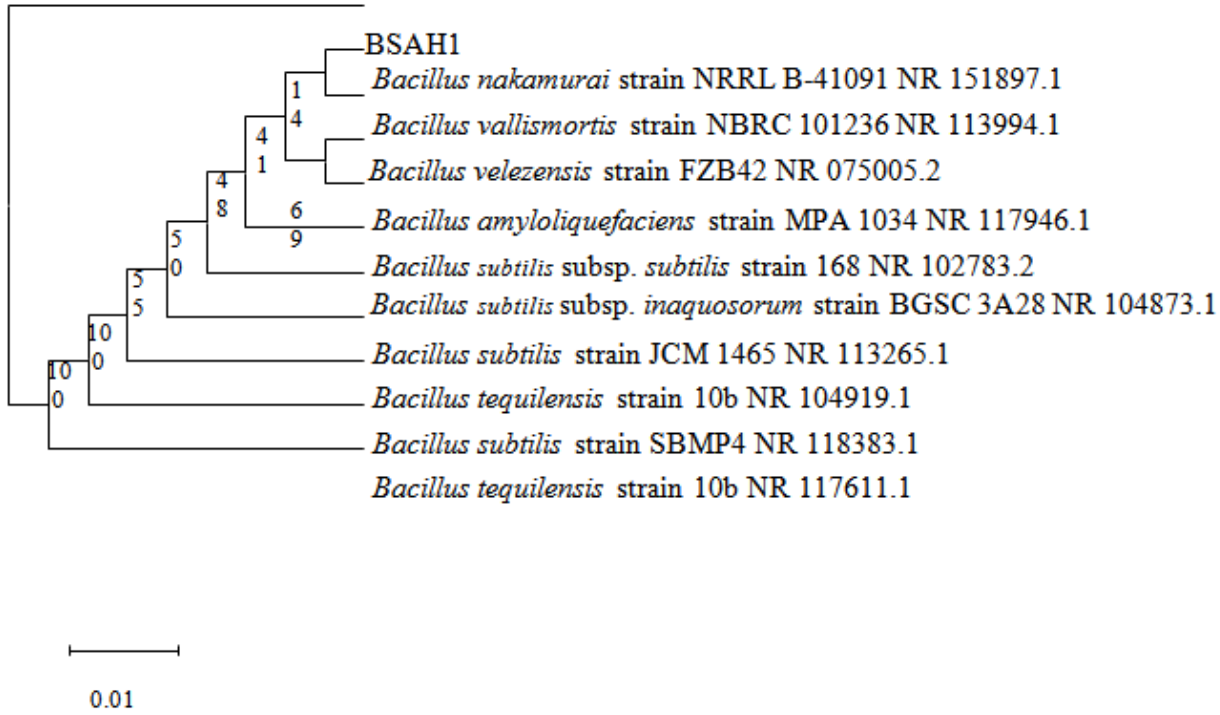

Figure no 1: Dendogram of phylogenetic tree showing the relationship of Bacillus species from Amanranthus hybridus 1(BSAH1) with selected closely related Bacillus species from NCBI Gene bank using the neighbor-joining method by Mega 6

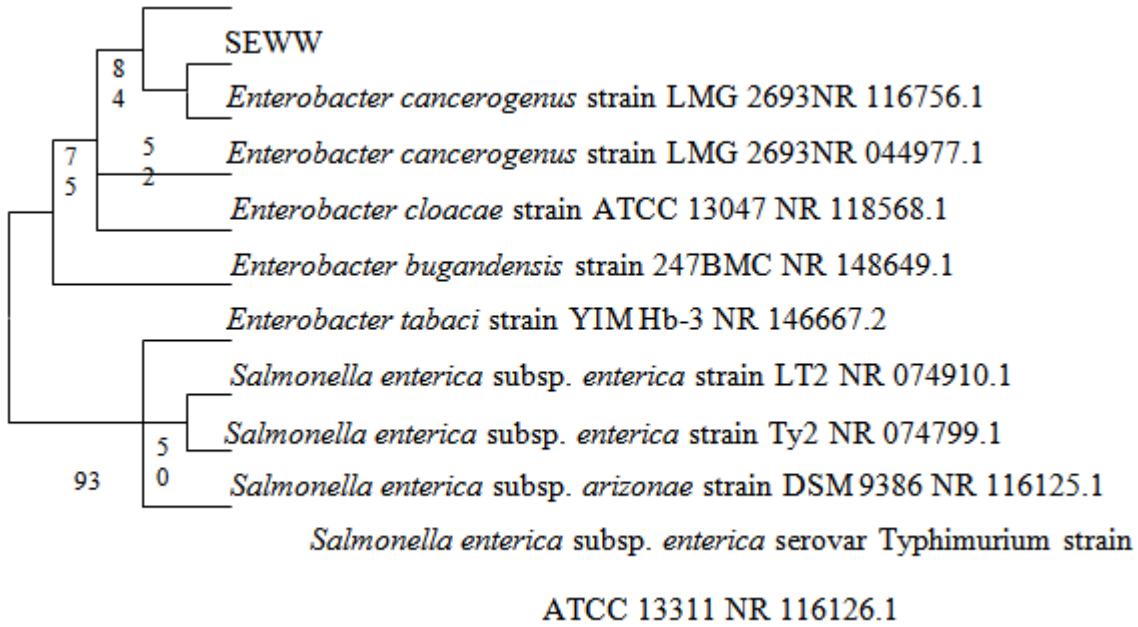

Figure no 2: Dendogram of phylogenetic tree showing the relationship of Salmonella enterica from well water (SEWW) with selected closely related enteric bacteria from NCBI Gene bank using the neighbor-joining method by Mega 6 software

