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Isolation and screening of soil bacteria with the potential to produce antibiotics

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ABSTRACT

Antibiotics are one of the most important commercially exploited secondary metabolites produced by bacteria and employed in a wide range. Soil samples were collected from five different locations in the school compound of Federal University Of Technology Minna, Niger State (Bosso campus). The total viable bacterial counts ranged from 2.11 x 10^5 - 4.45 x 10^5 cfu/ml. The bacterial isolates were identified as *Bacillus subtilis, Bacillus licheniformis, Bacillus polymxa* and *Pseudomonas aeruginosa* with *Bacillus subtilis* and *Bacillus polymxa* having the same frequency of occurrence of 30% while *Bacillus licheniformis* and *Pseudomonas aeruginosa* having the same freguency of 20%. The bacteria isolates were then screened for the potential to produce antibiotics. The bacteria isolated shows zone of inhibition on *Staphylococcus auereus, Escherichia coli*, *Salmonella* sp. and *Pseudornonas aeruginosa*, which suggest the evidence of antibiotics produced by those isolates from the soil.

KEYWORDS

Isolation;
Screening;
Soil bacteria;
Potential;
Produce antibiotics.

INTRODUCTION

Soil is the major depository of micro organisms that produces antibiotics capable of inhibiting the growth of other microorganisms. Clinically useful antibiotics have been isolated from four major groups of soil microorganisms; Streptomyces, Bacillus, Penicillium and Cephalosporium. The microbial types isolated include, Actinomycetes, Bacteria and molds[1]. Antibiotic production is a feature of several kinds of soil bacteria and fungi and may represent survival mechanisms whereby organisms can eliminate competition and colonize a niche[2]. Antibiotics first became widely available in the 1940s with the use of penicillin and sulfonamides. Since that time, the pharmaceutical industry has developed more than 100 varieties of these drugs, with 150 million prescriptions being written for antibiotics annually in the United States alone. This growth in antibiotic usage has been paralleled by the ability of bacteria to resist being killed by these agents, and has resulted in a steady decline in the number of effective antibiotics each year. At its most extreme, the acquisition of antibiotic resistance genes has resulted in at least four species of bacteria for which there are no effective forms of conventional therapy available[2]. In order to combat these infections, new antibiotics will need to be developed to which bacteria are less likely to become resistant. One approach taken by many pharmaceutical companies is to focus

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on the identification of antimicrobials with narrow specificities restricted to a single genus or species rather than the broad spectrum approaches of the past[2]. With the rapid biotechnological advances in infectious disease management threat posed by the emergence of highly resistant infectious agents become the next challenge. The antibiotic earlier shown to be effective in controlling a microorganism is no longer able to be so. The strike back of pathogens has revitalized the search for new antibiotics to counter drug resistant bacteria, fungi and viruses. In this respect, the new antibiotics obtained from Actinomycetes and other bacteria, having inhibiting spectra for gram positive and gram negative organisms, should not be toxic to human being, plants and animals[2]. The mass production of antibiotics began during World War 11 with the invention of streptomycin and penicillin. Their specific action against particular group of organisms made their use more important in medical, veterinary and agricultural practices. But more vexing problem is the emergence of resistant strain among the micro organisms that were sensitive to antibiotics before the drug became widely used. This phenomenon tends to limit severely the useful life of any new antibiotics, requiring the pharmaceutical industry to come up with new compounds continually. The need for new antibiotics is especially acute because of the following unfortunate situation. In any modern hospital, huge amount of antibiotics are used in the treatment as well as the prevention of infectious disease. As a result, the hospital environment becomes highly enriched for microorganisms that are resistant to those antibiotics. At the same time, the immune and other defense mechanisms of the body are not functionally well in many hospitalized patients, who are thus especially vulnerable to 'nosocomial' (hospital acquired) infection by these resistant bacteria. Scientists all over the world are constantly working to discover newer effective antibiotics to combat resistant strains[2]. Serious infections caused by bacteria that have become resistant to commonly used antibiotics have become a major global healthcare problem in the 21stcentury[3]. *Staphylococcus aureus,* for instance, a virulent pathogen that is associated with a wide range of infections including pimples, pneumonia, osteomyelitis, endocarditis and bacteremia, and bacteremia, has developed resistance to most classes of antibiotics[4]. For more than two decades, clinicians and public health officials gave faced hospital acquired methicilin-resistant *S. aureus* (MRSA), which also bears resistance tomany antibiotics. During such times, vancomycin has been the therapeutic answer to MRSA, but it use as the drug for choice has changed since vancomycin resistant strains have emerged clinically[5,6]. Vancomycin-resistance, but also because of resistance to many other antibiotics, including aminoglycosides, macrolides, and fluoroquinolones, fortunately, newer therapeutic agents, daptomycin, linezolid, and a streptograming combination (quinupristin/dalfopristin) have entered the clinical arena in the past few year[7,8]. This research is aimed at isolating and screening of soil bacterial with the potential to produce antibiotics.

