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Antibacterial Effect Of Bacillus species Biomolecules (Metabolites, Silver Nanoparticles, And Functionalized Silver Nanoparticles) Against Multi-Drug Resistance Listeria species

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ABSTRACT

The study investigated the anti-listeria activity of *Bacillus subtilis* (BsaM), *Bacillus licheniformis* (BlbM) and *Bacillus megaterium* (BmcM) against seven *Listeria* species. Metabolites from *Bacillus* spp. were used for silver nanoparticles (BsaSNPs, BlbSNPs, and BmcSNPs) biosynthesis. The Silver Nanoparticles (SNPs) characterization was done using visual observation, UV-Visible Spectrophotometric, FTIR, SEM, and EDXRF. The anti-listeria synergistic potential of the biosynthesized SNPs and functionalized SNPs against Multidrug resistance (MDR) *Listeria* strains was evaluated. The BsaM, BlbM, and BmcM had a varied anti-listeria effect against the *Listeria* spp. 71.43, 100, and 5.71 % of the *Listeria* spp. were susceptible to the BsaM, BlbM, and BmcM metabolites respectively. The metabolite bio-actively reduced $AgNO_3$ for BsaSNPs, BlbSNPs, and BmcSNPs production. Surface Plasma Resonance (SPR) peaks of 600 nm, 400 and 600 nm, and 600 nm were recorded for BsaSNPs, BlbSNPs, and BmcSNPs. The shape of the SNPs were aggregated, rods, and crystalline. The functional groups present in the nanoparticles were carboxylic acid, amino acid, alcohol, esters, and aldehydes had the highest intensity. BsaSNPs, BlbSNPs, and BmcSNPs had the highest anti-listeria potential of 20.0, 16.0 and 22.0 mm against *Listeria innocua* LA22A and *Listeria ivanovii* LA6. Functionalized BsaSNPs, BlbSNPs, and BmcSNPs had the highest anti-listeria activity compared to the metabolites and commercial antibiotics. Ciprofloxacin-BsaSNPs, Erythromycin-FBlbSNPs, and Erythromycin-FBmcSNPS had the highest antagonistic activity (28, 26, and 27 mm) against *Listeria ivanovii* LA6 respectively. The *Bacillus* strains' metabolites, the SNPs, and functionalized SNPs exhibit antimicrobial activities against Multidrug resistance *Listeria* species. Functionalization improved the anti-listerial ability of the antibiotics.

Keywords: *Bacillus* species, Anti-listeria activity, SNPs, MDR *Listeria* species, Functionalization.

1. INTRODUCTION

Antibiotic resistance (ATR) poses serious and dangerous menace to food security, populace health and global development. Geometrical increase in ATR and occurrence and prevalence of new emerging and re-emerging infections is of great public health concern (WHO, 2018). The emergence and re- emergence of Multi-Drug Resistant (MDR) are posing a big challenge to global community health (Halawani, 2016; Kamani and Lim, 2013). Recent researches have raised a red flag on the increasing zoonotic, nosocomial, and community-acquired infections due to the appearance of MDR pathogenic microorganisms which currently defy antibiotic therapies. Antimicrobial resistance as a result of improper utilization of antibiotics and the formation of resistant genes is now one of the major threats to human health (Rai et al., 2014; Halawani, 2016; Adebami et al., 2020). Antimicrobial resistance (AMR) is a threat to the prophylaxis and treatment of different microbial infections. Listeriosis is an important emerging zoonotic disease common in humans after consumption of contaminated animal food products (Van de Venter, 1999). Listeria infection is a disease caused by *Listeria* species and *Listeria monocytogen* is an etiological agent of Listeriosis in humans and some mammals (Garedew, 2015). Cases of listeriosis from ready–to–eat (RTE) food have been reported worldwide (SU, 2018).

As a result of the dangerous increase in ATR and the spread of new emerging and re-emerging infections globally growing number of infections are difficult to treat due to the ineffectiveness of antibiotics which causes longer hospitalization, expensive medication, and higher morbidity and mortality rate (WHO, 2018). Hence a need to gear research towards the development of new and broad spectrum antimicrobials to combat the ATR problems. Some species from the genus Bacillus were reported as a producer of secondary metabolites that have antimicrobial activity against diseases causing microorganisms (Katz and Demain 1977). The use of antimicrobial producing *Bacillus* spp. or their bioactive metabolites may be a supplementary means to synthetic chemicals for human protection (Leifert et al. 1995; Berger et al. 1996). Their antibiotics have a broad spectrum of antimicrobial, anti- viral, anti-ameobocytic, and anti-mycoplasma activities against microbial infections (Steller et al., 1999).

Bioactive metabolites from *Bacillus* strains have a broad use in the medicine and pharmaceutical industry as antimicrobials and bio-control agents in the treatment of plants, animals, and human diseases (Leifert et al., 1995). Nanoparticles have found a wide application in various fields of science and biomedical (Herrera et al., 2001). As a result of increasing drug resistance and growing concern regarding the over-prescription of antibiotics, has led to an emerging interest in the use of silver nanoparticles as antimicrobial agents. Unlike antibiotics, silver appears to be immune to resistance (Rai et al., 2009). The strong bactericidal potential of silver and its nanoparticles on MDR strains is of great help in tackling Antimicrobial resistance (ATR) (Rai et al., 2009).

The prevalence of microbial resistance to available antibiotics is at an alarming rate most especially in developing countries. As a result of this, therapeutic options have appeared more limited than ever. The emergence and re-occurrence of Multi drug resistance disease causing microorganisms have made research interest gear towards the development of efficient and safe antimicrobials. There's a dearth of information on the anti-listeria potential of SNPs against MDR *Listeria* strains. Functionalization of the existing antibiotics with nanoparticles will be of added advantages to improve their efficacy and effectiveness, hence a necessity for this research. The research aimed at evaluation of *in-vitro* anti-listeria potential of *Bacillus* species metabolites, its SNPs, and antibiotics functionalized SNPs against MDR *Listeria* strains.