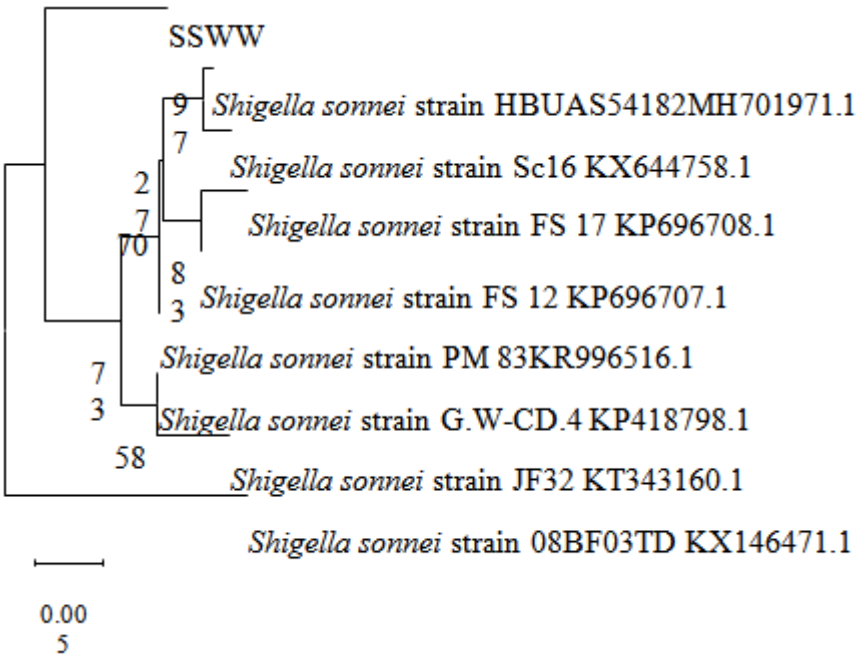

Figure no 3: Dendogram of phylogenetic tree showing the relationship of Shigella sonnei from well water (SSWW) with selected closely related Shigella sonnei from NCBI Gene bank using the neighbor-joining method by Mega 6 software

Nanoparticles synthesized from *Bacillus* species

Table no 5 shows reduction of aqueous silver ions to silver nanoparticles when added to all the *Bacillus* species isolated in separate flasks. This was indicated by the change in colour of the medium from yellow to brown after incubation. The UV-visible spectroscopy results showed that the absorption bands were observed for all the biosynthesized nanoparticles near UV region. The isolate with the highest wavelength 0f 293.50 was *Bacillus* species from *Zea mays* (BSZM1) while the least wavelength of 264.00 was recorded for *Bacillus* species from *A. hybridus* (BSAH2).

Table no 5: Wavelength and absorbance of colour formed during biosynthesis of nanoparticles by different species of *Bacillus*

Bacterial species	Maximum wavelength (ʎ) of each isolate	Absorbance (nm) at the highest wavelength
BSAH1	267.00	3.99
BSAH2	264.00	3.98
BSMP	273.50	4.00
BSME	267.50	3.98
BSZM1	293.5	3.93
BSZM2	265.00	3.99

Key: BS**:** *Bacillus* species from rhizosphere of: AH: *Amaranthus hybridus* L, MP: *Musa paradisiaca* L; ME: *Manihot esculenta* Crantz; ZM: *Zea mays* L

Figures nos 4-6 show the FT-IR spectra of some of the nanoparticles. *Bacillus* species from *M. paradisiaca* (BSMP) had the highest absorbance of 4.00 nm. The FT-IR spectra of nanoparticle from BSAH2 was observed at 1651/cm and 3300/cm which are assigned to bending vibrations of amides I of proteins and N-H stretching vibrations (Figure 4). The FTIR of nanoparticle from BSZM1 peaked at 1550/cm and 3350/cm which correspond to the bending vibrations of amides II of proteins and N-H stretching vibrations respectively (Figure 5). Nanoparticle from *Bacillus* species from *Z. mays* 2 (BSZM2) peaked at 1650/cm and 3400/cm which correspond bending vibrations of amides I of proteins and O-H stretch of carboxylic acids respectively (Figure 6).

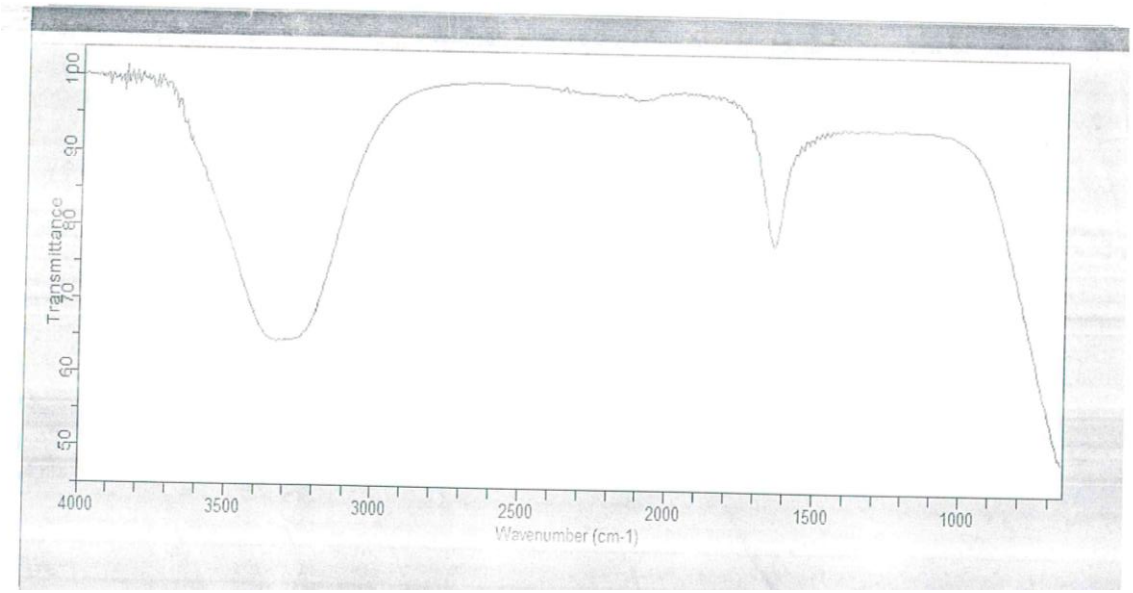

Figure no 4: FTIR spectra of nanoparticle from *Bacillus* species from *Amaranthus hybridus* (BSAH2)

