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Antibacterial Screening of Soil Bacteria Isolates from Guimaras Island, Philippines against Escherichia Coli and Staphylococcus Aureus

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Abstract

This study was conducted to screen and determine the antibacterial effects of soil bacterial isolates from three sampling sites of Sinapsapan, Jordan, Guimaras against Staphylococcus aureus and Escherichia coli. Test bacteria were isolated and characterized based on colonial morphology and cell characteristics. Soil bacteria isolates were screened for their antibacterial properties against S. aureus and E. coli using agar disc diffusion method. Each treatment was done in three replicates and trials. There were six bacterial isolates in each sampling site obtained and exhibited antibacterial properties on S. aureus and E. coli after 72 hours of incubation. For S. aureus, I4 (M=35.33 mm, active) from high sampling site; I2 (M=29.44 mm, partially active) from intermediate sampling site, and I1 (M = 38.00 mm, active) from low sampling site while for E. coli, I4 (M= 34.22, active) from high sampling site, I6 (32.78 mm, active) from intermediate sampling site, and I5 (M=34.78, active) from low sampling site had the highest zones of inhibition.

Keywords: *soil bacteria, agar disc, Guimaras, Escherichia coli, Staphylococcus aureus*

1. Introduction

1.1 Background of the Study

Soil is a natural reservoir for microorganism and their antimicrobial products [1]. A significant number of these organisms are undocumented and some of them may have an effect on pathogenic bacteria [22]. In a single gram of soil, there can be billions of bacteria [19]. It is estimated that there are about 60,000 different bacteria species, most which have yet to be named, and each has its own particular roles and capabilities [19]. Most bacteria live in the top 10cm of soil where organic matter is present [19]. Soil bacteria perform important services related to disease suppression [15].

Barangay Sinapsapan is located in Jordan, Guimaras Island, bordering Panay Gulf with approximate coordinates of N 010 35'.00 E 122 30'.47. The distance from the sea is about 270 meters at an elevation of about 30 meters above sea level. The terrain is a typical young coral island with the land rising rapidly to about 30-40 meters elevation when proceeding inland from the coastland some 100 meters. The inland elevation varies up and down forming valleys and ridges [8].

Barangay Sinapsapan has many unexplored territories which is most likely to yield purposeful results towards isolation of new antibiotics. There are areas that are not inhabited by people. Most of the soils in the area are rich in organic matter since leaves and twigs cover most of the land. The texture of the soil is primarily coarse. The

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arrangement of the soil particles is in different aggregates wherein the soil granules clump or bind together [9].

Bacteria are capable of causing disease. Humans are generally most interested in the species of pathogenic bacteria in humans, although these bacteria can also infect other animals and plants. Some notable pathogenic bacteria include *Staphylococcus aureus* and *Escherichia coli*. *Staphylococcus aureus*, often referred to simply as "staph", are gram-positive spherical bacteria that occur in microscopic clusters resembling grapes. Bacteriological culture of the nose and skin of normal humans invariably yields staphylococci [25]. *Escherichia coli* is the head of the large bacterial family, *Enterobacteriaceae*, the enteric bacteria which are facultatively anaerobic gram-negative rods that live in the intestinal tracts of animals [25].

The science of antibiotics has remained and will remain for many years, one of the most interesting natural sciences, in both theoretical and practical aspects. Microbial natural products still appear as the most promising source of the future antibiotics that society is expecting [16].

For the past years, pathogens underwent mutation which enabled them to resist antimicrobials, thereby threatening millions of people worldwide [1,14]. The antibiotic resistance problem demands to discover new antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. Majority of the bacteria in soil that have the potential for drug sources remain uncultivable, and thereby inaccessible for novel antibiotic discovery [1, 12, 16]. Thus, it is imperative to screen more and more bacteria from different soil samples for antimicrobial activity with a hope of getting some bacterial strains that produce antibiotics that have not yet been discovered and active against drug resistant pathogens.

1.2. Objectives of the Study

This study was conducted to screen and determine the antibacterial effects of soil bacterial isolates from three sampling sites of Sinapsapan, Jordan, Guimaras against *S. aureus* and *E. coli*. Furthermore, soil bacterial isolates with antibacterial properties against the test pathogenic bacteria were characterized. Specifically, it has the following specific objectives:

1. To determine the number of bacterial isolates present in the three sampling sites of Sinapsapan, Jordan, Guimaras.
2. To characterize (colonial morphology and gram staining reaction) bacterial isolates that have antibacterial properties against *S. aureus* and *E. coli*.
3. To determine the effects of the bacterial isolates on *S. aureus* and *E. coli* after 72 hours of incubation.
4. To determine significant differences on the zone of inhibition of bacterial isolates from the three sampling sites against *S. aureus* and *E. coli*.

2. Methodology

2.1. Sampling Site

The sampling sites were located in Sinapsapan, Jordan, Guimaras Island, in the land bordering Panay Gulf with approximate coordinates of N 010 35'.00 E 122 30'.47. The distance from the sea is about 270 meters at an elevation of about 30 meters above sea level [8].