2. MATERIALS AND METHODS

2.1 Culture collection

Cultures of *Bacillus subtilis, Bacillus licheniformis*, and *Bacillus megaterium* were gotten from the Microbial biotechnology Unit culture collection in the Department of Microbiology, University of Ibadan, Ibadan, Nigeria. Multi-Drug Resistant (MDR) *Listeria* species (*Listeria* sp. LB18, *Listeria ivanovii* LA6, *Listeria ivanovii* LA20A, *Listeria* spp. LA16, *Listeria innocua* LA22A, *Listeria monocytogenes* LA1X1, and *Listeria innocua* LB21X) were obtained from the Environmental Unit Culture Collection in the Department of Microbiology, University of Ibadan, Ibadan.

2.2 Production of metabolites from the Bacillus species and antagonistic activity against the test pathogens

Bioactive metabolites were produced using *Bacillus* spp. The pure culture of the isolates was inoculated in sterile Nutrient broth (NB). The broths were kept at 35°C for 72 hrs. The supernatant obtained from the centrifuged (5000 rpm for 20 minutes) fermentation broth was used as bioactive metabolites (BsaM, BlbM, and BmcM). The anti-listeria potential of the BsaM, BlbM, and BmcM against the MDR *Listeria* species was done using Agar Well Diffusion method (Balashanmugam et al., 2013).

Cell suspension of the isolate was prepared according to McFarland standard. An aliquot of the suspension was spread on the surface of Mueller Hinton agar plates with a sterile cotton swab. Wells were bored on the dried inoculated plates using a sterile cork borer (6 mm). The wells was filled with 100 µL of the bioactive metabolites and incubated at 37°C for 24 hrs. Zones of inhibition (ZOI) around the well were recorded.

2.3 Green synthesis and characterization of SNPs using BsaM, BlbM, and BmcM

SNPs were produced by adding 1mL of the BsaM, BlbM, and BmcM to 10 mL of 10 mM $AgNO_3$ solution respectively. The reaction mixture was incubated in the dark at room temperature for 3 days for biological reduction of $AgNO_3$ for silver nanoparticles synthesis. After 24 hrs incubation, the colourless solution turned darkish brown which indicates BsaSNPS, BlbSNPs, and BmcSNPs formation (Balashanmugam et al., 2013). Visual observation of the green produced nanoparticles was done by checking the reaction mixture for changes in colour to darkish brown which indicates SNPs formation in comparison to the control. The reduction of Ag^+ to Ag^0 was observed using UV-Visible spectrophotometer (a Lambda 25-Perkin Elmer, Waltham, MA, USA). 1 mL of each of the samples was withdrawn after 24, 48 and 72 hrs and absorbance was measured at wavelengths of 200 – 1000 nm with a resolution of 0.5 nm.

The functional groups present in the SNPs were determined using FTIR spectroscopy (Shimadzu). The dried samples and KBr pellets were pressed into a mold. The spectra were taken at a wave range of 500-4000 cm^{-1} and a resolution of 4 cm^{-1}. The size, shape, and morphology of the nanoparticles were determined by subjecting the SNPs aqueous solution to Scanning Electron Microscopy (SEM). The purity of the nanoparticles was determined and metal components distribution within the biosynthesized SNPs was determined using EDX.

2.4 Anti-listeria activity of the biosynthesized SNPs against the MDR Listeria species

Anti-listeria potential of the biosynthesized SNPs against the MDR *Listeria* species was evaluated using the agar diffusion method as previously described.

2.5 Functionalization of the SNPs with some antibiotics and synergistic effect of the functionalized nanoparticles

BsaSNPS, BlbSNPs, and BmcSNPs were functionalized with some antibiotics (Chlortetracycline (30 µg), Amoxicillin (10 µg), Colistin (10 µg), Oxytetracycline (30 µg), Gentamycin (10 µg), Erythromycin (15 µg). and Ciprofloxacin (5 µg)) respectively. For the production of functionalized nanoparticles, 0.1 mL of antibiotic solution was mixed with 2 mL of the biosynthesized silver nanoparticles. The reaction mixture was incubated at 35°C for 24 hrs for proper adhesion of the antibiotics to the surface of the nanoparticles.

The anti-listeria potential of nanoparticles, the functionalized BsaSNPS, BlbSNPs, and BmcSNPs, and antibiotics alone against the MDR *Listeria* spp. was evaluated using Agar Well Diffusion method. The lawn of the indicator strain was spread plated on Mueller Hinton agar. The holes (6 mm) were filled with SNPs, antibiotics only and functionalized samples. The plates were kept at 37°C for 24 hrs and zone of clearance around the wells were recorded. Clear zone around the well indicates antibacterial activity (Begum et al., 2009).

2.6 Determination of Minimum Inhibitory Concentration (MIC) of the biosynthesized SNPs

The MIC of the nanoparticles was determined using the Agar Well Diffusion method (Begum et al., 2009). Two fold dilutions of the nanoparticles was done using distilled water to prepared different concentrations (100, 50, 25, 12.5, 6.25, and 3.125 %) of the SNPs. Cell suspension of *Listeria* spp. was spread plated on Mueller Hinton agar. Sterile cork-borer was used to punch holes (6 mm) in the agar. The hole was filled with 100 µL of the synthesized SNPs (BsaSNPS, BlbSNPs, and BmcSNPs). The lowest dilution of the SNPs (BsaSNPS, BlbSNPs. and BmcSNPs) at which zones of inhibition were recorded against the test microorganisms was taken as the MIC for each SNPs. The positive control was ciprofloxacin.

3. RESULTS

3.1 Production of bioactive metabolites and anti-listeria potential of Bacillus species against MDR Listeria species

Productions of bioactive metabolites (BsaM, BlbM, and BmcM) were done using the Bacillus spp. (Bsa, Blb, and Bsc). Table 1 shows the anti-listeria potential of the metabolites against the MDR *Listeria* spp. The metabolites from the *Bacillus* species exhibited varied anti-listeria activity against the 7 Multidrug resistance *Listeria* spp. The anti-listeria activity of BsaM, BlbM, and BmcM ranged from 5.0 – 16.0 mm, 10.0 – 20.0 mm, and 6.0 – 14.0 mm. The highest antagonistic activity was against *Listeria ivanovii*LA20A follow in order by *Listeria innocua* respectively. 71.43, 100, and 5.71 % of the *Listeria* spp. were susceptible to the BsaM, BlbM, and BmcM metabolites respectively. Comparatively, BlbSNPs metabolite had the highest antagonistic potential against the *Listeria* species.