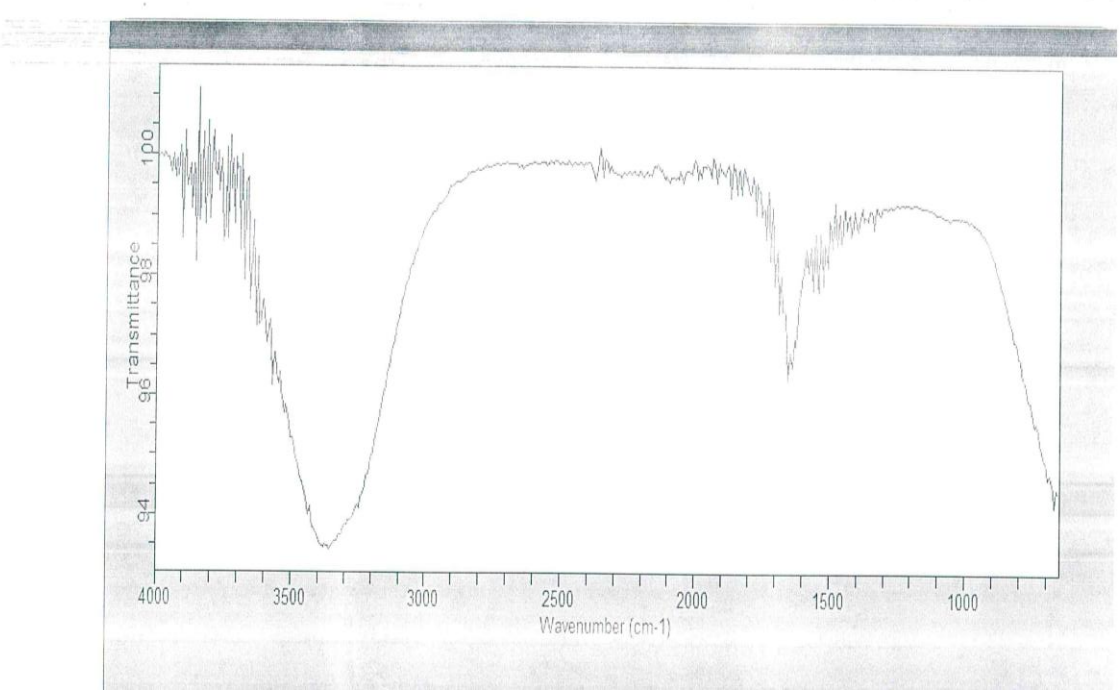

Figure no 5: FTIR spectra of nanoparticle of *Bacillus* species from *Zea mays* (BSZM1)

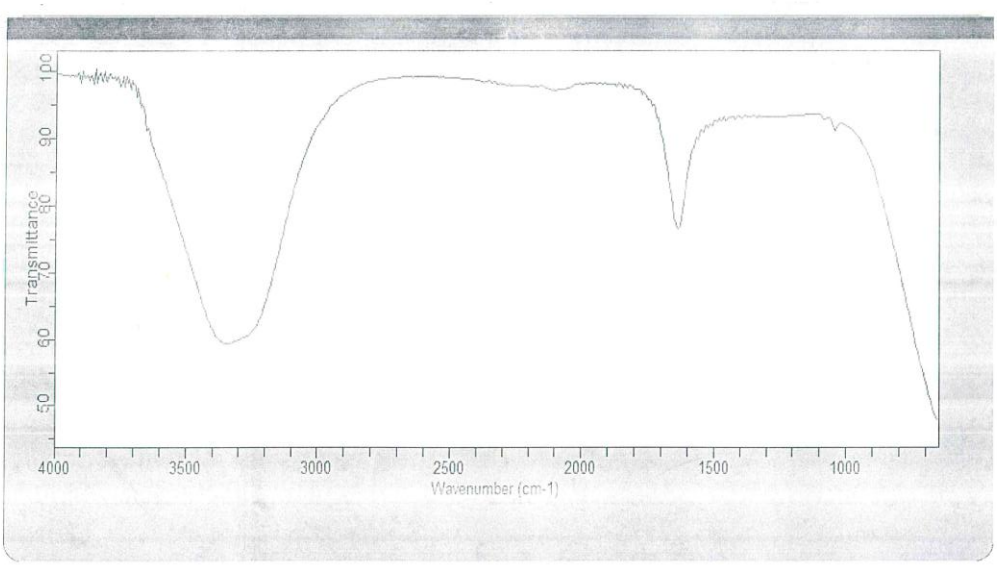

Figure no 6: FTIR spectra of nanoparticle from *Bacillus* species from *Zea mays* (BSZM2)

Table no 6 shows the antibacterial activities of silver nanoparticles against the test organisms. All the biosynthesized silver nanoparticles from *Bacillus* species showed antibacterial activities against the enteric bacteria obtained from the water samples. Generally, the antibacterial assay of nanoparticle *Bacillus* species from *Zea mays* (BSZM2) was significantly different from others at 95% confidence limit. BSZM2 showed the highest antibacterial potency against *Escherichia coli* from Microbiology laboratory, ABUAD (ECMB) with zones of inhibition of 36.33 ± 1.26^{a}mm at AgNp concentration of 0.8 mL. The least antibacterial effect of the nanoparticle was obtained BSAH1, BSME and BSZM1 at AgNp concentration of 0.8 mL with zones of inhibition of 8.67 ± 1.26^{def}mm, 8.33 ± 1.26^{ef}mm and 4.33 ± 1.26^{g}mm respectively (Table 6). The highest zone of inhibition was produced by nanoparticle from *Bacillus* species obtained from *Z. mays* (BSZM2) at 0.8 mL on *Escherichia coli* from Microbiology laboratory, ABUAD (ECMB). However, there were no significant differences in the antibacterial activities of the different concentrations of BSZM2 except on ECMB. There was no significant difference in the antibacterial effect of different volumes of BSAH1 on PVMB.