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Figure 1. Barangay Sinapsapan, Jordan, Guimaras (Photo courtesy of Eckerwall, 2013)

2.2. Soil Sample Collection

Twenty-seven soil samples were collected from three sampling sites of Brgy. Sinapsapan, Jordan, Guimaras. The three sampling sites were based on their elevation and labeled as high, intermediate and low. Each collection was done from 10-15 cm depth of the soil. Approximately, 10 g of soil was scooped and placed into sterile plastic bags.

2.3. Isolation and Culture Condition

For each collected soil sample, 1g of the soil was suspended in 100 ml of normal saline solution. It was incubated in an orbital shaker incubator at 28^0C with shaking at 200 rpm for 30 min. Mixtures were allowed to settle, and serial dilutions up to 10^{-6} sterile normal saline solution and were agitated normally. An aliquot of 0.1 ml of each dilution specifically 10^{-2}, 10^{-4}, and 10^{-6} were taken and spread evenly over the surface of nutrient agar (NA) medium. The culture medium was added with Fluconazole (75 mg/ml) to inhibit fungal contamination. Plates were incubated at 35^0C, and were monitored after 24 hours. Repeated streaking on NA agar plates of isolated colonies was done to purify bacterial colonies and then incubated for 18-24 hours. The procedure was repeated three times to ensure purification and obtain well-isolated bacterial colonies. The purified colonies were described based on colony characteristics on agar media as seen with the naked eye [10]. These were described according to form, elevation, margin, pigmentation or color, appearance, optical property and texture. The isolated strains were preserved at 4^0C overlaid with sterile mineral oil for further use and were maintained for longer period by serial subculture. Isolates with antibacterial properties in the different sampling sites were provided with code as H1 to H6 for high sampling site bacterial isolates, I1 to I6 for intermediate sampling site bacterial isolates, and L1 to L6 for low sampling site bacterial isolates.

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2.4. Test Organisms

Test organisms were the gram-positive bacterium, *Staphylococcus aureus* BIOTECH 1582 and the gram-negative bacterium, *Escherichia coli* BIOTECH 1634. The test bacteria were purchased from the Philippine National Collection of Microorganisms (PNCM), University of the Philippines Los Baños (UPLB) Biotech in Laguna. A letter for requisition of purchase of bacteria was made prior to the antimicrobial assay. The bacteria cultures were overlaid with sterile mineral oil and were sub-cultured for further use.

2.5. Broth Culture of Test Isolates and Test Pathogens

A loopful of test pathogens and characterized test bacteria isolates were inoculated in each nutrient broth culture medium aseptically. Inoculation was done twice to ensure growth of bacteria in broth solution. All bacterial broth cultures were incubated at 37^0C for 24 hours. About 5 ml of the solution of the same proportions as those used in preparing the culture suspension was transferred in a 25 ml screw-cap tube. The culture tube was tightly sealed and stored in the dark at room temperature. Prior to use, the turbidity standard was shaken thoroughly. The 0.5 Mc Farland Standard was used to adjust the turbidity of the inoculum prior to microbial assay. The turbidity standard may contain approximately 1.5 x 10^8 CFU/ml of the cultured bacteria [18].

2.6. Preparation of Antibiotic Solution

An antibiotic ciprofloxacin was purchased from a local drug store. The preparation of the solution was based on the indicated concentration *i.e.*, 50 mg/ml [3], wherein 500 mg of the antibiotic tablet was pulverized and dissolved in 10 ml sterile distilled water.

2.7. *In vitro* Screening of Soil Bacterial Isolates for Anti-bacterial Activity

Morphologically distinct colony of bacterial isolates was subjected for antibacterial screening against the test pathogens using the modified agar disk diffusion method. The test isolate was inoculated in an agar disk with a thickness of 2mm and a diameter of 10 mm on a plate of screening media spread-plated with the test pathogen. The plates were incubated for 24 h at 37 ^{0}C. The procedure was also employed in making agar disc as the positive control. Antagonism was measured by the size of the inhibition zone [14]. The presence of zone of inhibition of the test isolate was derived using the formula as indicated below and evaluated according to the observed and corresponding modified inferences [18]:

$$Zone\ of\ Inhibition = \frac{(X-Y)}{Z}$$

where X = highest mean diameter of the zone of inhibition (mm); Y = diameter of the agar plate disc (mm); and Z = number of descriptive scale used.

Table 1. Descriptive Inferences of the Zone of Inhibition

Measurement	Description
0-21.16 mm	Inactive
21.17-32.33 mm	Partially active
32.34-43.50 mm	Active
43.51-54.67 mm	Very active

Each bacteria isolate was tested on the test organism in three replicates and in three trials. Zone of inhibition was recorded every after 24 hours of incubation for three consecutive days.

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2.8. Gram Staining of Bacterial Isolates with Antibacterial Properties

A smear of bacteria isolate was taken in a clean glass slide and heated gently over a flame. The smear was covered with a thin film of crystal violet for 1 min and was washed gently in slow running tap water. Gram's iodine solution was flooded over the smear for 1 min and was washed with tap water. A concentration of 80% ethanol was used to decolorize the smear until the violet color ceased to flow away. The slide was flooded with water and counter stained with safranin for 2 min. The slide was washed, drained, air dried, and viewed under high power objective. The culture retaining the violet color indicates a gram-positive organism while the culture with pink color indicates a gram-negative organism [10].

