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Optimizing the production and efficacy of antimicrobial compounds obtained from *bacillus* species isolated from agricultural sites in Port Harcourt, Nigeria

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Abstract
Antimicrobial resistance has become a global health challenge because infectious microbes continue to develop resistance against available antibiotics. Hence, the urgent need to search for novel antibiotic drugs. This study is aimed at optimizing the production and efficacy of antimicrobial compounds obtained from *Bacillus* species isolated from agricultural sites in Port Harcourt, Nigeria. *Bacillus* species were isolated from agricultural sites and identified using standard culture techniques. These *Bacillus* species were stressed at 30^0C, 40^0C and 45^0C at the pH of 4.0 and 8.0 for 72 hours. The bacterial extract obtained were screened for antimicrobial activity using agar well diffusion technique of the Modified Kirby-Bauer antibiotic susceptibility test against already identified microorganism including *Escherichia coli, Klebsiella* species, *Pseudomonas* species *and Staphylococus aureus*. Visible zone of inhibitions were measured with reference to standard antibiotics used as control. The result of total heterotrophic bacteria counts showed that there was no significant difference in the total heterotrophic bacteria counts of the different depths sampled (p=0.091). At 72hours of incubation, the highest mean of zone of inhibition was recorded at 30^0C (15.36mm) which is moderately sensitive while at 48hours the lowest mean of zone of inhibition was recorded at 45^0C (12.49mm). The secretory molecules produced at acidic and alkaline pH by *Bacilli* isolated from soil surface and 15cm soil depth were moderately sensitive. The mean of zone of inhibitions (ZI) showing the effect of time on isolates from different soil depth showed that the highest mean of ZI was spotted in soil surface at 48hours and 72hours of incubation with an increase in ZI of 2.14mm (ZI 14.36 and 16.50 respectively) after 72 hours of incubation while 5cm depth had the lowest mean of ZI at 48 hours and 72hour (12.17 and 12.98 respectively) with an increase in ZI of 0.81mm after 72hours of incubation. It was discovered that increase in incubation time did not significantly increase the zone of inhibition. Gas Chromatography - Mass Spectrometry (GC-MS) analysis of the bacteria extracts revealed compounds contained in Bacteria extract as Cyclohexasiloxane, dodecamethyl, Dodecanoic acid, 1H-Pyrrolo[3,4-d]pyrimidine-2,5-di one, 4,6-bis(4-hydroxyphenyl), Methyl tetradecanoate, 1H-Pyrrolo[3,4-d]pyrimidine-2,5-di one, 4,6, bis(4-hydroxyphenyl), Cyclododecane, 1-Cyclopenteneacetic acid, 5-oxo-Bicyclo[4.1.1]oct-2-ene. In conclusion, the secretory molecules produced by *Bacilli* isolated from the soil sampled in this study have antibiotic potential and are chemically similar to the molecules found in phytochemicals such as flavonoids, alkaloids, terpenoids, and phenolic compounds.

Keywords: Optimization, production, efficacy, antimicrobial, bioactive, *bacillus* species, agricultural sites

Introduction
Antimicrobials- including antibiotics, antivirals, antifungals, and antiparasitics are medicines used to prevent and trait infectious diseases in humans, animals and plants.
Antimicrobial Resistances (AMR) occurs when bacteria, viruses, fungi and parasites no longer respond to antimicrobial medicines. As a result of drug resistance antibiotics and other antimicrobial medicines become ineffective and infective and infections become difficult or impossible to treat, increasing the risk of disease spread, severe illness, disability and death.
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 (de Kraker *et al.*, 2016) [30]. 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 the overuse and misuse of antimicrobials (Salam *et al.*, 2023) [29], 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, Millar & Moore, 2023) [20]. 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, Sameer,. 2019) [15]. 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, Michael. 2019) [28]. 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) [16]. 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) [8]. However, recent reviews by Chassagne *et al.* (2020) [6] 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) [16] 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) [17]; it is a valuable food spice and lucrative commodity for income generation and its dependence is due to its medicinal properties. Agi & Azike (2019) [1] 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 Penicillum species.

