

# Journal of Advances in Microbiology Research



E-ISSN: 2709-944X

P-ISSN: 2709-9431

Impact Factor (RJIF): 6.2

JRM 2025; 6(2): 117-124

© 2025 JAMR

[www.microbiojournal.com](http://www.microbiojournal.com)

Received: 10-06-2025

Accepted: 08-07-2025

**Easter Godwin Nwokah**  
Department of Medical  
Microbiology, Faculty of  
Medical Laboratory Science,  
Rivers State University,  
Nkpolu-Oroworukwo, Port  
Harcourt, Nigeria.

**Gloria Chile Amadi-Wali**  
Department of Medical  
Microbiology, Faculty of  
Medical Laboratory Science,  
Rivers State University,  
Nkpolu-Oroworukwo, Port  
Harcourt, Nigeria.

**Vivian Nkemkanma Agi**  
Department of Medical  
Microbiology, Faculty of  
Medical Laboratory Science,  
Rivers State University,  
Nkpolu-Oroworukwo, Port  
Harcourt, Nigeria

## Correspondence

**Gloria Chile Amadi-Wali**  
Department of Medical  
Microbiology, Faculty of  
Medical Laboratory Science,  
Rivers State University,  
Nkpolu-Oroworukwo, Port  
Harcourt, Nigeria

## Biodiscovery of antibiotics-producing *Bacillus* species from soil samples obtained from waste dump sites in Port Harcourt, Nigeria

**Easter Godwin Nwokah, Gloria Chile Amadi-Wali and Vivian  
Nkemkanma Agi**

DOI: <https://www.doi.org/10.22271/micro.2025.v6.i2b.244>

### Abstract

*Bacillus* species have got considerable attention worldwide due to their ability to produce useful bioactive metabolites; these bioactive metabolites can be harnessed to tackle the current antimicrobial resistance the world is currently facing. The aim of this study was to evaluate antimicrobial potentials of some metabolites from *Bacillus* species recovered from soil samples obtained from waste dump site within Port Harcourt, Nigeria. *Bacillus* species isolated from soil samples obtained from waste dump site were subjected to different stress condition such as time, temperature and pH for the production of bioactive metabolites. Soil samples were collected from waste dump sites and transported to laboratory for sample processing. Parent culture of *Bacillus* species obtained from different soil depth were prepared for the exposure study by inoculating each *Bacillus* species into 1000µl of peptone water in test tubes and the pH of the medium was adjusted to 4.0 and 8.0 with HCl and were subjected to temperatures of 4°C, 40°C, and 45°C and then incubated for 72 hours. Already purified standard organisms were plated on Mueller Hinton agar plate and bacteria extract obtained from exposure study were dropped in the wells on the agar plate which and incubated for 24 hours at 37°C and the diameter of inhibition zones was measured in millimeter. There was no significant difference in the total heterotrophic bacteria count. *Bacillus* species and *Staphylococcus* species were consistent in the entire soil depths sampled. The result of antimicrobial susceptibility test showed that secretory molecule produced by *Bacillus* species isolated from 15cm soil depth were sensitive at the temperature of 4°C, 40°C and 45°C and at pH 8.0. The bacteria extract obtained from *Bacillus* species in this study exhibited strong antibacterial activity against *Escherichia coli*, *Klebsiella* species, *Pseudomonas* species and *Staphylococcus aureus* as seen in the antimicrobial susceptibility test. Gas chromatography/Mass spectrometry (GC-MS) analysis conducted on the bacteria extract identified these compounds Pentadecanoic acid, 14-methyl-, methyl ester, 9-Octadecenoic acid, methyl ester, 9,17-Octadecadienal, (Z), Squalene, Pentacosanoic acid, methyl ester, 9-Octadecenal (Z), 9,12-Octadecadien-1-ol, (Z,Z)- BicycloPentylidene, 1,E-8,Z-10-Tridecatriene, 1-Hexadecyne, 1,5,9,13-Tetradecatetraene and Hexacosanoic acid, methyl ester which have been proven to exhibit antimicrobials activity against several pathogenic organisms. It was concluded that Waste dump sites has the ability to harbour *Bacilli* with bioactive metabolites and these bioactive metabolites produced have antimicrobial potentials.

**Keywords:** Biodiscovery, Antibiotics, Bioactive metabolites, *Bacillus* species, waste dump site

### Introduction

A number of *Bacillus* species have been reportedly associated with bioactive secondary metabolites called antibiotics that have transformed healthcare by combating infectious disease (Kaspar *et al.*, 2019) [20]. Antibiotics are important and commercially exploited secondary metabolites produced by fungi and bacteria, majority of these antibiotics produced are used in recent times in treatment of life threatening infections and diseases. Research conducted on the antimicrobial activity of *Bacillus* species suggest that the antimicrobial activity of some *Bacillus* species could be due to the production of hydrolytic enzymes such as chitinases, chitosanases, cellulases, proteases, lipases and glucanases which effectively hydrolyse the major components of the fungal and bacteria cell wall (Milijakovic, *et al.*, 2020) [32]. *Bacillus* species are also known for their ability to produce different types of antibiotics with wide range of antimicrobial activity such as bacitracin, gramicidin etc. *Bacillus licheniformis* is known for its ability to produce bacitracin and *Bacillus brevis* for the production of gramicidin which is a linear polypeptide antibiotic.

Soil microbes (bacteria and fungi) are in charge of biomass breakdown, biogenic element circulation, thus, making nutrients available to plants, impurity biodegradation, and soil structure preservation (Furtak & Gajda, 2018) <sup>[17]</sup>. The presence of microorganisms in soil is determined by the chemical composition, moisture content, pH and structure of the soil. The soil house majority of bacteria but *Bacillus* are said to be predominant in the soil this could be because of their ability to form resistant endospore and produce vital antibiotics such as bacitracin among many others found inhibiting the growth of other organisms (Abdulkadir & Waliyu., 2012).