2.9. Data Collection Procedure and Analyses

The data were collected based on the presence of zone of inhibition of bacterial isolates on *S. aureus and E. coli.* The zone of inhibition was measured with the aid of a vernier caliper. Each bacterial isolate was tested in three replicates and in three trials. The measurement of the zone of inhibition on the test bacteria was recorded and subjected for statistical analysis.

Descriptive Statistics. The mean zone of inhibition on each test bacterium was tabulated. Furthermore, the zone of inhibition of each bacterial isolate was evaluated with the aid of a modified descriptive scale on the zone of inhibition [18].

Inferential Statistics. One-Way Analysis of Variance with repeated measures (rANOVA) was used to compare the average zone of inhibition of the different bacterial isolates against *E. coli* BIOTECH 1634 *and S. aureus* BIOTECH 1582 over a seventy-two hours incubation period. *Post Hoc* test using Least Significant Difference (LSD) for pairwise comparison among the isolates was used. The level of significance was set at 0.05.

3. Results

3.1. Bacterial Isolates in the Three Sampling Sites of Sinapsapan, Jordan, Guimaras

Table 2 shows six bacterial isolates obtained from the three sampling sites of Sinapsapan, Jordan, Guimaras.

Table 2. Bacterial Isolates Obtained in the Three Sampling Sites of Brgy. Sinapsapan, Jordan, Guimaras

Sampling Site	Number of Isolates
High Area	6
Intermediate Area	6
Low Area	6

3.2. Characteristics of Bacterial Isolates in the Different Sampling Sites in Barangay Sinapsapan, Jordan, Guimaras

Table 3 shows the characteristics of the six bacterial isolates in each sampling site as characterized according to shape, elevation, margin, color, pigmentation, texture, cell arrangement, and Gram staining reaction.

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Table 3
Characteristics of Bacterial Isolates in the High, Intermediate and Low Area Sampling Sites of Brgy. Sinapsapan, Jordan,Guimaras

Isolate Number	Sampling site	Colony Characteristics	Cell Shape & Cell Arrangement	Gram Staining Reaction
H1	High	Irregular; Flat; Undulate; Dull; Opaque; Non- pigmented; Smooth	Bacilli occur singly	Gram negative (-)
H2	High	Circular; Flat; Entire; Dull; Opaque; Non- pigmented; Smooth	Cocci occur singly	Gram negative (-)
H3	High	Punctiform; Flat; Entire; Dull; Transluscent; Non- pigmented; Smooth	Bacilli in clusters	Gram positive (+)
H4	High	Rhizoid; Flat; Filamentous; Dull; Transluscent; Non-pigmented; Smooth	Cocci in clusters	Gram positive (+)
H5	High	Circular; Flat; Entire; Dull; Opaque; Yellow; Smooth	Cocci in chain	Gram positive (+)
H6	High	Circular; Flat; Entire; Dull; Opaque, Orange; Smooth	Cocci in clusters	Gram positive (+)
I1	Intermediate	Rhizoid; Flat;Filamentous; Dull; Transluscent; Non- pigmented; Smooth	Diplobacilli	Gram positive (+)
I2	Intermediate	Circular; Flat; Entire; Dull; Opaque; Non- pigmented; Smooth	Cocci occur singly	Gram negative (-)
I3	Intermediate	Punctiform; Flat; Entire; Dull; Transluscent; Non- pigmented; Smooth	Cocci in clusters	Gram negative (-)
I4	Intermediate	Irregular; Flat; Undulate; Dull; Opaque; Non- pigmented; Smooth	Cocci in clusters	Gram positive (+)
I5	Intermediate	Filamentous; Flat; Filamentous; Dull; Opaque; Non- pigmented; Smooth	Cocci in clusters	Gram positive (+)
I6	Intermediate	Circular; Flat; Entire; Dull; Opaque; Yellow; Smooth	Cocci occur singly	Gram negative (-)
L1	Low	Rhizoid; Flat;Filamentous; Dull;Opaque; Non- pigmented; Smooth	Bacilli occur singly	Gram positive (+)
L2	Low	Filamentous; Flat; Filamentous; Dull; Opaque; Non- pigmented; Smooth	Cocci in clusters	Gram positive (+)
L3	Low	Punctiform; Flat; Entire; Dull; Transluscent; Non- pigmented; Smooth	Cocci in clusters	Gram negative (-)
L4	Low	Irregular; Flat; Undulate; Dull; Transluscent; Non- pigmented; Smooth	Bacilli occur singly	Gram positive (+)
L5	Low	Circular; Flat; Entire; Dull; Opaque; Yellow; Smooth	Cocci occur singly	Gram negative (-)
L6	Low	Circular; Flat; Entire; Dull; Opaque; Orange; Smooth	Cocci occur singly	Gram negative (-)

3.3. Zone of Inhibition of Bacterial Isolates in the Three Sampling Sites against *Staphylococcus aureus* BIOTECH 1582

Figure 2 shows the mean zone of inhibition of highland soil bacterial isolates against *S. aureus* BIOTECH 1582 after 24, 48 and 72 hours of incubation. Bacterial isolate H4 (M=25.89mm, SD=6.41) and H1 (M=34.22mm, SD=5.91; M=35.33mm, SD=4.97) have the highest zone of inhibitions and described as partially active after 24, 48 and 72 hours of incubation among the isolates, respectively. However, the positive control antibiotic, ciprofloxacin has the highest zone of inhibition after 24, 48 and 72 hours (M=28.55 mm, SD=10.33; M=49.67 mm, SD= 9.12; 54.67 mm, SD=10.78), respectively.