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) [3, 4, 5]. 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.

Unfortunately, improper stewardship of antimicrobial agents has helped lead to the tremendous resistance issue that we now face. Factors that have contributed to the growing resistance problem include: increased consumption of antimicrobial drugs, both by humans and animals; and improper prescribing of antimicrobial therapy. Overuse of many common antimicrobials agents by physicians may occur because the choice of drug is based on a combination of low cost and low toxicity (Griffith et al, 2012) [14]. There may also be improper prescribing of antimicrobials drugs, such as the initial prescription of a broad-spectrum drug that is unnecessary, or ultimately found to be ineffective for the organism (s) causing the infection (Victor, 2011) [27]. The danger is that excessive use of antibiotics in humans leads to emergence of resistant organisms. In addition, prior use of antimicrobial drugs puts a patient at risk for infection with a drug resistant organism, and those patients with the highest exposure to antimicrobials are most often those who are infected with resistant bacteria (Griffith *et al.*, 2012) [14].

2. Materials and Methods

2.1 Study Area

This study was conducted in Oro-Igwe in Obio/Akpor Local Government Areas in Port Harcourt in Rivers State Nigeria. The study area, 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 area in Rivers State, Nigeria.

2.3 Sample Collection

Soil samples were obtained from two different locations within the waste dump site and from each location loopful of soil sample were obtained from soil surface, 5cm and 15cm soil depth into universal containers and transported to the laboratory in a cold chain using Geostyle vaccine carrier for sample processing.

2.4 Sample Analysis: One gram of the soil sample was weighed and made up to 10ml of normal saline and shaken vigorously for 2 minutes and was ten-fold serially diluted with normal saline up to 106. 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 37oC 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 37oC. 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 30oC, 40oC, and 45oC for 72hours. After 48hours 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 for the antimicrobial susceptibility testing. After 72 hours of incubation, this process was repeated.

2.6 Antimicrobial Activity Testing

From University of Port Harcourt teaching hospital microbiology laboratory already characterised standard microorganisms were obtained which includes Pseudomonas species Escherichia coli, Klebsiella species, and Staphylococcus aureus were inoculated in peptone water and incubated at 37oC for 48hours. A cork borer was used to create three (3) wells of 8mm in diameter on already prepared Mueller Hinton agar plate 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 37oC 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 Wallis tests were also used. Probability values less than or equal to 0.05 ($p \leq 0.05$) were considered statistically significant.

3.0. Results

Total heterotrophic bacteria count

The result of total heterotrophic bacteria count show that 6(37.5%) Bacillus species and 10(62.5%) Staphylococcus species were isolated from 15cm soil depth while 6(37.50%) Bacillus species and 6(75.0%) Staphylococcus species were isolated from 5cm depth. A total of 18 isolates were obtained from soil surface sample of which 18(100.0%) were Staphylococcus species as seen in table 3.1below.

Table 3.1: Total heterotrophic bacteria count

Soil Depth (cm)	Isolates	Frequency	Percentage
15	*Bacillus* species	6	37.5
	Staphylococcus species	10	62.5
5	*Bacillus* species	6	37.5
	Staphylococcus species	10	62.5
Surface	*Staphylococcus* species	18	100.0

Mean Total Heterotrophic Count of Bacteria Isolates by Soil Depth: The comparison of the mean total heterotrophic count of samples by soil depth is shown in table 3.2 below. The mean ± SD of the surface, 5cm and 15cm depths were 1908.33±1443.538 CFU/ml, 1937.50±1426.153 CFU/ml and 1745.63±1257.267 CFU/ml respectively. There was no significant difference in the total heterotrophic bacteria counts of the different depths (p=0.091) as represented in table 3.1

Table 3.2: Mean Total Heterotrophic Count of Bacteria Isolates by Soil Depth

Soil Depth	Mean ± SD (CFU/ml)
Surface (N=18)	1908.30 ± 1443.50
5cm (N=16)	1937.50 ± 1426.10
15cm (N=16)	1745.60 ± 1257.20
Total (N=50)	1865.60 ± 1355.10
p-value	0.091
F-value	0.913