Susceptibility patterns of the test bacteria to commercial antibiotics

Table no 7 shows the results of susceptibility patterns of the test bacteria to different antibiotics. The results showed that there were significant differences in the antibacterial activities of the antibiotics. The diameter of the zones of inhibition ranged from 12.00 ± 2.00^{a}mm to 35.55 ± 3.21^{a}mm for *S. enterica* from well water and *P. vulgaris* from Microbiology laboratory respectively. There was no significant difference in the antibacterial effects of ciprofloxacillin (CIP) and tarvil (TAR) on ECMB. Also, no significant difference was observed in the antibacterial effects of septrin (SEP) and amoxicillin (AMX) on ECMB. PVMB was most susceptible to pefloxacin (PEF) and least susceptible to septrin (SEP) and chloramphenicol (CHL) with zones of inhibition of 35.55 ± 3.21^{a}mm and 24.00 ± 2.00^{b}mm as well as 24.00 ± 3.61^{b}mm respectively.

SFWA was most susceptible to augmentin (AUG) with zone of inhibition of 29.00 ± 1.00^{a}mm. ECWA was most susceptible to tarvil (TAR) with zone of inhibition of $32.33\ \pm1.53^{d}$mm which was not significantly different from that obtained from augmentin (AUG). Also, the antibacterial effects of septrin (SEP) and ciprofloxacillin (CIP) on ECWA was not significantly different from each other. SEWA was most susceptible to septrin (SEP) and least susceptible to gentamicin (GEN) with zones of inhibition of 33.00 ± 3.00^{a}mm and 13.33 ± 1.16^{c} mm respectively. SEWW was most susceptible to pefloxacin (PEF) and least susceptible to amoxicillin (AMX) with zones of inhibition of 32.67 ± 0.58^{a} mm and 12.00 ± 2.00^{c} mm respectively. SFWW and ECWW were most susceptible to ciprofloxacillin (CIP) with zones of inhibition of 32.00 ± 2.00^{a}mm and 34.00 ± 2.00^{a} mm respectively. They were least susceptible to sparfloxacillin (SPA) with zones of inhibition of $15.00\ \pm1.00^{cd}$ mm and 12.33 ± 3.21^{c} mm respectively. SSWW was most susceptible to streptomycin (STR) and least susceptible to amoxicillin (AMX) with zones of inhibition of $25.67\ \pm1.53^{a}$ mm and 13.00 ± 1.00^{c} mm respectively.

Table no 8 shows the multiple resistance pattern of enteric bacterial isolates to the selected antibiotics. The results showed that ECMB, PVMB, SFWA were susceptible to all antibiotics. However, ECWA showed 40% resistance to SEP, CIP. GEN and STR. In the same vein, SEWA showed 50% resistance to SEP, CHL,

AUG, GEN and PEF. Also, ECWW showed 40% resistance to SEP, SPAR, GEN and STR while SSWW showed similar multiple resistance to SEP, CIP, AMX and GEN. ECMB, PVMB and SFWA showed 100% sensitivity to all the antibiotics while the rest enteric bacteria showed between 50% and 70% sensitivity to the antibiotics

Table no 9 shows the comparative antibacterial activities of silver nanoparticles and antibiotics against the test bacteria. Ciprofloxacillin showed potency against 7 (ECMB, PVMB, ECWA, SFWA, SEWA, SEWW and ECWW) out of the 9 test bacteria and it was used for the comparative study. The inhibitory effect of the nanoparticles obtained from BSZM2 was generally significantly higher than those obtained from other nanoparticles and ciprofloxacillin on all the test bacteria at $P \leq 0.05$. However, there was no significant difference in the antibacterial activities of BSZM2 and ciprofloxacillin (CIP) on ECMB with zones of inhibition of 36.33 ± 1.26^{a} and 35.00 ± 1.26^{a} mm respectively. Zone of inhibition of 35.00 ± 1.26^{a}mm exhibited by CIP on ECMB was significantly higher than that of nanoparticle obtained from BSAH1 having 8.67 ± 1.26^{a}mm. The table also shows that antibacterial activity of nanoparticle from BSZM2 on PVMB with zone of inhibition of 34.33 ± 2.05^{a}mm was significantly higher than that of CIP having 25.67 ± 2.05^{a}mm. The results show that the antibacterial properties of BSZM2 and ciprofloxacillin on SEWA, SFWW and ECWW were not significantly different from each other at $P \leq 0.05$.

Table no 6: Antibacterial activities of different concentrations of nanoparticles produced from *Bacillus* species on enteric bacteria