Table-1: Antibacterial activity of *Bacillus subtilis* (BsaM), *Bacillus licheniformis* (BlbM), and *Bacillus megaterium* (BmcM) metabolites against some MDR *Listeria* species

Listeria species	Antibacterial activity (mm) of the metabolites		
	BasM	BlsM	BmcM
Listeria sp. LB18	0	11.0	7.0
Listeria ivanovii LA6	16.0	18.0	14.0
Listeria ivanovii LA20A	9.0	20.0	8.0
Listeria spp. LA16	0	13.0	11.0
Listeria innocua LA22A	10.0	15.0	13.0
Listeria monocytogenes LA1X1	5.0	14.0	6.0
Listeria innocua LB21X	8.0	10.0	0

The ability of the metabolites of *Bacillus* species to inhibit the test isolates may be due to the presence of bioactive compounds present in the metabolites. Antagonistic activity of *Bacillus* species against some of the test pathogens agrees with the report of Ashim et al. (2002) on the antimicrobial activity of *B. megaterium* against some pathogens. Similarly, Silambarasan and Abraham, (2012) reported the antimicrobial activity of *Bacillus* sp. against some pathogens.

3.2. Characterization of the greenly synthesized SNPs

3.2.1 Visual detection

The visual observation of the BsaSNPs, BlbSNPs, and BmcSNPs was shown in Figure 1 a-c. The reaction mixture turned darkish-brown after keeping for 2 hrs respectively which indicate the formation of SNPs.

Figure-1: Visual characterization of (a) *Bacillus subtilis* (BsaSNPs), (b) *Bacillus licheniformis* (BlbSNPs), and (c) *Bacillus megaterium* (BmcSNPs) produced at 72 hrs

3.2.2. UV–visible determination of the BsaSNPs, BlbSNPs, and BmcSNPs

The UV –visible spectra of the BsaSNPs, BlbSNPs, and BmcSNPs at different incubation times are shown in Figure 2 a-c. BsaSNPs had a broad spectrum range between 400 to 800 nm at 24 – 72 hrs. Surface Plasmon Resonance (SPR) peak was observed at 600 nm. BlbSNPs had an SPR peak at 400, 600, and 800 nm at 24, 48, and 72 hrs respectively. BmcSNPs had an SRP of 600, 600, and 800 nm at 24, 48, and 72 hrs respectively.