Mean Detection of Secretory Molecule/Metabolites under one Stress Time: The Bacillus species isolated were stressed at different time and temperature and under this unfavorable condition they released some secretory molecules which were tested for antibiotic potentials by testing them against already identified standard microorganism and zone of inhibition (ZI) measured in millimeter and recorded according to CLSI standard. A comparison of mean of time and soil depth showed that secretory molecules produced by Bacillus species isolated from 5cm soil depth had the lowest mean of zone of inhibition (ZI) at 48 hours and 72hour (12.17mm and 12.98mm respectively). The highest mean of ZI was spotted in secretory molecules produced by Bacillus species isolated from Surface sample at 48hours and 72hours (14.36mm and 16.50mm respectively) as represented in table 4.7 below.

Table 3.3: Mean Detection of Secretory Molecule/Metabolites under one Stress Time

Time	Soil surface	5cm	15
Zone of Inhibition (mm)			
48hr	14.38	12.17	13.94
72hr	16.50	12.98	14.83

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 30oC, 40oC and 45oC is 14.37, 14.51 and 12.49 respectively while for 72hours, the mean of Zone of Inhibition at 4o30oC, 40oC and 45oC is thus 14.96, 15.36, 14.28 and 15.35 respectively. The highest mean of ZI (15.36) was seen at 72hours and within the temperature of 30oC while the lowest mean of ZI was recorded at 48hours within the temperature of 45oC.

Table 3.4 Detection of Secretory Molecules/Metabolites under Two

Time	30^{0C}	40^{0C}	45^{0C}
Zone of Inhibition (mm)			
48hr	14.37	14.51	12.49
72hr	15.36	14.96	15.35

Detection of Secretory Molecules/Metabolites under Two Combined Stress Conditions

The combination of stress temperature and pH showed that the highest mean of zone of inhibition was recorded at pH of 4.0 and temperature of 300C (16.06mm) while the lowest mean of zone of inhibition was recorded at pH 4.0 and temperature of 450C (13.80mm). At pH 8.0, the lowest mean of zone of inhibition was recorded at 300C (13.27mm) while at 400C and 450C the mean of zone of inhibition of 14.05mm and 14.58mm respectively was recorded.

Table 3.5: Detection of Secretory Molecules/Metabolites under Two

Temperature	4	8
Zone of Inhibition (mm)		
30	16.06	13.27
40	14.66	14.05
45	13.80	14.58

Detection of Secretory Molecules under Two (Double) Combined Stress Conditions

The highest mean of zone of inhibition was recorded at 72 hours and at pH 4.0 while the lowest mean of zone of inhibition was recorded at 48 hours and at pH 8.0 as represented the table

Table 3.6: Detection of Secretory Molecules under Two (Double) Combined Stress Conditions

Time	4	8
Zone of Inhibition (mm)		
48hr	14.20	13.35
72hr	15.53	14.40

Gas Chromatography: Mass Spectrometry (GC/MS) Report of Bacteria Extract Obtained from Bacillus Species Isolated from Agricultural 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 agricultural 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 as shown by the GC-MS is represented in the table 3.7 below.