The need to expand the available pharmaceutical repertoire is underlined by several recent reports, including the 2019 Antibiotic Resistance Threat Report by the Centers for Disease Control and Prevention; this document states that in the United States alone, more than 2.8 million antibiotic-resistant infections and more than 35,000 related deaths occur each year (Kadri, 2019) <sup>[19]</sup>. These fatal infections are most frequently caused by the 18 species of bacteria and fungi listed as current urgent, serious, or concerning human health threats (Cox, 2019) <sup>[34]</sup>. Despite the continuous popularity of herbal medicine across the globe, traditional antibiotics have previously overshadowed the exploration of plant-based products as therapeutics. Notably, 26% of all new approved drugs and 33% of all new small-molecule approved drugs between 1981 and 2014 were botanical drugs, unaltered natural products, or derivatives thereof (Newman & Cragg, 2016) <sup>[25]</sup>. This abundance underscores the vast, untapped potential of plants around the world to yield desperately needed novel drugs. In fact, only around 6% of the 300,000 species of higher plants have been pharmacologically investigated (Cragg & Newman, 2013) <sup>[12]</sup>. However, recent reviews by Chassagne *et al.* (2020) <sup>[10]</sup> have highlighted the increasingly evident antibacterial properties of various plant species and phytochemicals. Indeed, mainstream medicine is increasingly receptive to the use of natural sources and plant-derived drugs, especially those to which antimicrobial resistance is more difficult or unlikely to develop (Newman & Cragg, 2016) <sup>[25]</sup> an example is garlic (*Allium sativum*) a widely consumed vegetable in Nigeria and a popular item in the diet of the Nepalese (Panthee *et al.*, 2006) <sup>[26]</sup>; it is a valuable food spice and lucrative commodity for income generation and its dependence is due to its medicinal properties. Agi & Azike (2019) <sup>[3]</sup> conducted a study on the antifungal action of garlic (*Allium sativum*) and ginger (zingiber officinale) on some pathogenic fungi and reported that garlic inhibited the growth and survival of pathogenic fungi species *Candida albicans*, *Aspergillus* species and *Penicillium* species. Furthermore, there is an increasing amount of evidence that suggests that phytochemicals may be used in conjunction with current antimicrobials to obtain synergistic effects (Borges *et al.*, 2016; Barbieri *et al.*, 2017; Ayaz *et al.*, 2019) <sup>[6, 8, 37]</sup>. Recent study found out that crude extracts of multiple plant species showed *in vitro* synergistic activity with existing antibiotics when used against two multidrug-resistant enteric bacterial species. Thus, the combination of phytochemicals and antibiotics may help to combat resistance to conventional monotherapies for many diseases. As evidence for the use of natural products to treat human disease continues to accumulate, it will become increasingly important to perform in-depth safety studies on the identified extracts, compounds, and their derivatives. So far,

there is a general consensus that natural products, as compared to synthetic drugs, have relatively low toxicity to mammals and have less harmful effects on non-target beneficial organisms which is yet another appealing aspect of utilizing plant species for the identification of effective pharmaceuticals.

Antimicrobial resistance (AMR) has now emerged as a chronic public health problem globally, with the forecast of 10 million deaths per year globally by 2050. AMR occurs when viruses, bacteria, fungi and parasites do not respond to antimicrobial treatments in humans and animals, thus allowing the survival of the microorganism within the host. The prominent cause contributing to the current crisis remains to be the overuse and misuse of antimicrobials, particularly the inappropriate usage of antibiotics, increasing the global burden of antimicrobial resistance. The global consumption and usage of antibiotics are therefore closely monitored at all times (Tang *et al.*, 2023) <sup>[31]</sup>.

The microbial world is diverse, prolific, and ubiquitous, consisting of all species that are too small to be seen with the naked eye. The microbial world is the foundation of global ecology, containing not just bacteria and viruses but also large numbers of many different forms of multicellular creatures. These presumably invisible organisms can be found in every ecological niche on the earth, including every human's surface, cavity, and cellular environment. Through their interactions with the wider ecology, the majority of these microbial communities are largely benign or even beneficial to their human hosts. However, a small number of predators are active, causing harm, sickness, and even death. Infectious diseases are still the most obvious impact of these infections (Michael *et al.*, 2014) <sup>[22]</sup>.

Antimicrobial agents, a class of compounds that are effective at limiting, preventing, or eliminating the growth of microbial predators, have been developed to battle infectious disease. The majority of these antimicrobials have their origins in natural goods, where they were first utilized to guard against microbial attack by diverse organisms (D'Costa *et al.*, 2011; Moellering, 2011) <sup>[36]</sup>. Many of these "natural" compounds have been changed by humanity to provide additional or amplified antibacterial action after being isolated and described (D'Costa *et al.*, 2011) <sup>[35]</sup>. Many of these antimicrobial drugs have activities that are unique to specific types of pathogens, while others may affect a broad spectrum of bacteria.

## 2. Materials and Methods

### 2.1 Study Area

This study was conducted in Nkpolu, Port harcourt, Rivers State. Port Harcourt is the capital and largest city of Rivers State, Nigeria. It is situated in the South-South geopolitical zone of Nigeria and lies between latitude 4°15' and 5°45' North, and longitude 6°20' and 7°35' East of the equator.

### 2.2 Study Design

This study employed experimental study design to determine the antimicrobial efficacy of secondary metabolites produced by *Bacillus* species isolated from different soil depth in waste dump site within Port Harcourt in Rivers State, Nigeria.