Figure 3 shows the mean zone of inhibition of intermediate land soil bacteria isolates against *S. aureus* BIOTECH 1582 after 24, 48 and 72 hours of incubation. Bacterial isolate I5 (M=28.67mm, SD=10.31) and I2 (M=33.34mm, SD=4.21; M=29.44mm, SD=3.91) have the highest zone of inhibitions and described as partially active after 24, 48, and 72 hours of incubation among the isolates, respectively. However, the positive control antibiotic ciprofloxacin has the highest zone of inhibition after 24, 48 and 72 hours (M=28.55 mm, SD=10.33; M=49.67 mm, SD=9.12; 54.67 mm, SD=10.78), respectively.

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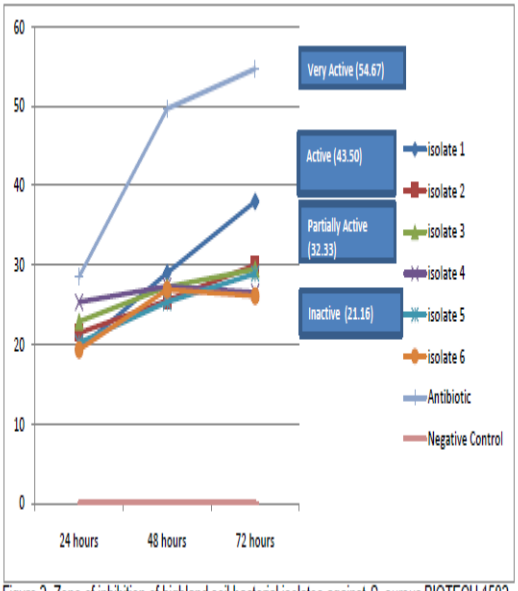

Figure 2. Zone of inhibition of highland soil bacterial isolates against *S. aureus* BIOTECH 1582

Figure 4 shows the mean zone of inhibition of lowland soil bacterial isolates against *S. aureus* BIOTECH 1582 after 24, 48 and 72 hours of incubation. Bacterial isolate L4 (M=25.33, SD=2.92) and L1 (M=29, SD=3.04; M=38, SD=5.79) have the highest zone of inhibitions and described as partially active after 24, 48, and 72 hours of incubation among the bacterial isolates, respectively. However, the positive control antibiotic ciprofloxacin has the highest zone of inhibition after 24, 48 and 72 hours (M=28.55 mm, SD=10.33 M=49.67 mm, SD=9.12; 54.67 mm, SD=10.78), respectively.

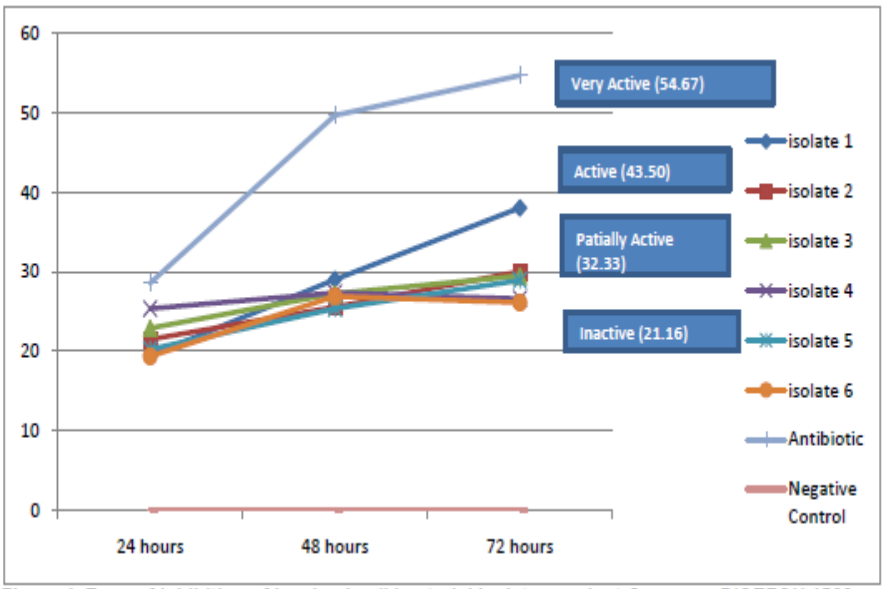

Figure 4. Zone of inhibition of low land soil bacterial isolates against *S. aureus BIOTECH 1582*

3.4. Zone of Inhibition of Bacterial Isolates in the Three Sampling Sites Against *Escherichia coli* BIOTECH 1634.

Figure 5 shows the mean zone of inhibition of highland soil bacterial isolates against *E. coli* BIOTECH 1634 after 24, 48 and 72 hours of incubation. Bacterial isolates H2 (M=20.11mm, SD=9.37) and H4 (M=26.22mm, SD=3.67; M=34.22mm, SD=5.29) have

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the highest zones of inhibitions and described as partially active after 24, 48, and 72 hours of incubation among the isolates, respectively. However, positive control antibiotic has the highest zone of inhibition after 24, 48 and 72 hours of incubation (M=22.33 mm, SD=3.39; 30.33 mm, SD=6.84; 36.65 mm, SD=5.66), respectively.