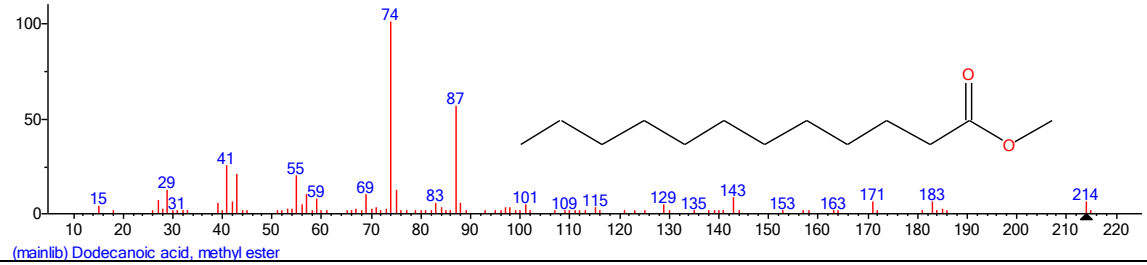
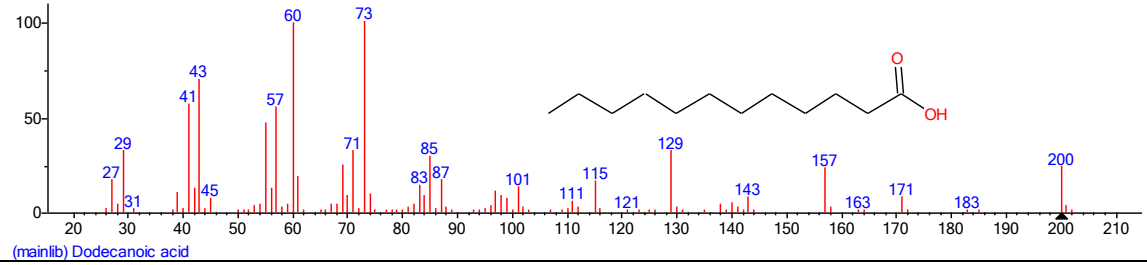
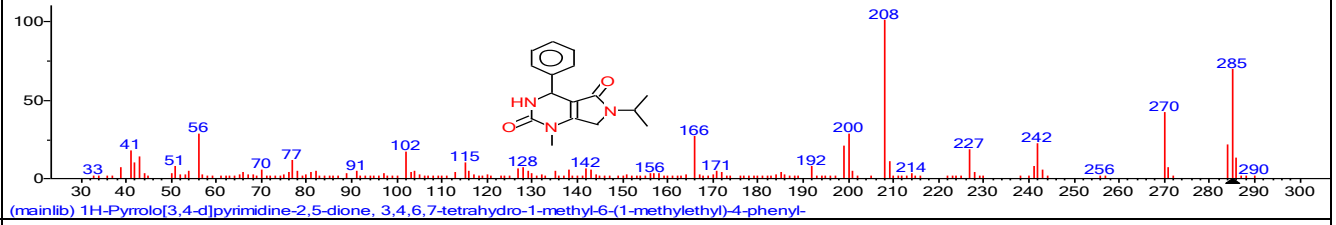
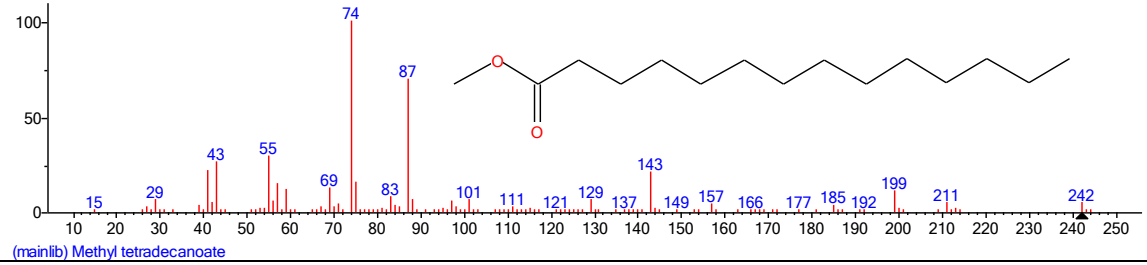
4. Discussion