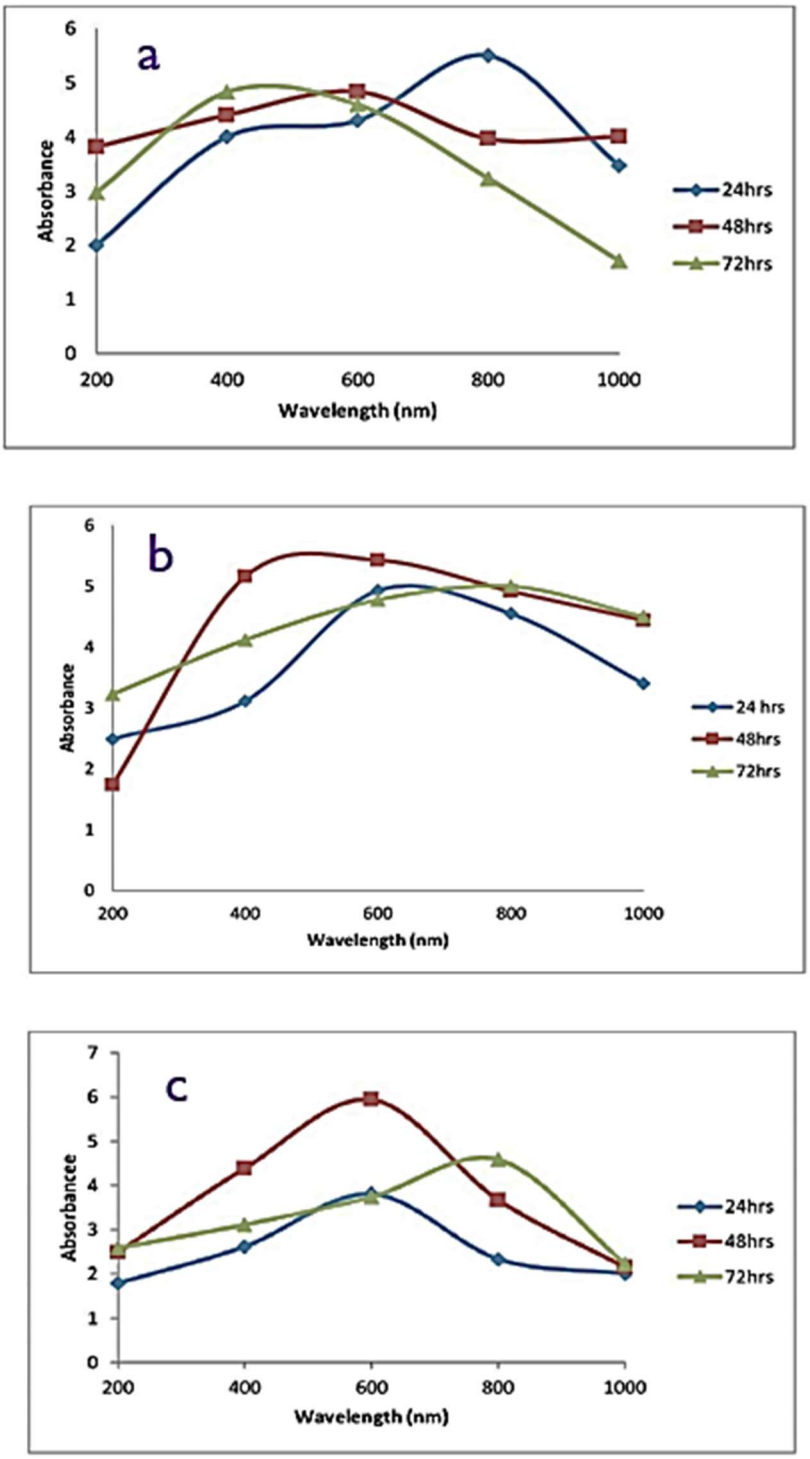

Figure-2a-c: UV-Visible spectra of (a) *Bacillus subtilis* Silver Nanoparticles (BsaSNPs), (b) *Bacillus licheniformis* Silver Nanoparticles (BlbSNPs), and (c) *Bacillus megaterium* Silver Nanoparticles (BmcSNPs) at different incubation time.

3.2.3. Fourier Transform Infra-Red (FTIR) determination of BsaSNPs, BlbSNPs, and BmcSNPs

BsaSNPs was characterized using FTIR and 7 bands were present at 3448.00, 2937.14, 1638.92, 1552.59, 1404.40, 1103.65, 607.24 cm^{-1} as shown in Figure 3a. The peak indicated O-H stretch of alcohol, presence of C-H symmetrical stretch, presence of amide and NH bend respectively. The presence of C=O stretch was shown by the peak at 1435.09 cm^{-1}. The peak at 1103.65 cm^{-1} indicated the presence of a C-O stretch of alcohol. The peak at 607.24 cm^{-1} signified the presence of acetylenic CH of alkynes. The functional groups observed generally signified the presence of amino acids, alcohol, aldehydes and carboxylic acid in the sample may be responsible for the reduction of silver nitrate to SNPs (Figure 3a).

BlbSNPs were characterized by FTIR as shown in Figure 3b. The spectrum has 9 peaks ranging between 3423.00, and 352.84 cm^{-1}. The peak at 3423.00 cm^{-1} could be attributed to the OH stretch vibration of alcohol. The peak at 2948.57 cm^{-1} indicated the stretching vibration of symmetrical C-H. The peak at 1641.33 and 1566.83 cm^{-1} corresponded to the C=O stretch of carboxylates and NH stretch of secondary amides. The absorption peak at 1412.23 cm^{-1} indicated the presence of O-H bends of esters, phenol, and tertiary alcohol or C-C bend of aldehyde. The absorption peaks at 1266.67 cm^{-1} and 1108.18 cm^{-1} signified C-O stretching vibration of carboxylic esters and C-O stretching vibration of a secondary alcohol. The peak at 610.24 cm^{-1} indicated the presence of acetylenic CH of alkynes. The peak at 352.84 cm^{-1} depicts the presence of a weak peak of an aromatic benzene ring. Therefore from the FTIR spectrum observed, it was clear that the SNPs were surrounded by carboxylic acid, aldehyde, esters, protein, and amino acids which may be responsible for the biosynthesis and stability of the SNPs (Figure 3b).

BmcSNPs were characterized by FTIR and the spectrum observed is shown in Figure 3c. The spectrum showed 12 absorption peaks which ranged between 3760.00 and 361.39 cm^{-1}. The peak at 3760.00 cm^{-1} signified the presence of an O-H stretch-free, strong alcohol. The absorption peak at 3417.00 cm^{-1} could be attributed to O-H stretch vibration of alcohol and 2938.61 cm^{-1} could be attributed to C-H symmetrical stretching. The peak at 2114.28 cm^{-1} indicated the carbonyl stretching of transition metals. The absorption peak at 1648.28 and 1575.38 cm^{-1} corresponded to the NH bend of amide and NH stretch of secondary amides respectively. The absorption peaks between 1266.70 and 1110.12 cm^{-1} indicated a C-O stretch of esters and carboxylic acids. The absorption peak at 612.00 cm^{-1} indicated the presence of acetylenic CH of alkynes. The peak at 361.39 cm^{-1} indicated weak aromatic benzene. The functional groups observed generally indicated the presence of amino acids, alcohol, aldehydes and carboxylic acid in the sample may be responsible for the reduction of silver nitrate to SNPs (Figure 3c).