### 2.3 Sample Collection

Soil samples were obtained from two different locations within a particular waste dump site in Nkpolu. A loopful of

soil sample were obtained from soil surface, 5cm and 15cm soil depth and transferred into universal containers and transported to the laboratory for sample processing in a cold chain using Geostyle vaccine carrier.

## 2.4 Sample Analysis

One gram of the soil sample was weighed and diluted 10ml of normal saline and shaken vigorously for 2 minutes and was ten-fold serially diluted with normal saline up to  $10^6$ . A 0.1ml aliquot of ten-fold serial dilution of the sample was inoculated onto the Sabarouad, MacConkey agar and Nutrient agar plates using pour plate method. The plates were incubated at 37°C for 24 hours. Observations were made for bacteria growth. Visible colonies on the plates were counted (30-300) and recorded, based on the dilution factor used. Biochemical tests such as coagulase test, catalase test, gram staining, indole test, oxidase test, motility test, urease test, germ tube test, capsule stain test and KOH test were carried out.

## 2.5 Exposure Study

A parent culture of the *Bacilli* for exposure study was made by inoculating each *Bacilli* into 1000µl of peptone water in test tubes and incubated for 24 hours at 37°C. Each *Bacilli* was placed into two groups by transferring four millilitres (4mls) of the parent culture into two sterile test tubes containing peptone water, fructose, and maltose as carbon sources. The pH of group A and B were adjusted to 4.0 and 8.0 with hydrogen chloride (HCl) and were subjected to temperatures of 4°C, 40°C, and 45°C for 72 hours. After 48 hours of incubation 1.5ml of the parent culture were transferred into 2mls sterile test tubes using a sterile syringe and centrifuged at 3000rpm for ten minutes to obtain the supernatant (Bacteria extract) for the antimicrobial susceptibility testing, this process was repeated after 72 hours of incubation.

## 2.6 Antimicrobial Activity Testing

Standard strains of *Pseudomonas* species *Escherichia coli*, *Klebsiella* species, and *Staphylococcus aureus* obtained were inoculated in peptone water and incubated at 37°C for 48 hours. A cork borer was used to bore three (3) wells of 8mm in diameter on already prepared Mueller Hinton agar plate and using spread plate technique; the known organisms were plated on the Mueller Hinton agar plate. A 0.2ml of the supernatant obtained from the exposure study was dropped into the wells made on the Mueller Hinton agar plate using sterile 2mls syringe then incubated at 37°C for 24 hours. Zone of inhibitions were measured and recorded according to Clinical and Laboratory Standard Institute (CLSI) with reference to standard antibiotics used as control.

Standard antibiotics used as control include Ciprofloxacin (CIP 5ug), Piperacillin-tazobactam (TZP110ug), Levofloxacin (Lev 5ug), Cefuroxime (CXM 30ug), Meropenem (MRP 10ug) and Nitrofurantoin (F100ug).

## 2.7 Data Analysis

Statistical package for social sciences (SPSS) version 21.0 Statistical package was used for data analysis. Descriptive Statistical tools such as mean and standard deviation, Mann-Whitney U and Kruskal Wallistests were also used. Probability values less than or equal to 0.05 ( $p \leq 0.05$ ) were considered statistically significant.

## 3.0 Result

The result of total heterotrophic bacteria count show that 2(33.3%) *Bacillus* species and 4(66.7%) *Staphylococcus* species were isolated from 15cm soil depth while 2(25.0%) *Bacillus* species and 6(75.0%) *Staphylococcus* species were isolated from 5cm depth. A total of 8 isolates were obtained from soil surface sample of which 8(100.0%) were *Staphylococcus* species as seen in table 3.1 below.

**Table 3.1** Total heterotrophic bacteria count

Soil Depth (cm)	Isolates	Frequency	Percentage
15	<i>Bacillus</i> species	2	33.3
	<i>Staphylococcus</i> species	4	66.7
5	<i>Bacillus</i> species	2	25.0
	<i>Staphylococcus</i> species	6	75.0
Surface	<i>Staphylococcus</i> species	8	100.0

**Effect of secretory molecules produced at different stress temperature and pH on standard microorganisms as seen in antimicrobial susceptibility testing measured by zone of inhibition (ZI):** The result of antimicrobial susceptibility test showed that secretory molecule produced at 4°C and pH 4.0 by *Bacillus* species isolated from 15cm soil depth and soil surface were sensitive and intermediately sensitive with mean of ZI 15.33 and 14.53 respectively but secretory molecule produced at 4°C and pH 4 by *Bacillus* species isolated from 5cm soil depth did not cause inhibition while secretory molecule produced at 4°C and pH 8 by *Bacillus* species isolated from soil surface and 15cm soil depth were sensitive with mean of ZI of 15.17 but secretory molecule produced at 4°C and pH 8 by *Bacillus* species isolated from 5cm soil depth produced an intermediate result with the mean of ZI 12.38.

Secretory molecule produced at the temperature of 40°C and pH 4 by *Bacillus* species isolated from soil surface was sensitive with mean of ZI 17.35 while those isolated from 15cm and 5cm were resistant with mean of zone of

inhibition 11.00 and 10.75 respectively. Secretory molecule produced at the temperature of 40°C and pH 8 by *Bacillus* species isolated from 15cm soil depth intermediate while that of 5cm and soil surface were resistant with mean of zone of inhibition of 10.50 and 13.50 respectively.

Secretory molecule produced at the temperature of 45°C and pH 4 by *Bacillus* species isolated from 5cm soil depth did not cause inhibition while *Bacillus* species isolated from soil surface and 15cm soil depth were resistant with mean of zone of inhibition 12.88 and 14.42 respectively while at the same temperature and pH 8 Secretory molecule produced by *Bacillus* species isolated from 15cm soil depth produced a sensitive result with mean of zone of inhibition measuring 19.00 while those isolated from 5cm and soil surface were resistant with mean of zone of inhibition of 11.50 and 14.25 respectively as seen in antimicrobial susceptibility testing with reference to standard antibiotics used as control according to CLSI standard all is represented in the table 3.2 below.