Figure 6 shows the mean zone of inhibition of intermediate land soil bacterial isolates against *E. coli* BIOTECH 1634 after 24, 48 and 72 hours of incubation. Bacterial isolates I4 (M=19.44mm, SD=4.00), I1 (M=27mm, SD=3.50), and I6 (M=32.78mm, SD=6.65) have the highest zones of inhibition and described as partially active after 24, 48, and 72 hours of incubation, respectively. However, positive control antibiotic has the highest zone of inhibition after 24, 48 and 72 hours of incubation (M=22.33 mm, SD=3.39; 30.33 mm, SD=6.84; 36.67mm, SD=5.66), respectively.

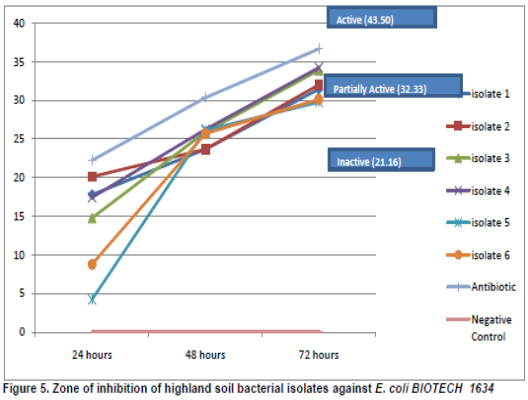

Figure 5. Zone of inhibition of highland soil bacterial isolates against *E. coli BIOTECH 1634*

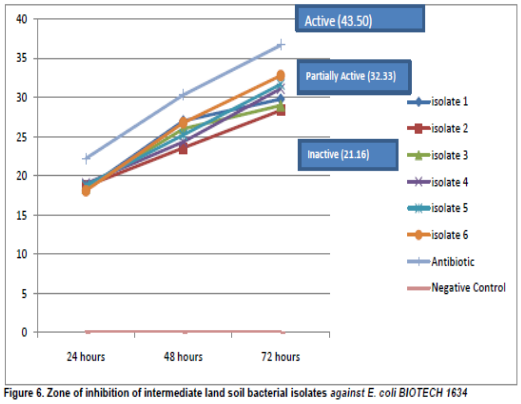

Figure 6. Zone of inhibition of intermediate land soil bacterial isolates *against E. coli BIOTECH 1634*

Figure 7 shows the mean zone of inhibition of lowland soil bacterial isolates against *E. coli* BIOTECH 1634 after 24, 48 and 72 hours of incubation. Bacteria isolates L1 (M=23mm, SD=10.77), L4 (M= 30.89mm, SD=4.88), and L5 (M=34.78mm, SD=3.60) have the highest zones of inhibitions and described as partially active after 24, 48 and 72 hours of incubation, respectively. However, positive control antibiotic has the highest zone of inhibition after 72 hours of incubation (M=36.67 mm, SD=5.66).

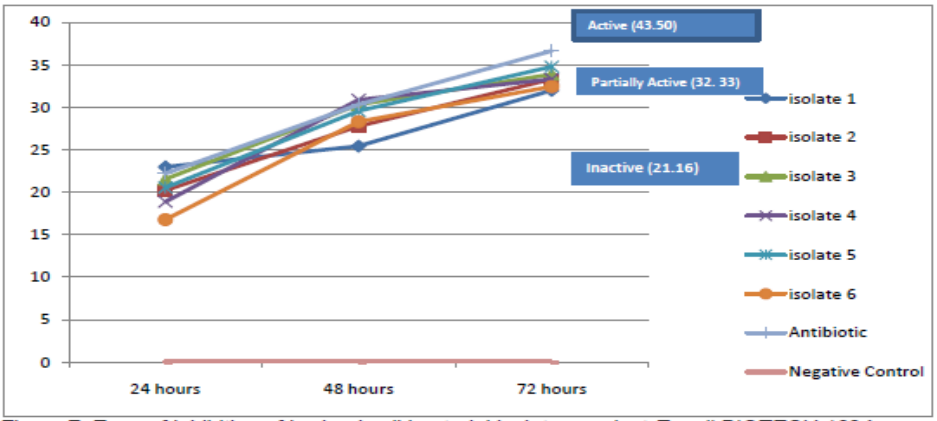

Figure 7. Zone of inhibition of lowland soil bacterial isolates against *E. coli BIOTECH 1634*

3.5. Inferential Data Analysis

Tests of Within-Subjects Contrasts on the Zone of Inhibition of Bacterial Isolates in the Different Sampling Sites against *Staphylococcus aureus* BIOTECH 1582 and *Escherichia coli* BIOTECH 1634. Table 4 shows no significant difference in the analysis of variance with repeated measures for the tests of within-subject contrasts on the zone of inhibition of bacterial isolates in the different sampling sites against *Staphylococcus*