Source/Volume of nanoparticles (ml)	Nanoparticles from *Bacillus* spp	Test bacteria/Zone of inhibition (mm)								
		ECMB	PVMB	SEWA	ECWA	SFWA	SEWW	SFWW	ECWW	SSWW
M. esculenta										
0.2	BSME	3.33 ± 1.26^{g}	1.33 ± 2.05^{e}	3.33 ± 1.65^{e}	1.67 ± 0.70^{h}	2.00 ± 2.32^{f}	3.00 ± 1.42^{g}	2.00 ± 1.93^{g}	1.67 ± 1.01^{e}	8.00 ± 2.08^{cdef}
0.4	BSME	3.00 ± 1.26^{g}	3.33 ± 2.051^{de}	4.67 ± 1.65^{e}	3.33 ± 0.70^{gh}	3.00 ± 2.32^{f}	4.33 ± 1.42^{ef}	2.33 ± 1.93^{fg}	2.67 ± 1.01^{de}	12.33 ± 2.08^{bcde}
0.8	BSME	8.33 ± 1.26^{ef}	10.00 ± 2.05^{bcd}	5.67 ± 1.65^{de}	6.33 ± 0.70^{de}	6.00 ± 2.32^{def}	5.67 ± 1.42^{ef}	4.33 ± 1.93^{efg}	3.33 ± 1.01^{de}	8.00 ± 2.08^{cdef}
A. hybridus										
0.2	BSAH1	3.33 ± 1.26^{g}	4.00 ± 2.05^{de}	3.00 ± 1.65^{ef}	1.67 ± 0.70^{h}	5.00 ± 2.32^{ef}	3.33 ± 1.42^{g}	6.67 ± 1.93^{defg}	3.00 ± 1.01^{de}	6.67 ± 2.08^{cdef}
0.4	BSAH 1	3.67 ± 1.26^{g}	4.33 ± 2.05^{de}	3.67 ± 1.65^{ef}	4.00 ± 0.70^{fg}	3.33 ± 2.32^{f}	4.33 ± 1.42^{ef}	4.00 ± 1.93^{efg}	4.00 ± 1.01^{de}	6.00 ± 2.08^{def}
0.8	BSAH1	8.67 ± 1.26^{def}	5.33 ± 2.05^{de}	7.67 ± 1.65^{cde}	6.00 ± 0.70^{cdef}	6.67 ± 2.32^{def}	8.33 ± 1.42^{de}	9.00 ± 1.93^{bcde}	5.33 ± 1.01^{d}	5.67 ± 2.08^{ef}
0.2	BSAH2	13.00 ± 1.26^{c}	12.67 ± 2.05^{bc}	11.67 ± 1.65^{c}	12.00 ± 0.70^{c}	11.67 ± 2.32^{bcde}	13.00 ± 1.42^{bc}	11.33 ± 1.93^{bcd}	13.00 ± 1.01^{c}	12.00 ± 2.08^{bcde}
0.4	BSAH 2	12.33 ± 1.26^{cd}	13.67 ± 2.05^{bc}	13.00 ± 1.65^{b}	13.00 ± 0.70^{bc}	13.67 ± 2.32^{bcd}	$14.67^{b} \pm 1.42^{c}$	15.00 ± 1.93^{b}	14.00 ± 1.01^{bc}	13.33 ± 2.08^{bc}
0.8	BSAH 2	15.33 ± 1.26^{c}	16.67 ± 3.51^{b}	14.33 ± 1.65^{b}	14.67 ± 0.70^{bc}	14.67 ± 2.32^{bc}	15.67 ± 1.42^{b}	14.00 ± 1.93^{bc}	16.33 ± 1.01^{b}	15.00 ± 2.08^{b}
M.parasidiaca										
0.2	BSMP	11.67 ± 1.26^{cde}	13.00 ± 2.05^{bc}	13.33 ± 1.65^{b}	11.67 ± 0.70^{c}	12.00 ± 2.32^{bcde}	11.33 ± 1.42^{bcd}	13.33 ± 1.93^{bc}	13.33 ± 1.01^{bc}	12.67 ± 2.08^{bcd}
0.4	BSMP	13.00 ± 1.26^{c}	15.00 ± 2.05^{b}	13.00 ± 1.65^{b}	13.33 ± 0.70^{bc}	16.67 ± 2.32^{b}	13.33 ± 1.42^{bc}	14.00 ± 1.93^{bc}	14.67 ± 1.01^{bc}	13.33 ± 2.08^{bc}
0.8	BSMP	15.67 ± 1.26^{c}	15.33 ± 2.05^{b}	15.00 ± 1.65^{b}	16.33 ± 0.70^{b}	17.00 ± 2.32^{b}	15.00 ± 1.42^{bc}	13.33 ± 1.93^{bc}	16.00 ± 1.01^{bc}	15.00 ± 2.08^{b}
Z.mays										
0.2	BSZM1	3.33 ± 1.26^{g}	7.33 ± 2.059^{cde}	14.00 ± 1.65^{b}	3.33 ± 0.70^{gh}	9.00 ± 2.32^{cdef}	14.33 ± 1.42^{bc}	12.33 ± 1.93^{bcd}	4.67 ± 1.01^{de}	5.00 ± 2.08^{f}
0.4	BSZM1	6.00 ± 1.26^{fg}	4.33 ± 2.05^{de}	10.00 ± 1.65^{bcd}	4.67 ± 0.70^{efg}	8.33 ± 2.32^{cdef}	13.00 ± 1.421^{bc}	8.33 ± 1.93^{cdef}	2.67 ± 1.01^{de}	4.00 ± 2.08^{f}
0.8	BSZM1	4.33 ± 1.26^{g}	7.67 ± 2.05^{cde}	10.00 ± 1.65^{bcd}	7.00 ± 0.70^{d}	8.67 ± 2.32^{cdef}	10.33 ± 1.42^{cd}	10.67 ± 1.93^{bcd}	4.67 ± 1.01^{de}	7.67 ± 2.08^{cdef}
0.2	BSZM2	31.67 ± 1.26^{b}	31.67 ± 2.05^{a}	34.00 ± 1.65^{a}	33.33 ± 0.70^{a}	32.33 ± 2.32^{a}	32.00 ± 1.42^{a}	35.33 ± 1.93^{a}	35.33 ± 1.01^{a}	33.00 ± 2.08^{a}
0.4	BSZM2	33.33 ± 1.26^{ab}	33.00 ± 2.05^{a}	33.67 ± 1.65^{a}	33.00 ± 0.70^{a}	35.67 ± 2.32^{a}	34.33 ± 1.42^{a}	34.67 ± 1.93^{a}	36.00 ± 1.01^{a}	32.67 ± 2.08^{a}
0.8	BSZM2	$36.33 \pm 1.$	$34.33^{a} \pm 2.$	$33.67 \pm 1.$	$35.00 \pm 0.$	$37.00 \pm 2.$	$33.67 \pm 1.$	$33.67 \pm 1.$	$35.00 \pm 1.$	$33.00 \pm 2.$

	26[a]	05[a]	65[a]	70[a]	32[a]	42[a]	93[a]	01[a]	08[a]

Note: Values followed by similar alphabets along the same column are not significantly different from each other; **Key:** BS: *Bacillus* species from rhizosphere of: AH: *Amaranthus hybridus* L, MB: *Musa paradisiaca* L; ME: *Manihot escuenta* Crantz; ZM: *Zea mays* L ; ECMB: *Escherichia coli* from Microbiology laboratory, ABUAD; PVMB: *Proteus vulgaris* from Microbiology laboratory, ABUAD; SEWA: *Salmonella enterica* from waste water; ECWA: *E. coli* from waste water; SFWW: *Shigella flexneri* from waste water, SEWW: *S. enterica* from well water; SFWW: *Sh. sonnei* from well water; SFWW: *Sh. flexneri* from well water; ECWW: *E. coli* from well water.