**Table 3.2:** Showing the effect of secretory molecules produced at different stress temperature and pH on standard microorganisms as seen in antimicrobial susceptibility testing measured by zone of inhibition (ZI)

Stress Temp (°C)	Stress pH	Soil Depth	ZI(mm) Mean	ZI(mm) Std. Error	95% Confidence Interval	
					Lower	Upper
4	4	15cm	15.33	1.53	12.32	18.35
		5cm	*	*	*	*
		Surface	14.15	0.95	12.28	16.03
	8	15cm	15.17	1.21	12.78	17.55
		5cm	12.38	1.37	9.68	15.07
		Surface	15.17	1.43	12.34	17.99
40	4	15cm	11.00	1.62	7.79	14.20
		5cm	10.75	1.15	8.49	13.01
		Surface	17.35	0.70	15.97	18.74
	8	15cm	15.70	1.25	13.24	18.16
		5cm	10.50	1.62	7.29	13.70
		Surface	13.50	1.82	9.92	17.08
45	4	15cm	12.88	0.77	11.37	14.40
		5cm	*	*	*	*
		Surface	14.42	0.64	13.17	15.68
	8	15cm	19.00	1.62	15.79	22.20
		5cm	11.50	2.29	6.97	16.03
		Surface	14.25	1.28	11.72	16.78

\* This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable. ZI = Zone of Inhibition

### Detection of Secretory Molecules/Metabolites under Two (Double) Combined Stress

#### Conditions

The mean of zone of inhibition of *Bacillus species* stressed for 48hours at the temperature of 4°C, 40°C and 45°C is

12.83, 20.00 and 12.00 respectively while for 72hours, the mean of ZI at 4°C, 40°C and 45°C is thus 15.00, 14.28 and 12.50 respectively. The highest mean of ZI (15.36) was seen at 72hours and within the temperature of 30°C while the lowest mean of ZI was recorded at 48hours and within the temperature of 45°C as seen in table 3.3 below.

**Table 3.3:** Detection of Secretory Molecules/Metabolites under Combined Stress Conditions

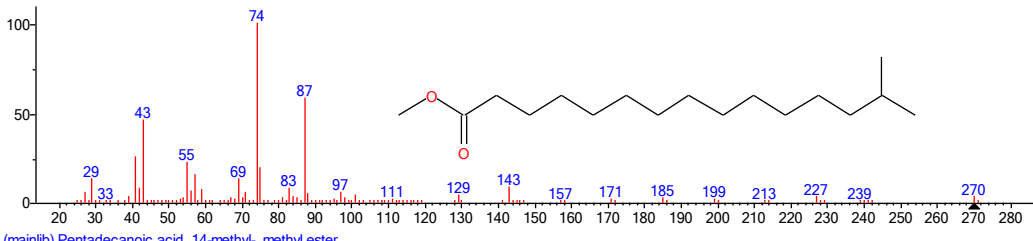
Time	Temp (°C)	ZI (mm)Mean	ZI (mm) Std. Error	95% Confidence Interval	
				Lower	Upper
48hr	4	12.83	1.53	9.82	15.85
	40	20.00	1.87	16.30	23.69
	45	12.00	2.29	7.47	16.53
72hr	4	16.50	1.25	14.04	18.96
	40	14.28	0.71	12.88	15.69
	45	12.50	1.62	9.29	15.70

### Gas Chromatography - Mass Spectrometry (GC/MS) Report of Bacteria Extract Obtained from *Bacillus Species* isolated from Waste Dump Site.