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aureus BIOTECH 1582 and *Escherichia coli* BIOTECH 1634 over 72 hours of incubation, F(9,77)=1.917, P=0.062>0.05, partial eta squared=0.183. The effect size is small indicating the measure of the degree to which variability among the observations among the soil bacteria isolates on the zone of inhibition of the test bacteria can be attributed to conditions controlling for the subjects effect that were unaccounted. This may indicate that soil bacterial isolates have varying effects on *S. aureus* and *E. coli* as indicated in the previous figures. However, the Least Significant Difference for pair wise comparison shows significant differences among the following: all isolates and the antibiotic ciprofloxacin (p=0.000); isolates 3 and 6 (p=0.001); isolates 4 and 6 (p=0.001), $p < 0.05$. This may indicate that some soil bacterial isolates may have comparable effects with the antibiotic ciprofloxacin.

Table 4. Analysis of Variance with Repeated Measures Tests of Within-Subjects Contrasts of Bacterial Isolates in the Different Sampling Sites against *Staphylococcus aureus* BIOTECH 1582 and *Escherichia coli* BIOTECH 1634

Source	Time	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
TIME * AREA * BACTERIA	Linear	80.850	2	40.425	3.588	.032	.085
TIME * ISOLATE * AREA * BACTERIA	Linear	194.343	9	21.594	1.917	.062	.183
Error(TIME)	Linear	867.504	77	11.266			

P > 0.05 is not significant.

Tests of Between-Subjects Effects on the Zone of Inhibition of Bacterial Isolates in the Different Sampling Sites against *Staphylococcus aureus* BIOTECH 1582 and *Escherichia coli* BIOTECH 1634. Table 5 shows a significant difference in the analysis of variance with repeated measures for the tests of between-subjects effects on the zone of inhibition and time of incubation of bacterial isolates in the different sampling sites against *Staphylococcus aureus* BIOTECH 1582 and *Escherichia coli* BIOTECH 1634 over 72 hours of incubation, $p < 0.05$. F(9,77)=3.890, P=0.000<0.05, partial eta squared=0.313. The effect size is small indicating the measure of the degree to which variability among the observations among the soil bacteria isolates on the zone of inhibition of *S. aureus* and *E. coli* can be attributed to conditions controlling for the subjects effect that were unaccounted. This may indicate that soil bacterial isolates have varying effects on *S. aureus* and *E. coli* as indicated in the previous figures. Furthermore, this is supported by the Least Significant Difference for pair wise comparison. There were significant differences among the following sampling sites: all isolates in the different sampling sites and the antibiotic ciprofloxacin (p=0.000), $p < 0.05$.

Table 5. Analysis of Variance with Repeated Measures Tests of Between-Subjects Effects of Bacterial Isolates in the Different Sampling Sites against Staphylococcus aureus BIOTECH 1582 and Escherichia coli BIOTECH 1634

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	208460.792	1	208460.792	19827.194	.000	.996
ISOLATE	261.034	5	52.207	4.966	.001	.244
AREA	251.008	2	125.504	11.937	.000	.237
BACTERIA	669.625	1	669.625	63.690	.000	.453
ISOLATE * AREA * BACTERIA	368.108	9	40.901	3.890	.000	.313
Error	809.569	77	10.514			

*P < 0.05 is significant.

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4. Discussion

A total of 18 bacterial isolates in the 3 sampling sites with variable colony and cell characteristics in each sampling site were collected. The bacterial isolates were all flat, dull, non-pigmented and smooth. Bacteria isolate I1 is a diplobacilli while others are either clustered or occurred individually. Among the different microorganisms inhabiting the soil, bacteria are the most abundant and predominant organisms [24]. Morphologically, soil bacteria are divided into three groups viz c*occi (*round/spherical), (rod-shaped) and s*pirilla/spirillum* (cells with long wavy chains) [24]. *Bacilli* are most numerous followed by cocci *and spirilla in* soil. The major soil factors which influence the microbial population, distribution and their activity in the soil are soil fertility, cultural practices, soil moisture, soil temperature, soil aeration, light, soil pH, organic matter, food and energy supply, nature of soil, and microbial associations [22]. All these factors play a great role in determining not only the number and type of organism but also their activities. Fertilizers and manures applied to the soil for increased crop production, supply food and nutrition not only to the crops but also to microorganisms in soil and thereby proliferate the activity of microbes [12]. Optimum soil moisture (range 20 to 60 %) must be there for better population and activity of microbes in soil [17]. Seasonal changes in soil temperature affect microbial population and their activity. The organic matter in soil being the chief source of energy and food for most of the soil organisms has an influence on the microbial population [17]. Organic matter influences directly or indirectly on the population and activity of soil microorganisms. It influences the structure and texture of the soil, thereby the activity of the microorganisms. Ecological relationships among soil organisms are influenced by soil structure. Changes in resource locations may allow for microorganisms to colonize another area [24].