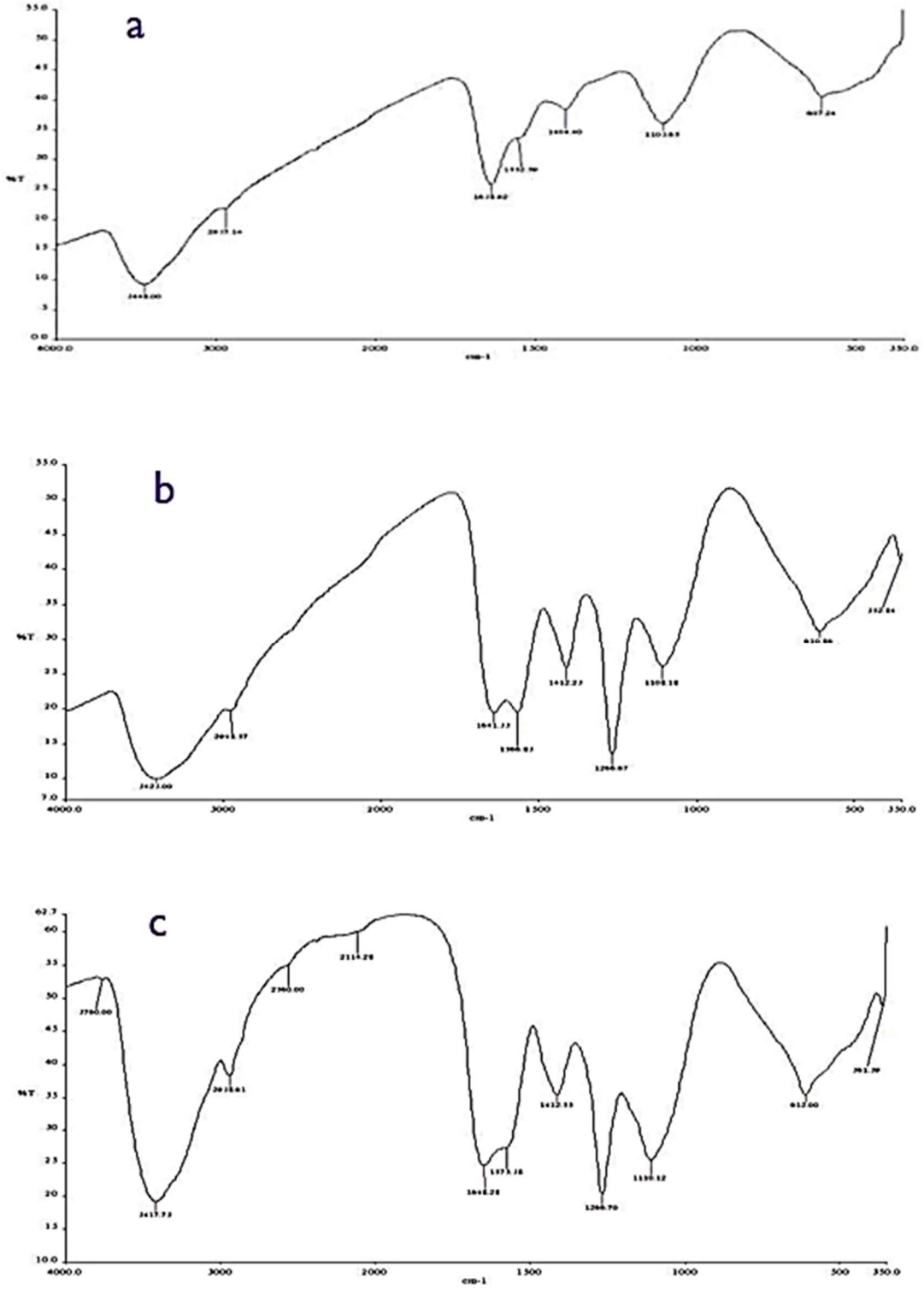

Figure-3a-c: FTIR spectra of (a) *Bacillus subtilis* Silver Nanoparticles (BsaSNPs), (b) *Bacillus licheniformis* Silver Nanoparticles (BlbSNPs), and (c) *Bacillus megaterium* Silver Nanoparticles (BmcSNPs)

3.4. Scanning Electron Micrograph of BsaSNPs, BlbSNPs, and BmcSNPs

The Scanning Electron Micrograph of BsaSNPs, BlbSNPs, and BmcSNPs are shown in Figure 4 a-c. BsaSNPs, BlbSNPs, and BmcSNPs were aggregate, rod, and crystalline in shape respectively.

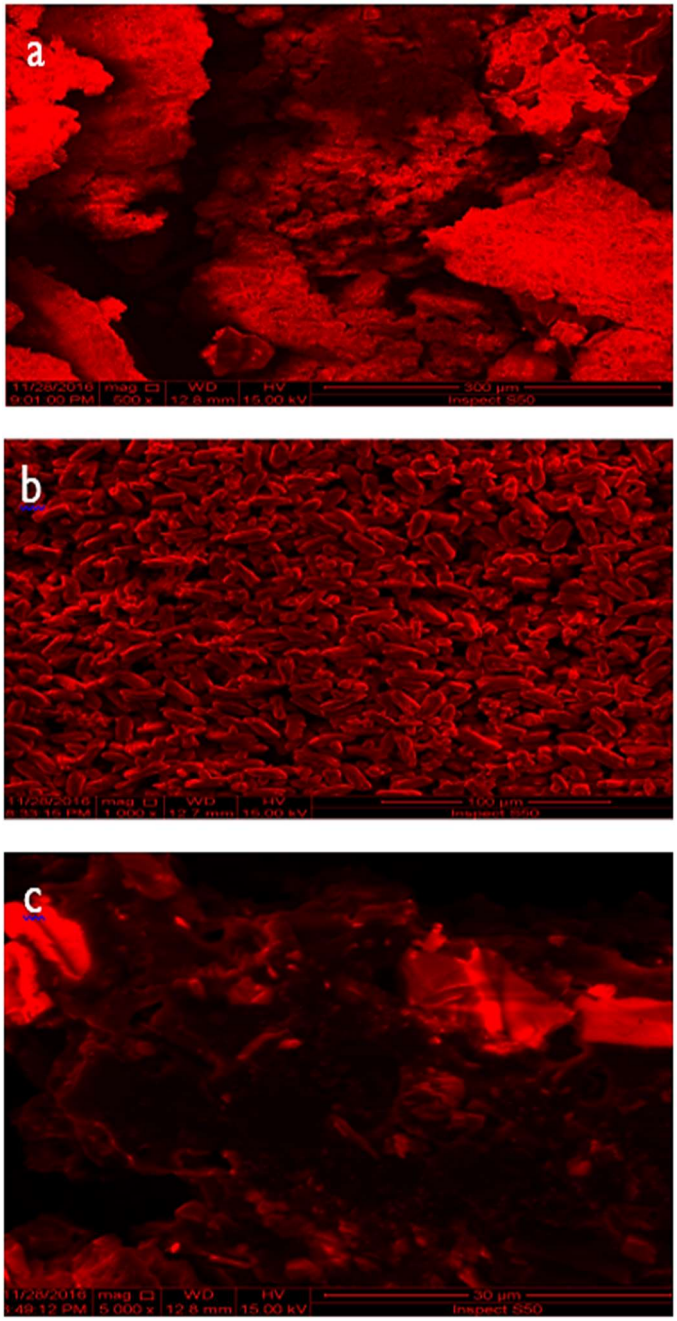

Figure-4 a-c: Scanning Electron Micrograph of (a) *Bacillus subtilis* Silver Nanoparticles (BsaSNPs), (b) *Bacillus licheniformis* Silver Nanoparticles (BlbSNPs), and (c) *Bacillus megaterium* Silver Nanoparticles (BmcSNPs)

3.5. Energy Dispersion X-Ray Fluorescence Analysis of the SNPs

Characterization of the biosynthesized silver nanoparticles using Energy dispersion X-Ray fluorescence (EDXRF) was done at a voltage of 40.0 KV and a current of 350 µA. EDX analysis of BsaSNPs, BlbSNPs, and BmcSNPs was shown in Figure 5a-c. The intensity ranged from 0.0001 – 0.2976, 0.0001 – 0.1318 and 0.0001 – 0.2500 respectively. Ag had the highest intensity. Elements such as Magnesium (Mg), Potassium (K), Chromium (Cr), Lead (Pb), Gold (Au), and Cadmium (Cd) had zero intensity and content. The intensity and content observed generally indicated the reduction of $AgNO_3$ and subsequent formation of SNPs.