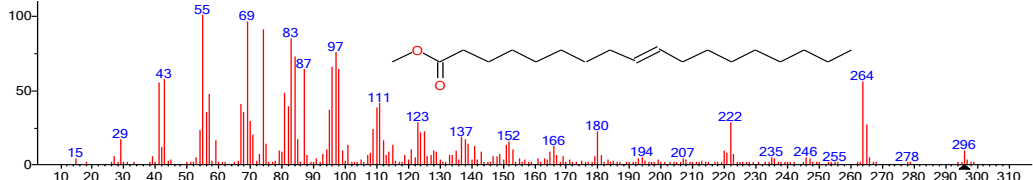
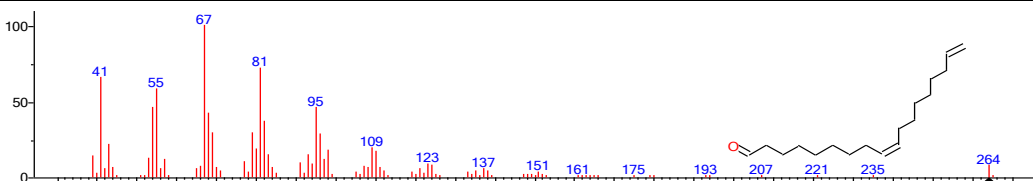
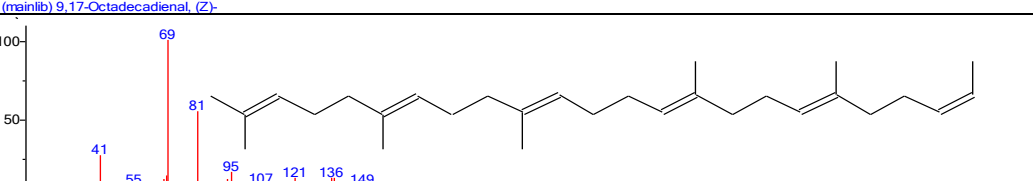
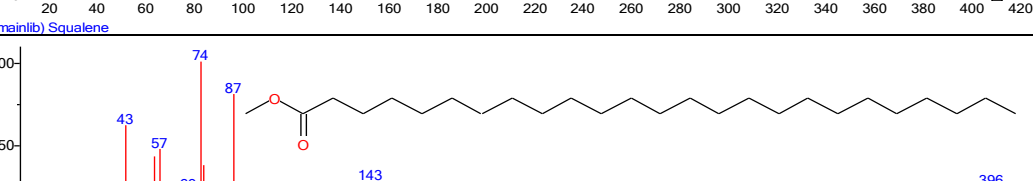
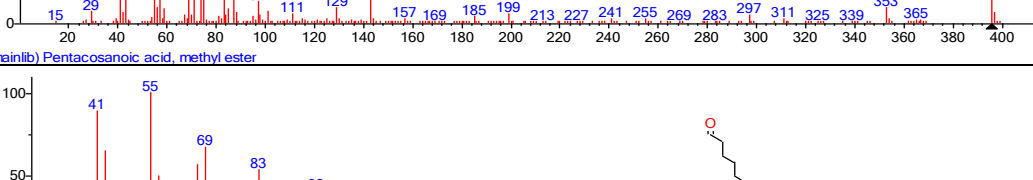
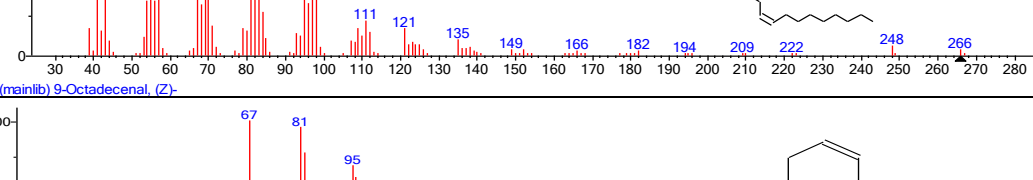
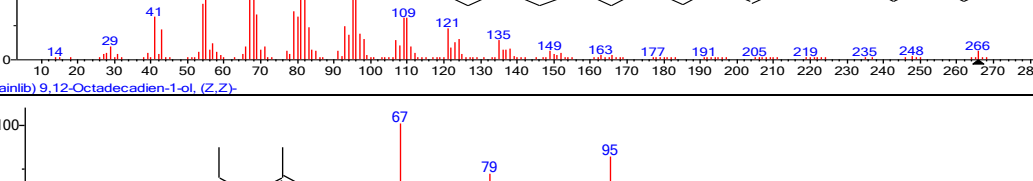
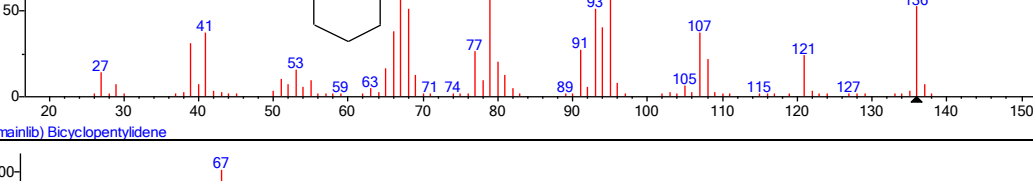
A thorough GC-MS analysis to identify the precise compounds present in the bacteria extract obtained from

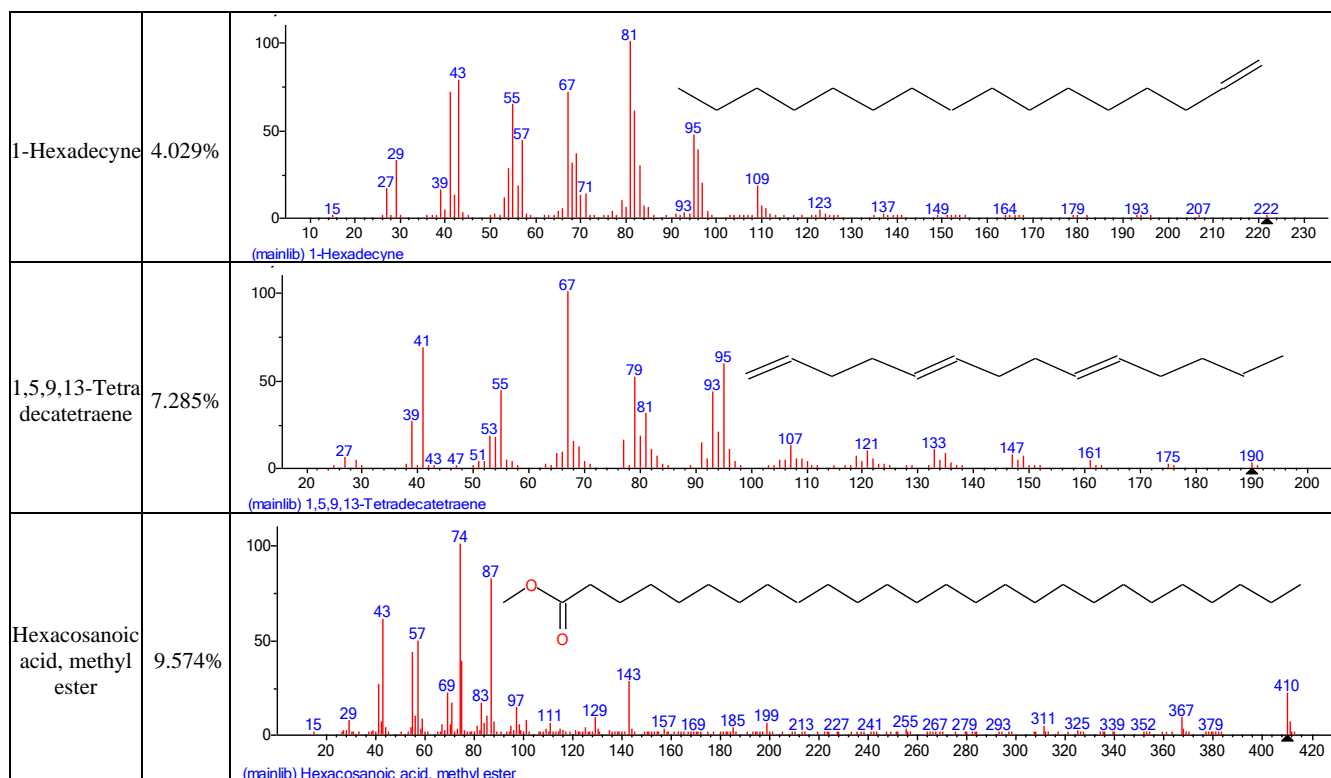
*Bacillus species* isolated from soil sample obtained from waste dump site in order to comprehend the compositions of the secondary metabolites produced by these *Bacillus species*. The quantities, structures and compounds contained in these bacteria extract is represented in the table 3.4below.