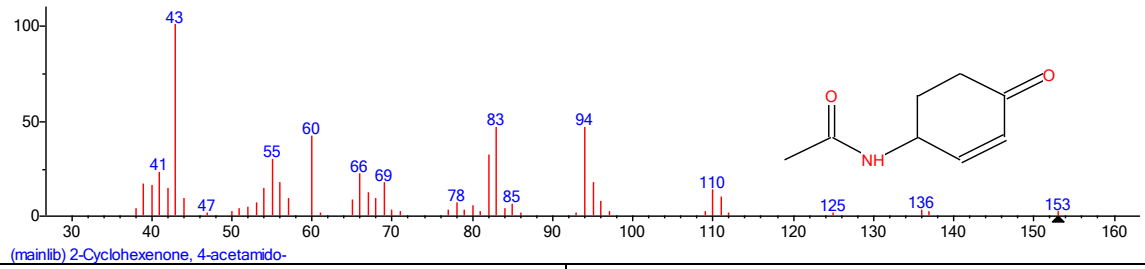
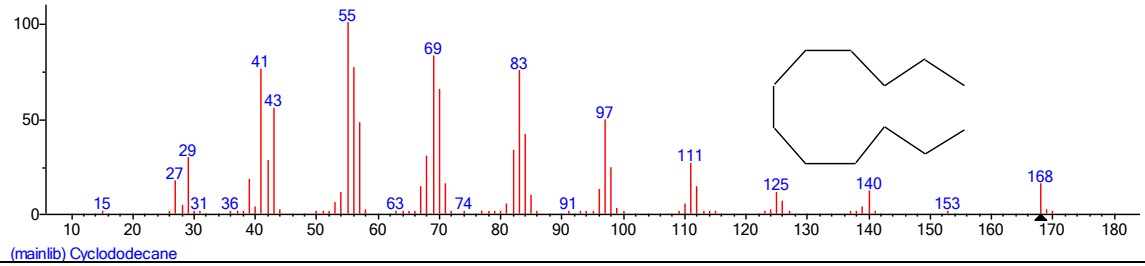
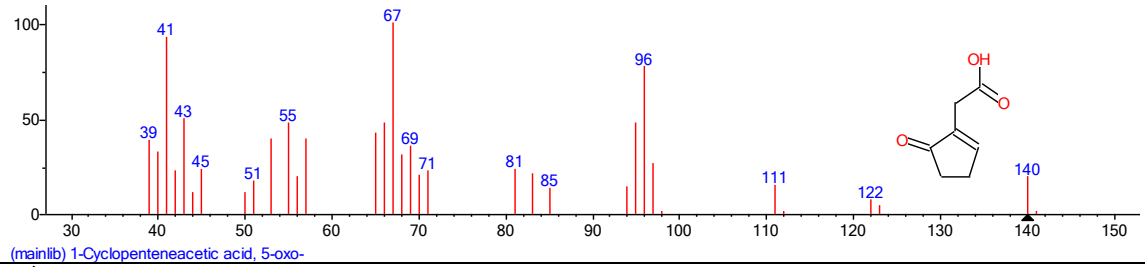
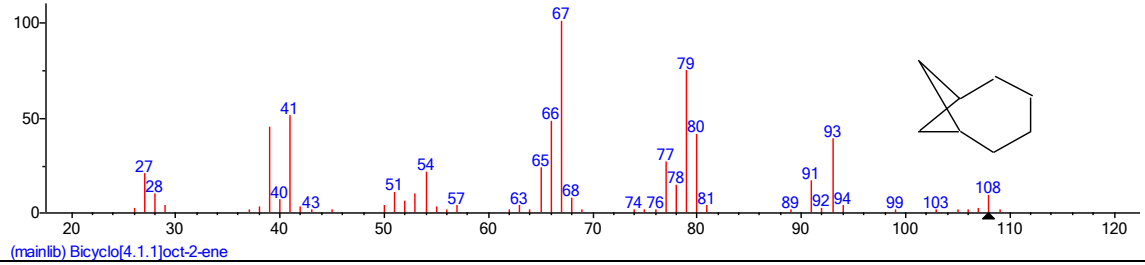
The increasing challenge of antimicrobial resistance across the globe has necessitated the search and discovery of natural sources of antibiotic substance. Certain chemicals of various compositions have been known to be produced by Bacillus species and majority of these compounds have demonstrated antimicrobial properties that have been widely employed against certain other microorganisms (Sethi *et al.*, 2013) [26]. In this present study, the antimicrobial potential of novel Bacillus species have been evaluated by isolating Bacillus species from soil samples obtained from agricultural site in Nkpoklu area in Port Harcourt, Rivers State Nigeria. Antimicrobial potentials of Bacillus species isolated from agricultural site were explored by analyzing the secondary metabilities produced by these Bacilli at different stress times, temperatures, and pH.