Table no 7: Comparative susceptibility patterns of bacterial isolates obtained from selected water samples to commercial antibiotics

Antibiotics (µg)	Test bacterial isolates/Zones of inhibition (mm)								
	ECMB	PVMB	SFWA	ECWA	SEWA	SEWW	SFWW	ECWW	SSWW
SEP (30)	22.00±2.00[b]	24.00±2.00[b]	21.00±1.00[bc]	13.67±1.52[de]	15.33±0.58[cd]	31.00±1.00[a]	22.00±1.00[d]	13.67±3.21[c]	14.33±3.21[c]
CHL (30)	33.67±2.08[a]	24.00±3.61[b]	22.00±2.00[bc]	22.00±1.00[c]	14.00±2.00[c]	12.00±2.00[e]	23.67±1.00[b]	23.67±1.53[b]	24.67±1.51[a]
SPA (10)	33.33±4.16[a]	27.33±2.08[b]	22.67±3.06[bc]	22.00±2.00[c]	22.00±2.00[b]	23.33±1.16[b]	15.00±1.00[cd]	12.33±3.21[c]	22.33±1.53[ab]
CIP (30)	35.00±1.00[a]	25.67±1.53[b]	26.00±2.00[ab]	12.00±2.00[e]	33.00±3.00[a]	25.00±2.00[b]	32.00±2.00[a]	34.00±2.00[a]	16.00±1.00[c]
AMX (30)	22.67±3.06[b]	26.00±2.00[b]	24.33±3.15[bc]	25.00±1.00[b]	24.00±2.00[b]	12.00±2.00[e]	24.67±3.06[b]	24.00±1.00[b]	13.00±1.00[c]
AUG (10)	34.00±4.00[a]	25.00±1.00[b]	29.00±1.00[a]	31.00±1.00[a]	14.00±2.00[c]	16.33±1.53[c]	22.33±1.53[bc]	24.33±3.79[b]	24.00±3.00[ab]
GEN (30)	23.00±2.00[b]	32.33±1.53[a]	24.00±2.00[bc]	15.33±1.53[d]	13.33±1.16[c]	31.00±1.00[a]	22.33±1.00[bc]	15.33±3.06[c]	14.00±2.00[c]
PEF (30)	26.00±2.00[b]	35.33±3.21[a]	24.67±3.06[bc]	24.00±2.00[bc]	15.67±1.53[c]	32.67±0.58[a]	13.00±2.65[d]	21.67±1.53[b]	21.00±1.00[b]
TAR (10)	34.67±3.06[a]	27.00±2.65[b]	24.00±1.00[bc]	32.33±1.53[a]	24.00±4.00[b]	15.33±1.16[cd]	12.33±1.53[d]	22.00±2.00[b]	21.00±1.00[b]
STR (30)	32.33±2.52[a]	27.33±2.08[b]	21.33±1.53[b]	14.00±2.00[de]	23.33±2.08[b]	13.33±1.16[de]	21.33±1.16[bc]	13.67±1.53[c]	25.67±1.53[a]

Note: Values followed by similar alphabets in the same column are not significantly different from each other.
Key: SEP: Septrin, CHL: Chloramphenicol, SPAR: Sparfloxacillin, CIP: Ciprofloxacillin, GEN: Gentamicin, AMX: Amoxycillin, PEF; Pefloxacin, TAR: Tarvil,
STR: Streptomycin, AUG: Augmentin ECMB: *Escherichia coli* from Microbiology laboratory, ABUAD; PVMB: *Proteus vulgaris* from Microbiology laboratory, ABUAD
ECMB: *Escherichia coli* from Microbiology laboratory, ABUAD; PVMB: *Proteus vulgaris* from Microbiology laboratory, ABUAD
SEWA: *Salmonella enterica* from waste water; ECWA: *E. coli* from waste water; SFWA: *Shigella flexneri* from waste water. SEWW: *S. enterica* from well water; SFWW: *Sh. sonnei* from well water; SFWW: *Sh. flexneri* from well water; ECWW: *E. coli* from well water

Table no 8: Sensitivity/Resistant patterns of the enteric bacteria to commercial antibiotics after determination of zones of inhibition

Antibiotics (µg)	**Sensitivity/resistivity pattern of isolates**								
	ECMB	PVMB	SFWA	ECWA	SEWA	SEWW	SFWW	ECWW	SSWW
SEP (30)	S	S	S	I	I	S	S	1	I
CHL (30)	S	S	S	S	I	R	S	S	S
SPA (10)	S	S	S	S	S	S	I	R	S
CIP (30)	S	S	S	R	S	S	S	S	I
AMX (30)	S	S	S	S	S	R	S	S	I
AUG (10)	S	S	S	S	I	I	S	S	S
GEN (30)	S	S	S	I	I	S	S	I	I
PEF (30)	S	S	S	S	I	S	I	S	S
TAR (10)	S	S	S	S	S	I	R	S	S
STR (30)	S	S	S	I	S	I	S	I	S
Sensitivity (%)	100	100	100	60	50	50	70	60	60
No of Multiple drug resistant isolates	0	0	0	4	5	5	3	4	4

Key: SEP: Septrin, CHL: Chloramphenicol, SPAR: Sparfloxacillin, CIP: Ciprofloxacillin, GEN: Gentamicin, AMX: Amoxycillin, PEF; Pefloxacin, TAR: Tarvil,