**Table 3.4:** Gas Chromatography - Mass Spectrometry (GC/MS) Report of Bacteria Extract Obtained from *Bacillus Species* isolated from Waste Dump Site.

Compounds Present in Bacteria Extract obtained from <i>Bacilli</i>	Quantity	Structure
Pentadecanoic acid, 14-methyl-, methyl ester	3.247%	 <p>(mainlib) Pentadecanoic acid, 14-methyl-, methyl ester</p>



9-Octadecenoic acid, methyl ester	7.352%	 <p>(mainlib) 9-Octadecenoic acid, methyl ester, (E)-</p>
9,17-Octadecadienal, (Z)-	2.779%	 <p>(mainlib) 9,17-Octadecadienal, (Z)-</p>
Squalene	26.224%	 <p>(mainlib) Squalene</p>
Pentacosanoic acid, methyl ester	8.946%	 <p>(mainlib) Pentacosanoic acid, methyl ester</p>
9-Octadecenal, (Z)-	19.692%	 <p>(mainlib) 9-Octadecenal, (Z)-</p>
9,12-Octadecadien-1-ol, (Z,Z)-	2.749%	 <p>(mainlib) 9,12-Octadecadien-1-ol, (Z,Z)-</p>
Bicyclo Pentylidene	3.861%	 <p>(mainlib) Bicyclopentylidene</p>
1,E-8,Z-10-Tridecatriene	2.282%	 <p>(mainlib) 1,E-8,Z-10-Tridecatriene</p>



#### 4. Discussion

Several *Bacillus* species have been applied as agent for control of plant pathogens and antimicrobial drugs (Mondol *et al.*, 2013) [24]. Certain chemicals of various compositions have been known to be produced by these *Bacillus* species and some of these compounds have demonstrated antimicrobial properties that have been widely employed against certain other microorganisms (Sethi *et al.*, 2013) [29]. In this present study, the antimicrobial potential of novel *Bacilli* have been evaluated by isolating *Bacillus* species from soil samples obtained from waste dump site in Nkpolu area in Port Harcourt, Rivers State Nigeria. We then analyzed the secondary metabolites produced by these *Bacillus* species and assessed their antibiotic capabilities when the *Bacillus* species are stressed at different times, temperatures, and pH. This was done in order to explore the antimicrobial potentials of *Bacillus* species isolated from waste dump site. Soil profiles are often many meters deep but with the majority of studies in soil microbiology focusing exclusively on the soil surface, we know little about the nature of the microbial communities inhabiting the deeper horizons. Fierer *et al.*, 2013 [16] used phospholipids fatty acid (PLFA) analysis to examine the vertical distribution of specific microbial abundance and community-level with the soil profile. The frequency of distribution of isolates from the various soil depths sampled in this study which include soil surface, 5cm and 15cm depth showed that *Bacillus* species and *Staphylococcus* species were consistent in the entire soil depths sample. (Cheng *et al.*, 2022) [11].

There was generally no significant difference in the total heterotrophic count of bacteria in the three different soil depths sampled. However, the surface sample produced the highest total heterotrophic count (THC) of bacteria, then the 5cm depth sample while 15cm soil depth produced the lowest THC. The observation is that, as the depth increased, the number of isolated bacteria decreased. The physico-chemical and structural characteristics of soil provide

suitable environments in which complex bacterial populations evolve (Ranjard & Richaume, 2018) [27].

Akeed *et al.* 2020 [4] investigated the influence of incubation temperature on enzyme production of *B. licheniformis* B<sub>307</sub>, results showed that temperature significantly influenced production and the study conducted by Sidorova *et al.*, (2020) [30] on optimization of laboratory cultivation conditions for the synthesis of antifungal metabolites by *Bacillus subtilis* strains showed that temperature and pH influence the synthesis of antifungal metabolites. In this study, *Bacillus* species were subjected to different stress time, temperature and pH and at these stress conditions the stressed *Bacilli* were able to release some secretory molecules which were measured by the various zone of inhibitions produced as evident in the antimicrobial susceptibility testing with reference to standard antibiotic used as control and recorded according to Clinical Laboratory Standard Institute (CLSI) standard. The result of antimicrobial susceptibility test showed that secretory molecule produced by *Bacillus* species isolated from 15cm soil depth were sensitive at the temperature of 40°C and 45°C and at pH 8.0 with mean of ZI (15.17, 15.70 and 19.00) while at pH 4.0 the same *Bacillus* species produced secretory molecule with sensitive result (mean of ZI 15.33) only at 4°C. Secretory molecule produced by *Bacillus* species isolated from soil surface were sensitive only at temperature of 40°C and pH 4 with mean of ZI 17.35 and temperature of 4°C and pH 8.0 with mean of ZI 15.17 while the lowest mean of ZI (12.38, 10.50 and 11.50) was recorded at temperature 4°C, 40°C, 45°C and pH 8.0 by secretory molecule produced by *Bacillus* species isolated from 5cm soil depth of which did not cause inhibition at temperature of 4°C, 40°C and pH 4.0 (Horak *et al.*, 2021, Sidorova *et al.*, 2020) [30, 38].