Soil is an ecosystem that can provide the nutrients required for the growth of living organisms. 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) [13].

The presence of microorganisms in soil is determined by the chemical composition, moisture content, pH and structure of the soil. Human activity has a significant impact on the creation of ecosystems. The various soil depths sampled in this study include soil surface, 5cm and 15cm depth and the frequency of isolates distribution show that Bacillus species and Staphylococcus species were consistent in the entire soil depths sampled (Cheng *et al.*, 2022) [7].

Table 3.7: Gas Chromatography - Mass Spectrometry (GC/MS) Report of Bacteria Extract Obtained from Bacillus Species isolated from agricultural Site

Extract 3 (Agricultural Site)	**Compounds Obtained in GC/MS**	**Quantity**	**Structure**
Compound 1	Cyclohexa siloxane, dodecamethyl-	4.786%	
Compound 2	Dodecanoic acid	8.776%	
Compound 3	1H-Pyrrolo[3,4-d]pyrimidine-2,5-di one, 4,6-bis(4-hydroxyphenyl)	16.094%	
Compound 4	Methyl tetradecanoate	3.172%	

Compound 5	2-Cyclo hexenone, 4-acetamido-	4.978%	
Compound 6	Cyclododecane	10.430%	
Compound 7	1-Cyclo penteneacetic acid, 5-oxo-	8.770%	
Compound 8	Bicyclo[4.1.1] oct-2-ene	8.776%	

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, while the total heterotrophic bacteria count for 5cm depth and15cm soil depth were the same. The observation is that, as the depth increased, the number of isolated bacteria decreased. The physico-chemical and structural characteristics of soil provide many microenvironments in which complex bacterial populations can evolve (Ranjard & Richaume, 2018) [18]. As well as the physical properties of the soil, bacteria are also influenced by nutrient availability resulting from rhizodeposition and decomposition (Lynch & Whipps, 2019). The diversity, abundance and activity of bacterial communities are therefore structured in relation to depth, since the primary source of nutrient input in grasslands is above ground.

Given that pathogenic bacteria are progressively developing resistance to commonly used curative agents, prospecting for novel antibiotics from natural sources is becoming more and more crucial for the pharmaceutical industry (Suzuki & Giovannoni, 2016) [23]. Many types of soil bacteria and fungi produce antibiotics which may be an adaptation strategy used by the microorganisms to drive out rivals and occupy a new niche (Von Wintzingerode *et al.*, 2017) [24].

The Bacillus species isolated were stressed at different time and temperature and under this unfavorable condition they released some secretory molecules which were tested for antibiotic potentials by testing them against already identified standard microorganism and zone of inhibition (ZI) measured in millimeter and recorded according to CLSI standard. A comparison of mean of time and soil depth showed that secretory molecules produced by Bacillus species isolated from 5cm soil depth had the lowest mean of zone of inhibition (ZI) at 48 hours and 72hour (12.17mm and 12.98mm respectively). The highest mean of ZI was spotted in secretory molecules produced by Bacillus species isolated from Surface sample at 48hours and 72hours (14.36mm and 16.50mm respectively). The highest mean of ZI (15.36) was seen at 72hours and within the temperature of 30oC while the lowest mean of ZI was recorded at 48hours within the temperature of 450C. At 450C, the Bacilli began to die due to high temperature and the production of secretory molecules was reduced hence mean of zone of inhibition at the lowest while at 30C, the Bacilli under stressed is still viable hence high mean of zone of inhibition. Akeed *et al.*, 2020 [2] investigated the influence of incubation temperature on enzyme production of B. licheniformis B307, results showed that temperature significantly influenced production and the study conducted by Sidorova *et al.*, (2020) [19] 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.