Bacteria are usually the most abundant group in soils in terms of numbers [2]. The genus *Bacillus* is very common in soil. *Bacillus*, a Gram positive, aerobic or facultative endospore forming motile bacteria belongs to family *Bacillaceae* [6]. Isolates H3, L1, and L4 reflect the cellular characteristics of the genus *Bacillus*. Thus, it may be implied that these isolates belong to this genus. *Bacillus* can resist and survive in a variety of environmental stresses and adverse conditions and considered as very important microbiota due to its diverse ecophysiology, direct and indirect functions such as N2 fixer [13], denitrifiers [23], antibiotic [4], and phytohormones [2] producers.

In general, the soil bacterial isolates exhibited an antimicrobial activity after 24 hours of incubation. Most of the zone of inhibition became even wider after 48 and 72 hours of incubation. The result of the study may contain soil bacterial isolates that could be producers of novel bioactive compounds [7]. Various antimicrobial substances from soil bacterial isolates have been isolated and characterized including aminoglycosides, anthracyclines, glycopeptides, betalactams, macrolides, nucleosides, peptides, polyenes, polyester, polyketides, actionomycins, and tetracyclines [17]. Most of the antibiotics are extracellular metabolites which are normally secreted in culture media and have been used as herbicides, anticancer agents, drugs, immunoregulators and antiparasitic drugs [12]. *Actinomycetes* are gram-positive rods grow as filaments, branching rods, and diphtheroidal rods [5]. One of the soil bacterial isolates, I1 has almost parallel characteristics to *Actinomycetes* as gram-positive, filamentous and diplobacilli. Thus, I1 could probably be an *Actinomycete* isolate.

Bacillus spp. is considered to be the safe microorganisms that hold remarkable abilities for synthesizing a vast array of beneficial substances which may have antibacterial properties [21]. They can produce IAA, siderophore, phytase, organic acid, ACC deaminase, cyanogens, lytic enzymes, oxalate oxidase, and solubilized various sources of organic and inorganic phosphates as well as potassium and zinc.

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The ability of microorganisms to produce enzymes that may have antibacterial properties is influenced by environmental conditions such as temperature, pH, and presence of inductors or repressors [20].

A common statement of the competitive exclusion principle is that a species whose use of resources is very similar cannot live in the same place for an extended period of time [11]. Thus, if two or more species eat the same thing, use the same hiding places, occupy the same habitats, etc. one species will be more efficient than the others and will fill the niche with its offspring leaving no resources for other species. In this way, the more efficient species will "competitively exclude" the less efficient species.

5. Conclusions and Recommendations

There were 6 soil bacterial isolates obtained in the high, intermediate, and low sampling sites in Sinapsapan, Jordan, Guimaras. Isolate I1 is the only bacterium that is diplobacilli while the rest are either clustered or occur singly. Isolates I1 and I5 were the most effective soil bacterial isolate against *S. aureus* and *E. coli*, respectively. This is may be due to the fact that they can degrade and inhibit bacterial growth due to their secretion of digestive enzymes. After 72 hours of incubation, all bacterial isolates exhibited variations on their antibacterial effect against *S. aureus* and *E. coli.* For *S. aureus*, the following isolates had the widest zone of inhibitions after 72 hours of incubation: in the highland sampling site, isolate H4; in the intermediate sampling site, isolate I2; and lowland sampling site, isolate L1. For *E. coli*, the following isolates had the widest zone of inhibitions after 72 hours of incubation: in the highland sampling site, isolate H4; in the intermediate sampling site, isolate I6; and lowland sampling site, isolate L5.

There was a significant difference on the zone of inhibition of the different bacterial isolates in the three sampling sites due to varying antibacterial effects of the isolates against *S. aureus* and *E. coli*. This may be due to differences on the amount of substrates produced by the different bacterial isolates which inhibit the growth of the test pathogens, *S. aureus* and *E. coli.*

The findings have shown that different isolates were more effective on *S. aureus* than on *E. coli*. It is therefore possible for the isolates to be considered as a prospective source of antibiotic against on *S. aureus* than *E. coli.* Searching for new possible source of antibiotics is necessary because it becomes a manner for bacteria to be resistant to certain antibiotics. Antibiotic resistant bacteria are increasing nowadays in number that makes the treatment for some diseases to be difficult.

Bacteria become resistant more quickly when antibiotics are used improperly. Since there are kinds of bacteria that are resistant to antibiotics and are untreatable, there is a greater must to search for more possible sources of antibiotics like from unexplored soil sources in the nearby locality.

The study was limited only on the characterization of the colonies and gram staining technique. It is recommended to do special staining methods such as flagella staining to determine the arrangement of the flagella, endospore staining to determine whether the isolates are spore-former and the position of their spores, capsule staining to determine whether the isolates are virulent or not, and negative staining to reveal the morphology of the cell.

The study did not determine the genera or the species of the isolates with antibacterial properties. It is recommended to do biochemical tests to identify the genus of each isolate and confirm using Bergey's Manual of Systematic Bacteriology for the identification of the isolate. Furthermore, molecular characterization of the isolates to reveal the identity of each species of the isolate is also suggested. Phylogenetic analysis should be done to determine the group of each isolate. This will also help to determine if the bacterial isolates that were used in this study are newly discovered or not.