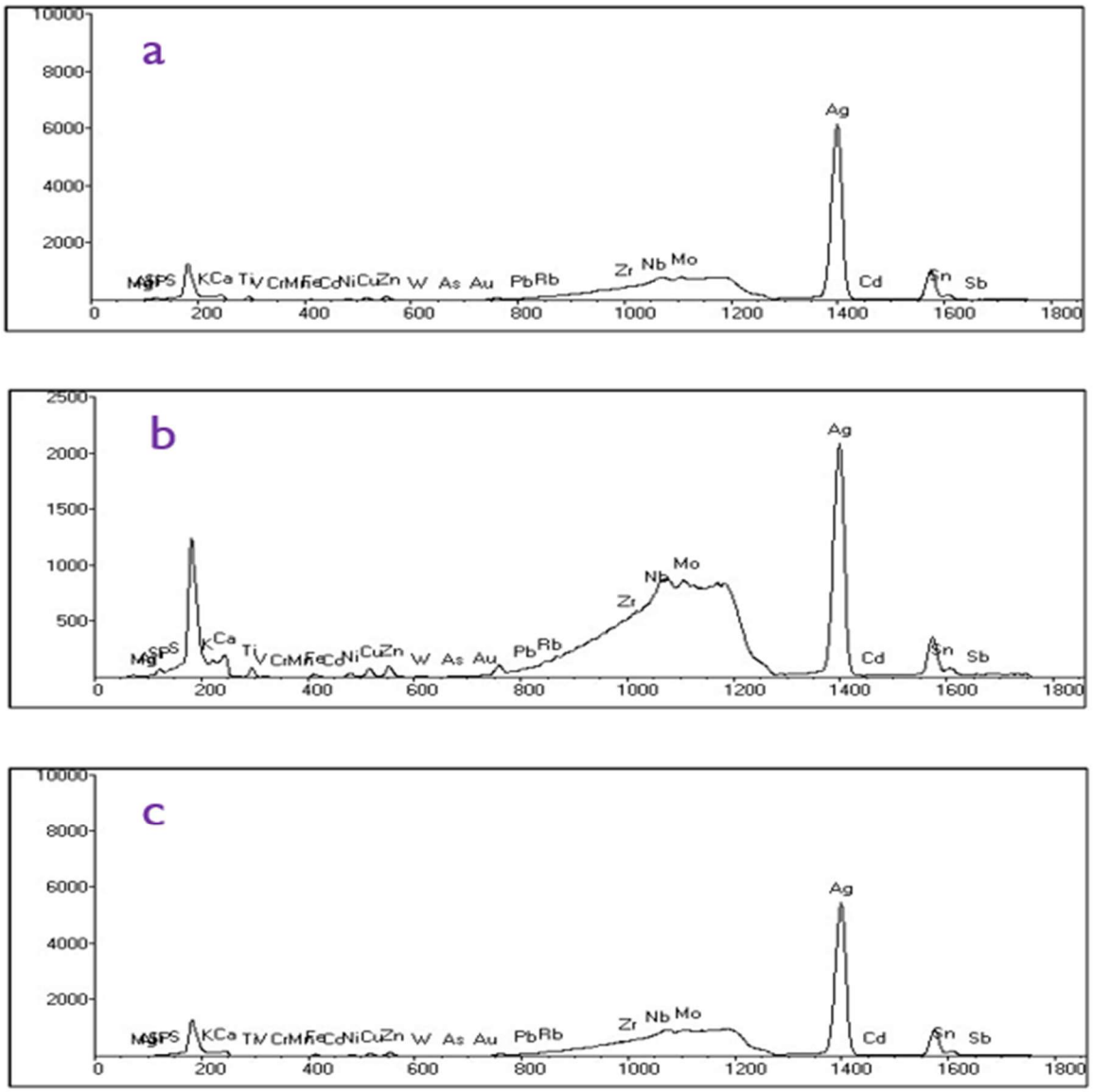

Figure-5a-c: EDX analysis of (a) *Bacillus subtilis* Silver Nanoparticles (BsaSNPs), (b) *Bacillus licheniformis* Silver Nanoparticles (BlbSNPs), and (c) *Bacillus megaterium* Silver Nanoparticles (BmcSNPs)

3.6. Antagonistic activity of the nanoparticles

Antibacterial activity of BsaSNPs, BlbSNPs, and BmcSNPs against the MDR *Listeria innocua* LA22A and *Listeria ivanovii* LA6 is shown in Table 2. The antagonistic activity ranged from 9.0 – 18.0 mm and 13.0-19.0 mm, *Listeria ivanovii* L6 and *Listeria innocua* L22A had the highest susceptibility to BlbSNPs.

Table 2: Antibacterial activity of the biosynthesized SNPs from the three (3) selected *Bacillus* species against the selected (MDR) *Listeria* spp.