The analysis of bacteria extracts through GC-MS indicated the presence of different compounds such as Pentadecanoic acid, 14-methyl-, methyl ester, 9-Octadecenoic acid, methyl ester, 9,17-Octadecadienal, (Z), Squalene, Pentacosanoic

acid, methyl ester, 9-Octadecenal (Z), 9,12-Octadecadien-1-ol, (Z,Z)- BicycloPentylidene, 1,E-8,Z-10-Tridecatiene, 1-Hexadecyne, 1,5,9,13-Tetradecatetraene and Hexacosanoic acid, methyl ester that have antimicrobial potentials (El Damhougy *et al.*, 2017)<sup>[14]</sup>. These compounds are similar to those identified by Sadiqi *et al* 2022<sup>[28]</sup>. The GC-MS analysis that was conducted by Sadiqi *et al.*, 2022<sup>[28]</sup> that researched on the molecular characterization of bacterial isolates from soil samples and evaluation of their antibacterial potential against multi-drug resistant strain (MDRS) identified antimicrobial compounds such as propanoic acid, oxalic acid, phenol and hexadecanoic acid. The study conducted by Sadiqi *et al* 2022<sup>[28]</sup> revealed that all bacteria extracts possess activity against Gram-negative and Gram-positive bacteria at a concentration of 5 mg/ml. These compounds have been shown to exhibit antimicrobials activity against *Escherichia coli*, *Lactobacillus spp.*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* (Cueva *et al.*, 2010, Sadiqi *et al* 2022)<sup>[13, 28]</sup>. The bacteria extract obtained from *Bacillus* species in this study exhibited strong antibacterial activity against *Escherichia coli*, *Klebsiella species*, *Pseudomonas species* and *Staphylococcus aureus* as seen in the antimicrobial susceptibility test. The medicinal qualities of compounds derived from microorganisms are now a crucial component of global healthcare (Kaspar *et al.*, 2019)<sup>[20]</sup>. Thus, novel bioactive compounds should be taken into account for their potential medicinal applications, including been used as antifungal and antibiotic drugs, as well as for protecting human and environmental health (Kumah *et al.*, 2017, Al-Ajlani & Hasnain, 2010)<sup>[5, 39]</sup>.

## Conclusion

This study revealed that secretory molecules produced by *Bacilli* isolated from waste dump site were able to inhibit the growth of pathogenic organisms and also *Bacillus* species with varying degrees of antibiotic potentials are domicile in waste dump site.

This study concluded that: time, temperature and pH play a crucial role in the production of bioactive metabolites and at high temperatures production of secretory molecules is reduced.

Gas chromatography/Mass spectrometry (GC-MS) analysis revealed the presence of compounds with antimicrobial properties in the bacteria extract.

## References

1. Abdulkadir M, Waliya S. Screening and isolation of soil bacteria for ability to produce antibiotics. *Eur J Appl Sci*. 2012;4(5):211-2115.
2. Abida H, Ruchaud S, Rios L, Humeau A, Probert I, De Vargas C, *et al.* Bioprospecting marine plankton. *Mar Drugs*. 2013;11(11):4594-611.
3. Agi VN, Azike CA. Antifungal action of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) on some pathogenic fungi. *Asian J Res Biochem*. 2019;4(4):1-6.
4. Akeed Y, Atrash F, Naffaa W. Partial purification and characterization of chitinase by *Bacillus licheniformis* B307. *Heliyon*. 2020;6(5):85-90.
5. Al-Ajlani MM, Hasnain S. Bacteria exhibiting antimicrobial activities; screening for antibiotics and the associated genetic studies. *Open Conf Proc J*. 2010;6(5):230-238.
6. Ayaz M, Ullah F, Sadiq A, Ullah F, Ovais M, Ahmed J, *et al.* Synergistic interactions of phytochemicals with antimicrobial agents: potential strategy to counteract drug resistance. *Chem Biol Interact*. 2019;308(12):294-303.
7. Baribieri R, Coppo E, Marchese A, Daglia M, Sobarzo Sanchez E, Nabavi SM. Phytochemicals for human disease: an update on plant derived compounds antibacterial activity. *Microbiol Res*. 2017;196(8):44-68.
8. Borges A, Abreu AC, Dias C, Saavedra MJ, Borges F, Simões M. New perspectives on the use of phytochemicals as an emergent strategy to control bacterial infections including biofilms. *Molecules*. 2016;21(7):877.
9. Chandra N, Kumar S. Antibiotics producing soil microorganisms. In: *Antibiotics and Antibiotics Resistance Genes in Soils: Monitoring, Toxicity, Risk Assessment and Management*. *Soil Biol*. 2017;51:1-18.
10. Chassagne F, Samarakoon T, Porras G, Lyles JT, Dettweiler M, Marquez, *et al.* A systematic review of plants with antibacterial activities: a taxonomic and phylogenetic perspective. *Front Pharmacol*. 2021;11(8):2069-83.
11. Cheng X, Zhang Y, Xing W. Depth-dependent patterns in the C:N:P stoichiometry of different soil components with reclamation time in coastal poplar plantations. *Soil Tillage Res*. 2022;22(3):105494-9.
12. Cragg GM, Newman DJ. Natural products as sources of novel drug leads. *Biochim Biophys Acta Gen Subj*. 2013;1830(6):3670-3695.
13. Cueva C, Moreno-Arribas MV, Martín-Alvarez PJ, Bartolomé B. Antimicrobial activity of phenolic acids against commensal, probiotic and pathogenic bacteria. *Res Microbiol*. 2010;161(5):3372-3382.
14. El Damhougy KA, Mansour AEB, Hussein AN, Hassan AHI, Fekry MAS. GC/MC analysis of bioactive components of *Callyspongia crassa* (Porifera) from Gulf of Aqaba Red Sea (Egypt). *Al Azhar Bull Sci*. 2017;9(2):111-8.
15. Elsayed TR, Galil DF, Sedik MZ, Hassan HM, Sadik MW. Antimicrobial and anticancer activities of actinomycetes isolated from Egyptian soils. *Int J Curr Microbiol Appl Sci*. 2020;9(9):1689-700.
16. Fierer N, Schimel JP, Holden PA. Variations in microbial community composition through two soil depth profiles. *Soil Biol Biochem*. 2013;35(1):167-76.
17. Furtak K, Gajda AM. Activity and variety of soil microorganisms depending on the diversity of the soil tillage system. *Sustainability Agroecosyst*. 2018;4(5):211-226.
18. Hark I, Van Rensburg PJJ, Claassens S. Effect of cultivation media and temperature on metabolite profiles of three nematocidal *Bacillus* species. *Nematology*. 2021;24(4):383-399.
19. Kadri SS. Key takeaways from the US CDC's 2019 antibiotic resistance threats report for frontline providers. *Crit Care Med*. 2020;48(7):939-945.
20. Kaspar F, Neubauer P, Gimpel M. Bioactive secondary metabolites from *Bacillus subtilis*: a comprehensive review. *J Nat Prod*. 2019;82(7):2038-2053.
21. Kumar S, Kumar PRK, Narasimhan B. Synthesis, antimicrobial evaluation and QSAR studies of propionic acid derivatives. *Arab J Chem*. 2013;10(1):2-7.