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References

[1] A. J. Alanis, "Resistance to Antibiotics: Are We in the Post-Antibiotic Era?, Archives Med Research, vol. 36, **(2005)**, pp. 697-705.
[2] F. F. Araujo, A. A. Henning and M. Hungria, "Phytohormones and antibiotics produced by Bacillus subtilis and their effects on seed pathogenic fungi and on soybean root development", World J. Microb. Biot., vol. 21, **(2005)**, pp. 1639-1645.
[3] L. V. Allen and H. C. Ansel, "Ansel's pharmaceutical dosage forms and drug delivery system", 10th ed. USA: Wolters Kluwer, **(2013)**.
[4] C. W. Bacon and D. M. Hinton, "Endophytic and biological control potential of Bacillus mojavensis and related species", Biol. Control, vol. 23, **(2002)**, pp. 274-284.
[5] G. H. W. Bowden, "Actinomyces, Propionibacterium propionicus, and Streptomyces", Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; Chapter 34, Retrieved from: http://www.ncbi.nlm.nih.gov/books/NBK8385, **(1996)**.
[6] D. Claus and R. C. W. Berkeley, "Genus Bacillus Cohn 1872, 174AL", Bergey's Manual of Systematic Bacteriology, vol. 2, **(1986)**, pp. 1105-1139.
[7] S. Das, P. S. Lyla and S. Ajmal Khan, "Marine microbial diversity and ecology: present status and future perspectives. Current Science", Department of Health (2009). National health data dictionary, vol. 90, no. 10, **(2006)**, pp. 1325-1335.
[8] Retrieved from:http://uhmis1.doh.gov.ph/standards/sdetails. php?data_element=BARANGAY
[9] Eckervall, Personal Communication, **(2013)** October 10.
[10] Española, Personal Communication, **(2013)** October 10.
[11] J. P. Harley, "Laboratory exercises in Microbiology", (6thed.) Boston: McGraw Hill Higher Education, **(2005)**.
[12] C. Hoagstrom, "Competitive exclusion principle", Retrieved from: http://www.eoearth.org/view/article/151404, **(2011)**.
[13] T. R. P. Kekuda, K. S. Shobha and R. Onkarappa, "Fascinating diversity and potent biological activities of Actinomycete metabolites", Journal of Pharmacy Research, vol. 3, no. 2, **(2010)**, pp. 250-256.
[14] X. Liu, H. Zhao and S. Chen, "Colonization of maize and rice plants by strain Bacillus megaterium C4", Curr. Microbio., vol. 52, **(2006)**, pp. 186-190.
[15] M. T. Madigan, J. M. Martinko, D. A. Stahl and D. P. Clark, "Brock's biology of microorganism", (13thed.) Boston: Benjamin Cummings, **(2012)**.
[16] J. P. Martin, "Soil Microbiology and Biochemistry", SSSA; Madison, WI, **(1976)**.
[17] F. Pela´ ez, "The historical delivery of antibiotics from microbial natural products—Can history repeat?, Biochemical Pharmacology, vol. 71, **(2006)**, pp. 981-990.
[18] T. R. Prashith Kekuda, K. S. Shobha, R. Onkarappa, S. A. Goutham and H. L. Raghavendra, "Screening biological activities of a Streptomyces species isolated from soil of Agumbe", Karnataka, India. Int. J. Drug Dev. & Res., vol. 4, no. 3, **(2012)**, pp. 104-114.
[19] E. A. Quinto and M. A. G. Santos, "A guidebook to plant screening: Phytochemical and biological", Microbiology section. España, Manila; University of Santo Tomas Publishing House, **(2005)**.
[20] G. Reid and P. Wong, "Soil bacteria", State of New South Wales Department of Primary Industries, **(2005)**.
[21] R. Sharma, Y. Chisti and U. C. Banerjee, "Production, purification, characterization, and applications of lipases", Biotechnology Advances, vol. 19, no. 8, **(2001)**, pp. 627-662.
[22] T. Stein, "Bacillus subtilis antibiotics: structures, syntheses and specific functions", MolMicrobiol, vol. 56, no. 4, **(2005)**, pp. 1669-1679.
[23] A. Stephanie, E. J. A. Breznak and T. M. Schmidt, "Isolation and characterization of soilbacteria that define ' Terriglobis ' gen. nov.", in the Phyllum 'Acidobacteria' Appl. And Env. Microbiology, vol. 73, no. 8, **(2007)**, pp. 2708-2717.
[24] Suharti, H. A. Heering and S. de Vries, "NO Reductase from Bacillus azotoformans is a bifunctional enzyme accepting electrons from menaquinol and a specific endogenous membrane-bound cytochrome c551", Biochem., vol. 43, **(2004)**, pp. 13487-13495.
[25] D. M. Sylvia, J. F. Fuhrmann, P. G. Hartel and D. A. Zuberer, "Principles and applications of Soil Microbiology", New Jersey, Pearson Education Inc., **(2005)**.

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[26] K. Todar, "An Introduction to Bacteriology: University of Wisconsin Inc.", Retrieved at http://textbookofbacteriology.net/index.html, **(2008)**.

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