Biosynthesized SNPs	Antagonistic activity (mm) of the nanoparticles against the MDR *Listeria* spp.	
	Listeria innocua LA22A	*Listeria ivanovii* LA6
BsaSNPs	9.0	17.0
BlbSNPs	18.0	19.0
BmcSNPs	11.0	17.0
Ciprofloxacin	10.0	12.24
$AgNO_3$	17.0	13.0

The MIC of BsaSNPs, BlbSNPs, and BmcSNPs against the MDR *Listeria innocua* LA22A and *Listeria ivanovii* LA6 is shown in Table 3. BsaSNPs, BlbSNPs, and BmcSNPs had a MIC of 12.5, 6.25, and 6.25 % against *Listeria innocua* LA22A and MIC of 6.25, 3.125, and 6.25 %, respectively, against *Listeria ivanovii* LA6. Generally, all the SNPs had the highest MIC while the least MIC of all the SNPs was on *Listeria innocua* LA22A.

Table 3: Determination of MIC of biosynthesized SNPs on the selected (MDR) *Listeria* species

MIC Concentration (%)	Antagonistic activity (mm) of the nanoparticles					
	MDR *Listeria innocua* LA22A			MDR *Listeria ivanovii* LA6		
	BsaSNPs	BlbSNPs	BmcSNPs	BsaSNPs	BlbSNPs	BmcSNPs
50	14.0	10.0	12.0	15.0	18.0	15.0
25	11.0	10.0	10.0	12.0	14.0	16.0
12.5	10.0	6.0	12.0	9.0	11.0	12.0
6.25	00	6.0	5.0	8.0	7.0	9.0
3.25	00	00	00	00	5.0	00
Ciprofloxacin	12.0	12.0	9.0	11.0	13.0	10.0

3.6. Anti-listeria potential of the nanoparticles, selected antibiotics, and functionalized antibiotics against the MDR Listeria species

The antagonistic activity of the silver nanoparticles (BsaSNPs/ BlbSNPs/ BmsSNPs), the antibiotics, and the functionalized nanoparticles (FSNPs) against *Listeria innocua* LA22A and *Listeria ivanovii* LA6 is shown in Table 4a. Antagonistic activity of BsaSNPs, the antibiotics, and the functionalized BsaSNPs against *Listeria innocua* LA22A and *Listeria ivanovii* LA6 ranged from 4.0-20.0 mm and 10.0 – 28.0 mm. LA22A had the highest susceptibility to Ciprofloxacin FBsaSNPs. *Listeria ivanovii* LA6 showed high susceptibility to Erythromycin, Amoxicillin, and Gentamycin-FBsaSNPs with antagonistic zones of 25.0 and 24.0 mm respectively. *Listeria innocua* LA22A had the lowest susceptibility to Chlorotetracycline-FBsaSNPs while LA6 had the lowest susceptibility to Oxytetracycline.

Listeria innocua LA22A was not susceptible to Oxytetracycline and Oxytetracycline-FBsaSNPs. *Listeria ivanovii* LA6 were susceptible to the nanoparticles, the antibiotics, and the functionalized nanoparticles. All the functionalized BsaSNPs had better antagonistic activity against *Listeria ivanovii* LA6. Antagonistic activity of BlbSNPs, the antibiotics, and the functionalized BlbSNPs against *Listeria innocua* LA22A and *Listeria ivanovii* LA6 ranged from 6.0 – 24.0 mm and 10.0 – 26.0 mm. Ciprofloxacin-FBlbSNPs had the highest antagonistic effect against *Listeria innocua* LA22A while Erythromycin-FBlbSNPs had the highest antagonistic activity against *Listeria ivanovii* LA6. The anti-listeria activity of the BlbSNPs had better anti- listeria activity against *Listeria innocua* LA22A compared to ordinary antibiotics and the functionalized antibiotics except for Ciprofloxacin-FBlbSNPs. Two of the antibiotics and four of the functionalized BlbSNPs exhibited a better antibacterial activity against *Listeria ivanovii* LA6. Functionalization improves the antagonistic potentials of some of the antibiotics.

Table 4a: The antagonistic activity of the SNPs (BsaSNPs / BlbSNPs / BmsSNPs), the antibiotics, and the functionalized SNPs from *Bacillus* spp. metabolites against *Listeria innocua* LA22A and *Listeria ivanovii* LA6

SNPs/Antibiotics/functionalized antibiotics	Antagonistic activity (mm)					
	BsaSNPs		BlbSNPs		BmsSNPs	
	Listeria innocua LA22A	*Listeria Ivanovii* LA6	*Listeria innocua* LA22A	*Listeria Ivanovii* LA6	*Listeria innocua* LA22A	*Listeria Ivanovii* LA6
SNPs(BsaSNPs/ BlbSNPs/BmsSNPs)	9.0	17	18	19	11	17
Chlorotetracycline	11	20	11	20	11	20
Amoxicillin	14	19	14	19	14	19
Colistin	8.0	15	8.0	15	0	15
Oxytetracycline	0	10	0	10	0	10
Gentamycin	12	21	12	21	12	21
Erythromycin	10	17	10	17	10	17
Ciprofloxacin	15	13	15	13	15	13
Chlorotetracycline FSNPs	4.0	22	6.0	19	10	25
Amoxicillin FSNPs	20	24	13	12	11	20
Colistin FSNPs	12	21	10	18	0	11
Oxytetracycline FSNPs	0	14	14	22	0	22
Gentamycin FSNPs	15	24	16	23	10	20
Erythromycin FSNPs	9.0	25	7.0	26	6.0	27
Ciprofloxacin FSNPs	16	28	24	24	10	30
$AgNO_3$	17	13	17	13	12	21

Antagonistic activity of BmcSNPs, the antibiotics, and FBmcSNPs against *Listeria innocua* LA22A and *Listeria ivanovii* LA6 ranged from 6.0 -15.0 mm and 10.0 – 30.0 mm. The highest antagonistic activity against *Listeria innocua* LA22A was recorded when Ciprofloxacin was used while Ciprofloxacin-FBmcSNPs had the highest activity against *Listeria ivanovii* LA6.

Erythromycin-FBmcSNPs and Oxytetracycline had the lowest antagonistic activity against *Listeria innocua* LA22A and *Listeria ivanovii* LA6 respectively. *Listeria innocua*LA22A was not susceptible to Colistin, Oxytetracycline, Colistin-FBmcSNPs, and Oxytetracycline-FBmcSNPs. *Listeria ivanovii* LA6 were 100 % susceptible to BmcSNPs, the antibiotics, and the FBmcSNPs respectively. 18.75 % (3) and 43.75% (7) of the antibiotics and the FBmcSNPs had higher activity against *Listeria ivanovii*LA6 compared to BmcSNPs.