22. Michael CA, Dominey-Howes D, Labbate M. The antimicrobial resistance crisis: causes, consequences, and management. *Front Public Health*. 2014;2(11):657-665.
23. Miljakovic J, Balesevic-Tubic S. The significance of *Bacillus* spp. in disease suppression and growth promotion of field and vegetable crops. *Microorganisms*. 2020;8(7):1037-1041.
24. Mondol MAM, Shin HJ, Islam MT. Diversity of secondary metabolites from marine *Bacillus* species: chemistry and biological activity. *Mar Drugs*. 2013;11(8):2846-2872.
25. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. *J Nat Prod*. 2016;79(3):629-661.
26. Panthee DR, Kc RB, Regmi HN, Subedi PP, Bhattarai S, Dhakal J. Diversity analysis of garlic (*Allium sativum* L.) germplasms available in Nepal based on morphological characters. *Genet Resour Crop Evol*. 2006;53:205-212.
27. Ranjard L, Richaume AS. Quantitative and qualitative microscale distribution of bacteria in soil. *Res Microbiol*. 2018;1(52):707-716.
28. Sadiqi S, Hamza M, Ali F, Alam S, Shakeela Q, Ahmed S, *et al*. Molecular characterization of bacterial isolates from soil samples and evaluation of their antibacterial potential against MDRS. *Molecules*. 2022;2(7):6281-9.
29. Sethi S, Kumar R, Gupta S. Antibiotic production by microbes isolated from soil. *Int J Pharm Sci Res*. 2013;4(8):2967-3000.
30. Sidorova TM, Asaturova AM, Homyak AI, Zhevnova NA, Shternshis MV, Tomashevich NS. Optimization of laboratory cultivation conditions for the synthesis of antifungal metabolites by *Bacillus subtilis* strains. *Saudi J Biol Sci*. 2020;27(7):1879-1885.
31. Tang KWK, Millar BC, Moore JE. Antimicrobial resistance (AMR). *Br J Biomed Sci*. 2023;80:387-395.
32. Milijaković D, Stanojkovic-Sebic A, Marjanovic M. Effect of pesticides on soil microorganisms. *Pestic Fitomed*. 2020;35(1):9-15.
33. Abdulkadir A, Waliyu S. Role of microbial biofertilizers in the development of sustainable agriculture in Nigeria. *Int J Agric Biosci*. 2012;1(5):80-83.
34. Cox C. Pesticides and health: What you need to know. *J Pestic Reform*. 2019;39(3):4-9.
35. D'Costa VM, King CE, Kalan L, Morar M, Sung WWL, Schwarz C, *et al*. Antibiotic resistance is ancient. *Nature*. 2011;477(7365):457-461.
36. Moellering RC Jr. Discovering new antimicrobial agents. *Int J Antimicrob Agents*. 2011;37(1):2-9.
37. Barbieri N, Mangiaterra G, Dominguez Rubio AP, Pérez Chaia A, Nader-Macías ME, Vinderola G. Safety evaluation and antimicrobial activity of lactic acid bacteria isolated from raw goat milk. *J Dairy Res*. 2017;84(4):445-456.
38. Horak I, Hlisnikovský L, Kintl A, Elbl J, Lošák T, Vítěz T. Effect of organic and mineral fertilizers on soil microbial communities: A review. *Sustainability*. 2021;13(7):3840.
39. Kumah DB, Akuffo SB, Aidoo R. Pesticide use in vegetable production and its impact on the health of farmers in selected districts of Ghana. *Afr J Agric Res*. 2017;12(24):2074-2080.

**How to Cite This Article**

Agi VN, Amadi-Wali GC. Biodiscovery of antibiotics-producing *bacillus* species from soil samples obtained from waste dump sites in Port Harcourt, Nigeria. *Journal of Advances in Microbiology Research*. 2025;6(2):117-124.

**Creative Commons (CC) License**

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 International (CC BY-NC-SA 4.0) License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.