The combination of stress temperature and pH showed that the highest mean of zone of inhibition was recorded at pH of 4.0 and temperature of 300C (16.06mm) while the lowest mean of zone of inhibition was recorded at pH 4.0 and temperature of 450C (13.80mm). At pH 8.0, the lowest mean of zone of inhibition was recorded at 300C (13.27mm) while at 400C and 450C the mean of zone of inhibition of 14.05mm and 14.58mm respectively. The highest mean of zone of inhibition was recorded at 72 hours and at pH 4.0 while the lowest mean of zone of inhibition was recorded at 48 hours and at pH 8.0. The result of the effect of time, temperature and pH on Bacillus species is in agreement with Sidorova *et al.*, (2020) [19] that conducted a research on optimization of laboratory cultivation condition for the synthesis of antifugal metabolites by Bacillus subtilis strains in Saudi Arabia, reported that maintaining the ideal time, temperature and pH is crucial for the synthesis of bioactive secondary metabolites; otherwise, growth may not occur, or production may fail. More specifically, the effect of temperature on cellular enzymes directly influences the growth of microorganisms. Enzyme activity raises with temperature until denaturation of the protein structure causes these molecules' three-dimensional configuration to disappear. On the other hand, enzyme inactivation takes place and cellular metabolism progressively decreases as the temperature drops toward the freezing point. Most cells stop their biochemical processes at 0°C. The vast majority of Streptomyces, Nocardia, and Micromonospora are typically incubated at a temperature range of 25-30°C for normal growth and maintenance (Waksman & LeChevalier, 2013) [25].

Compounds contained in the bacteria extract examined for antimicrobial capability includes Cyclohexasiloxane, dodecamethyl (4.786%), Dodecanoic acid (a saturated fatty acid with 12 carbon atom chain) (8.776%), Cyclododecane (10.430%), Pyroles: 1H-Pyrrolo [3,4-d]pyrimidine-2,5-dione, 4,6-bis (4-hydroxyphenyl)-1-methyl-3,4,6,7-tetrahydro- (13.941%, the most abundant), Pyrazol-5(4H)-one, 3-(4-nitropheny l)-1-phenyl-4- (2-thienylmethylene) (6.470%). The bacteria extract contain mainly Pyrrole and its derivatives according to the result of GC\MS analysis conducted which is contrary to the report of Elsayed *et al.*, (2020) [11] that researched on antimicrobial and anticancer activities of Actinomycetes isolated in Egyptians soil reported that Pyrrole and its derivates obtained from Actinomycetes exhibited antimicrobial activities against Escherichia coli strain that was resistant to some antibiotics while Pyrrole and its derivatives obtained from Bacilli in this study did not exhibit antimicrobial activity. Pyrrole is one of the six structural isomers of the bicyclic ring system containing a pyrrole moiety fused to a pyridine nucleus. Pyrrols have been shown to have antimicrobial qualities, which is one of their biological activities. The majority of its derivatives have also been investigated as sedatives and analgesics. Pyrrolopyridines have been demonstrated to be effective as antiviral and antimycobacterial agents in other biological studies (Veselov *et al.*, 2020) [22].

Conclusion

The surface sample produced the highest total heterotrophic count (THC) of bacteria and 15cm soil depth produced the lowest THC. The observation is that, as the depth increased, the number of isolated bacteria decreased.

This study also showed that most of the microorganisms were isolated from soil surface; however, the total heterotrophic counts of microorganisms isolated from all the soil depths did not significantly differ in number.

This study also revealed that soil samples from agricultural sites have the potential to harbour Bacillus species that have the potential to produce Pyrrole and its derivates. It was observed that temperature, time and pH influence the production of secretory molecules as evident in antimicrobial susceptibility test.

Majority of the compounds identified in the bacteria extract from Bacillus species isolated in this study were derivatives of volatile substances like alkaloids and phenolic compounds, according to the GC-MS data analysis.

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