5. DISCUSSION

The metabolites from *Bacillus* species biologically reduce $AgNO_3$ for silver nanoparticles production. Vithiya et al. (2014) reported *Bacillus* spp. produced silver nanoparticles extracellularly. El-Batal et al. (2013) reported the antimicrobial activity of the SNPs produced by *B. stearothermophilus.* Kamani and Lim, (2013) reported the biosynthesis and structural evaluation of silver nanoparticles using bacterial exopolysaccharides and their antimicrobial effect against food and Multi-Drug Resistant pathogens. Colour changes from darkish brown is an indication of the silver nanoparticles formation from *Bacillus* species. This is in agreement with the work of Nanda and Raghavan, (2014) on colour change of EPS supernatants to yellowish-brown. Furthermore, the green production of silver nanoparticles using *Bacillus subtilis* and its antimicrobial potential has been reported by Natarajan and Selvaraj (2010).

Characterization of silver solution exposed to the supernatant of *Bacillus* species by UV-Visible spectrophotometer confirmed the biological reduction of silver ions to silver nanoparticles. The Surface Plasmon Resonance peak recorded for the nanoparticles produced from the samples was within the range of 500-600nm. This observation is also in contrast to the work of Vithiya et al. (2014) that extracellular *Bacillus* mediated SNPs showed a strong SPR peak at 400-430 nm with broadband, indicating varied shape and size SNPs formation. The peak absorbance of SNP produced by *Bacillus subtilis* investigated by Natarajan and Selvaraj (2011) was 440 nm, this is contrary to the absorbance peak of 500-600nm which was evaluated in this present study. Shahverdi et al. (2011) proposed that as the particle size increments caused narrower peaks and reduction in bandwidth and increased band intensity. This work is in contrast to the report of Balashanmugam et al. (2013) who reported 386 nm SPR.

The FTIR spectra measurement was carried out to inspect the available functional groups of the *Bacillus* species metabolites responsible for the biological reduction and stabilization of the SNPs. FTIR is very useful for SNPs characterization as reported by many authors (Kanmanni and Lim 2013; Magdi et al., 2014). The formation and stabilization of SNPs may be due to the release of extracellular protein molecules from *Bacillus* spp. (El-Batal et al., 2013). Carboxylic acid, amino acid, alcohol, esters, and aldehydes were the functional group present in the production of SNPs. This is similar to the work of Chitra and Annadurai (2013) who reported these functional groups as the reducing agents responsible for silver nanoparticles production. The stabilization of the nanoparticles may be as a result of the presence of proteins and amino acids in the metabolites.

Scanning Electron Microscope was further used to characterize the SNPs. SEM is an important tool for SNPs characterization (Begum et al., 2009; Balashanmugam et al., 2013). The greenly synthesized SNPs were partially aggregated, rod-shaped, and crystalline. The aggregation recorded may be as a result of the drying process. Sadowski et al. (2008) reported the effect of drying on the shape and size of SNPs from *Penicillium* strains. BlbSNPs were observed to be rod-shaped; this is agrees with the work of Gardea-Torresday et al. (2007) on gold nanoparticles from *Triticum aestivum* leaves.

BmcSNPs was a crystalline shape, Nanda and Raghavan (2014) reported that the shape of the biosynthesized SNPs from exopolysaccharides (EPS) produced by *Bacillus subtilis* was dispersed. Energy dispersion X-ray fluorescence was further used to characterize the biosynthesized SNPs. EDXRF is an essential tool for SNPs characterization that the EDXRF pattern peak is metallic silver (Vithiya et al., 2014). Kamani and Lim, (2013) has reported that EPS–reduced SNPs exhibited intense peak of silver ions. EDXRF optical absorption peak observed at 3keV is typical for metallic $AgNO_3$ (Magdi et al., 2014). The BsaSNPs, BlbSNPs, and BmcSNPs had antibacterial activity against MDR *Listeria* spp. The antagonistic activity may be due to functional groups present in the stabilized silver nanoparticles by the bioactive metabolites. This work also agrees with the report of Kanmanni and Lim (2013) who investigated the antibacterial activity of EPS-mediated SNPs against the pathogen *Escherichia coli*, the food-borne pathogen *Listeria monocytogenes*, and the MDR *Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

MIC was checked with two MDR *Listeria* spp. The SNPs that were used to check the MIC was 10mM, different MICs were recorded for the two (MDR) *Listeria* spp. Balashanmugam et al. (2013) reported the highest MIC of their SNPs on some pathogens and *Escherichia coli* and *Bacillus subtilis*. The functionalization effect of the biosynthesized SNPs and some antibiotics was examined and the combined effect was more efficient than the SNPs alone and even the antibiotics alone. This result is in line with the findings of Krishna et al. (2015) using the biogenic synthesis of silver nanoparticles who reported an increase in who report an increase in antimicrobial activity of combination of antibiotics and silver nanoparticles. Yua et al. (2021) reported the enhanced antibacterial properties of silver nanoparticles from secondary metabolites of *Bacillus subtilis* Silver nanoparticles biosynthesis using *Bacillus licheniformis* isolated from Quail manure from Vietnam has been reported (Tan et al., 2021).

In conclusion, the *Bacillus* strains metabolites exhibit anti-listeria activity against the MDR *Listeria* species. The metabolites bio-reduce $AgNO_3$ for SNPs biosynthesis. The biosynthesized silver nanoparticles and combination of silver nanoparticles with some antibiotics had an antimicrobial effect on MDR *Listeria* strains. Hence, SNPs in combination with some antibiotics could be used as an alternative to commercial antibiotics to combat MDR *Listeria* species.

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Statement of Human and Animal rights

This article does not contain any studies with human and animal subjects performed by any of the authors and complied with all ethical standards.

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