STR: Streptomycin, AUG: Augmentin ECMB: *Escherichia coli* from Microbiology laboratory, ABUAD; PVMB: *Proteus vulgaris* from Microbiology laboratory, ABUAD
ECMB: *Escherichia coli* from Microbiology laboratory, ABUAD; PVMB: *Proteus vulgaris* from Microbiology laboratory, ABUAD
SEWA: *Salmonella enterica* from waste water; ECWA: *E. coli* from waste water; SFWA: *Shigella flexneri* from waste water. SEWW: *S. enterica* from well water; SFWW: *Sh. sonnei* from well water; SFWW: *Sh. flexneri* from well water; ECWW: *E. coli* from well water; S: Sensitive; I: Intermediate; R: Resistance

Table no 9: Comparative antibacterial activities of biosynthesized nanoparticles from *Bacillus* species and the antibiotics ciprofloxacillin

Antibacterial agents	Zone of inhibitions (mm) produced by nanoparticles and ciprofloxacillin against the test bacteria								
	ECMB	PVMB	SEWA	ECWA	SFWA	SFWW	SSWW	ECWW	SEWW
BSAH1	8.67±1.26^{c}	5.33±2.05^{e}	7.67±1.65^{e}	6.00±0.70^{d}	6.67±2.32^{e}	9.00±1.93^{bc}	5.67±2.08^{c}	5.33±1.01^{c}	8.33±1.42^{de}
BSAH2	15.33±1.26^{b}	16.67±2.05^{c}	14.33±1.65^{bc}	14.67±0.70^{b}	14.67±2.32^{cd}	14.00±1.93^{b}	15.00±2.08^{b}	16.33±1.01^{b}	15.67±1.42^{c}
BSMP	15.67±1.26^{b}	15.33±2.05^{cd}	15.00±1.65^{b}	16.33±0.70^{b}	17.00±2.32^{c}	13.33±1.93^{b}	15.00±2.08^{b}	16.00±1.01^{b}	15.00±1.42^{c}
BSME	8.33±1.26^{c}	10.00±2.05^{de}	5.67±1.65^{e}	6.33±0.70^{d}	6.00±2.32^{e}	4.33±1.93^{c}	8.00±2.08^{bc}	3.33±1.01^{c}	5.67±1.42^{e}
BSZM1	4.33±1.26^{c}	7.67±2.05^{de}	10.00±1.65^{cd}	7.00±0.70^{d}	8.67±2.32d^{e}	10.67±1.93^{b}	7.67±2.08^{bc}	4.67±1.01^{c}	10.33±1.42^{d}
BSZM2	36.33±1.26^{a}	34.33±2.05^{a}	33.67±1.65^{a}	35.00±0.70^{a}	37.00±2.32^{a}	33.67±1.93^{a}no	33.00±2.08^{a}	35.00±1.01^{a}	33.67±1.42^{a}
Ciprofloxacillin	35.00±1.26^{a}	25.67±2.05^{b}	33.00±1.65^{a}	12.00±0.70^{c}	26.00±2.32^{b}	32.00±1.93^{a}	16.00±2.08^{b}	34.00±1.01^{a}	25.00±1.42^{b}

Note: Values followed by similar alphabets in the same column are not significantly different from each other.
Key: BS: *Bacillus* species from the rhizosphere of:
AH: *Amaranthus hybridus* L, MB: *Musa paradisiaca* L; ME: *Manihot escuenta* Crantz; ZM: *Zea mays* L.
CIP: Ciprofloxacillin,
ECMB: *Escherichia coli* from Microbiology laboratory, ABUAD; PVMB: *Proteus vulgaris* from Microbiology laboratory, ABUAD
SEWA: *Salmonella enterica* from waste water; ECWA: *E. coli* from waste water; SFWA: *Shigella flexneri* from waste water.
SEWW: *S. enterica* from well water; SFWW: *Sh. sonnei* from well water; SFWW: *Sh. flexneri* from well water; ECWW: *E. coli* from well water

IV. Discussion

In this study, enteric bacteria such as *E. coli, Shigella* and *Salmonella* species were isolated from both well water and waste water samples. Salmonellae have been detected by several authors in surface waters [15]. It is generally accepted that *Salmonella* present in water can be traced back to its animal origins [16]. Outbreaks of *Shigella sonnei* associated with contaminated water have been documented in many developed countries[17]. Saima AS et al concluded that the presence of *Shigella* species in household water may be due to poor sanitation, mixing of sewage water with fresh water and contamination of fresh water with fecal material [18].

These enteric organisms have been shown in literatures to be pathogenic[19] although their pathogenicity was not tested. Most of these pathogenic bacteria are responsible for water-borne diseases causing gastrointestinal disturbances and having symptoms such as diarrhoea, fever, vomiting and abdominal pain [20]. Every year, millions of people die in developing countries mainly due to the diseases caused by water contamination [19] which is a serious concern not only in developing countries but also developed countries [21].

Bacillus species were isolated from the rhizosphere soil samples of the selected plants. Generally, the genus *Bacillus* has been designated as a group of soil inhabitants [22] . Virthiya K et al isolated *Bacillus* sp from the rhizosphere soil of carpet grass. Similarly, [24] isolated *Bacillus* species from the rhizosphere of tomato [23].

Conventional methods based on biochemical and phenotypic techniques for the identification of bacteria is the most common method used in the laboratory. However, due to the similarities among closely related species, species identification is sometimes difficult hence the need for molecular characterization [25]. In this study, the percentage similarities for *Bacillus* species obtained from the rhizosphere soil ranged from 81 to 97%. However, Hayat R et al reported between 98.8 and 99.6 % similarities for *Bacillus* species obtained from the rhizosphere of some legumes. Molecular characterization showed that *Bacillus nakamurai* and *B. wiedmannii* were isolated from the rhizosphere of *A. hybridus.* Also, *Bacillus wiedmannii* was isolated from the rhizosphere of *Musa paradisiaca* while *Bacillus cereus* was isolated from the rhizosphere of *Manihot esculenta* and *Zea mays* respectively [26].

With the rise in antibiotic resistance and the development of fewer new antibiotics, focus has been on the use of biosynthesized silver nanoparticles since they are known to be antibacterial and also have the ability to overcome the bacterial resistance [27]. Several bacteria are known to produce metal nanoparticles intracellularly or extracellularly by either reduction process or by both biosorption and reduction process[28] .

In this study, all the *Bacillus* species obtained from the rhizosphere of the selected plants produced extracellular nanoparticles indicated by change of colour of the culture medium from yellow to brown. Several researchers have reported production of extracellular silver nanoparticles from *Bacillus* species through visible colour change [28]. Vithiya K et al also reported the biosynthesis of silver nanoparticle from *Bacillus* sp obtained from rhizosphere soil sample around carpet grass (*Axonopus compressus*) in the premises of VIT University, Vellore, Tamil Nadu, India [23]. Also,[29] synthesized extracellular nanoparticle from thermophilic *Bacillus* sp. AZ1 that was isolated from a hot spring in Ardebil province.

Kalishwaralal K et al reported extracellular synthesis of silver NPs by bioreduction of aqueous Ag^+ ions with the culture supernatant of *Bacillus licheniformis* [30]. Moreover, well-dispersed silver nanocrystals were synthesized using *B. licheniformis* [31]. Silver NPs were biosynthesized by *Bacillus flexus* [32]. Wei X et al reported the synthesis of circular and triangular crystalline silver NPs by solar irradiation of cell-free extracts of *Bacillus amyloliquefaciens* and silver nitrate ($AgNO_3$) [33]..

The appearance of brown colour in $AgNO_3$ treated flask is attributed to the Surface Plasmon Resonance (SPR) suggesting formation of AgNPs[29] . It is reported that reduction of Ag^+ to Ag^0 occurs through reductase enzymes including nitrate reductase and other proteins. These enzymes released in the solution can reduce the silver nitrate to silver nanoparticles through capping agents such as proteins [34]. Several researchers have observed that biological method of synthesis of silver nanoparticles exhibit strong absorption of electromagnetic waves in the visible range between 400 and 430 nm [23]. Deljou A and Goudarzi S observed absorption peak of the biosynthesized nanoparticle from *Bacillus* sp. AZI at 425nm. However, in this study, the maximum wavelength was 295 nm which was obtained from *B. cereus* from the rhizosphere of maize [29]. The FTIR band observed at 1650/cm by the nanoparticles synthesized by *Bacillus* species can be assigned the bending vibrations of amides II of proteins while the band at 3350/cm corresponds to N – H stretching vibrations. Kumar A et al observed that the FTIR of the nanoparticles from *Brevibacillus* species peaked at 631, 1345, 1410, 1651 and 3626/cm[28].

The use of biosynthesized silver nanoparticles as antibacterial agents has attracted significant attention in recent years [29]. Nanoparticle synthesized from BSZM2 was most inhibitory to all the test bacteria ranging from 33.00 to 37.00mm. The inhibitory effect of nanoparticle obtained from *B. licheniformis* an isolate of soil sample on *E. coli* was 22.00mm as reported by [29]. In this study the diameter of zones of inhibition of all the *E. coli* was between 35.00 and 36.33mm which was produced by nanoparticle from BSZM2.

The mechanism of inhibitory action of AgNPs on microorganisms suggests that upon treatment, DNA loses its replication ability and expression of ribosomal subunit protein, as well as other cellular proteins and enzymes essential to ATP production hence microorganisms become inactivated [35]**.** Other studies proposed that, AgNPs may attach to the surface of cell membrane disturbing permeability and respiration functions of the cell or by interfering with components of the microbial electron transport system[36] . The enteric bacteria used in this study showed varied susceptibility to the antibiotics used. ECMB, PVMB and SSWW showed 100% susceptibility to all the antibiotics whereas ECWA was resistant to septrin, ciprofloxacin as well as streptomycin but showed intermediate sensitivity to gentamycin. Costa EC et al observed that all the *E. coli* obtained from beach waters and sewage from the municipality of Vila Velha-ES, Brazil showed 100% sensitivity to ciprofloxacin, chloramphenicol and gentamicin[37]. Ogu GI et al also reported the sensitivity of *Proteus* sp and *E. coli* isolated from drinking water sources in Amai Kingdom, Delta State to ciprofloxacillin, chloramphenicol, amoxicillin and gentamicin. SEWA showed resistance to gentamicin, intermediate sensitivity to septrin, augmentin and pefloxacillin and sensitivity to chloramphenicol, sparfloxacillin, ciprofloxacillin, amoxicillin, tarvil and streptomycin[38].

However, *Salmonella enterica* was resistant to chloramphenicol, amoxicillin and streptomycin.[8] recorded multiple antibiotic resistance of *Salmonella* species obtained from both clinical and different water samples ranging from well water, tap water to abattoir waste water.

V. Conclusion

Seven enteric bacteria; were obtained from well water and waste water samples collected from Onyearugbulem Market, Akure. Extracellular silver nanoparticles were synthesized from the supernatants of *Bacillus nakamurai, B. wiedmannii, B. cereus* and one unidentified *Bacillus* species obtained from the rhizosphere soils of *Amaranthus hybridus* L, *Musa paradisiaca* L., *Manihot escuenta* Crantz and *Zea mays* L in the presence of silver nitrate at room temperature in this study. All the biosynthesized nanoparticles showed antibacterial activities against enteric bacteria obtained from well water and waste water samples. However, nanoparticles from unidentified *Bacillus* species obtained from the rhizosphere of *Zea mays* was more potent in

inhibiting the growth of the test bacteria than other nanoparticles as well as antibiotics. Also, the test bacteria showed varied susceptibility to different antibiotics.

VI. Recommendation

Since the nanoparticles synthesized from unidentified *Bacillus* species *Zea mays* outcompeted the commercial antibiotics used in this investigation, it can be used in place of the antibiotics. Also, mass production of the biosynthesized nanoparticles should be encouraged since it does not need any hazardous, toxic, and expensive chemical materials for synthesis. However, the need to optimize culture conditions for maximum production cannot be over-emphasize

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