MATERIALS AND METHODS

Collection and processings of samples

The soil samples were collected from different soil sites. The samples was collected from depth of 20cm from the soil surface, placed in sterile polythene bags, closed tightly, labeled and taken back to the laboratory for analysis. The samples were collected from five different locations in the school compound of Federal University of Technology Minna, Niger State. Bosso Campus.

Isolation of bacteria

The pour plate method was used as described by[9]. Where 1ml of the serially diluted sample of 10^5 were transferred into well labeled clean sterile petri dish..

Characterization and identification of isolates

The bacterial isolates were characterized using Gram's reaction and biochemical tests including sugar utilization profiles. The colonial morphology of the isolates was examined and characteristic colonies were identified using special microscopic techniques and biochemical tests. The isolates were identified using the scheme of Cowan and Steel[10].

Screening of isolates for the potential to produce antibiotic

Nutrient agar medium was prepared and sterilized by autoclave. The media was allowed to cool to 45-50^{0}C and poured into the sterile Petri plates and allowed to solidify. Isolated colonies from slant culture was taken carefully with an inoculating wire loop and streaked on the solidified medium in the Petri dish. The

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Petri dishes were then incubated at 37°C overnight as described by[11].

Source of microorganisms

The microorganisms used for this study were *Salmonella* sp, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*. These organisms were obtained from the stock culture of Microbiology Department, Federal University of Technology, Minna, Niger State. The organisms were maintained on Nutrient Agar slant at 4°C prior to sub culture. Gram staining procedures were carried out on the organism to confirm their purity before transferring into slants and incubating at 35hours. Pure culture of test organisms were then transferred onto slants and stored at 4°C.

Assay of antibiotic activity of microorganisms by streak method

Nutrient agar medium was prepared, sterilized and poured into Petri plates under aseptic conditions. The organisms expected to be the antibiotic producer was streaked on the solidified agar plate dividing it into two halves and test organisms were streaked diagonally on either side of streak of selected culture. Next day the growth of the test organisms is checked for the inhibition.

RESULTS

Microbial count

The total viable bacteria counts ranged from 2.11 x $l0^5$ and 4.43 x10^5 colony forming unit per mililitre (cfu/ml) (TABLE 1).

Characterization and identification of isolates

The bacterial isolates were identified as *Bacillus subtilis*, *Bacillus lichenformis*, *Bacillus polyrnyxa* and *Pseudomonas aeruginosa*. *Bacillus subtilis* and *Bacillus polyrnyxa* having the same frequency of occurrence of 30% while *Bacillus licheniformis* and *Pseudomonas aeruginosa* having the same frequency of occurrence 20% (TABLE 2).

Screening of isolate for the potential to produce antibiotics

All the isolates screened for antibiotic activity has the potential to produce antibiotic, except *Pseudomonas aeruginosa*. This was determined by the absence of inhibition zone around the organisms which implies that, the organisms have antibiotics activity (TABLE 3).

Antibiotics activity of the isolates on some micro organisms

The preliminary screening of the potential of the isolates shows that they exhibit zone of inhibition on the growth of the test organisms (TABLE 3).

TABLE 1: Total viable bacteria count of soil sample.

Soil sample	Bacteria count (cfu/rnl)
A	3.67x $l0^5$
B	2.82 x 10^5
C	2.l1x10^5
D	3.66x10^5
E	4.45x10^5

KEY: A-New lecture hall 1; B-Microbiology Lab1; C-Cafeteria; D- School Clinic; E- Girls' hostel.

TABLE 2 : Percentage occurrence of bacteria isolate with antibiotic activity.

Bacteria isolate	Number of isolate	% occurrence
Bacillus subtilis	3	30
Bacillus licheniformis	2	20
Bacillus polymyxa	3	30
Pseudomonas aeruginosa	2	20
Total	10	100

TABLE 3 : Screening of bacteria isolates.

Bacteria isolate	Absence of clear zone	Presence of clear zone
Bacillus polymyxa		+
Bacillus subtilis		+
Bacillus lincheniformis		+
Pseudomonas aeruginosa	_	

KEY: +: antibiotic producers; - : non antibiotic producers.

TABLE 4 : Antibiotics suseptibility of isolates on some test organisms.

Bacteria isolates	*Staphylococcus aureus*	*Salmonella sp.*	*Escherichia coli*	*Pseudomonas aeruginosa*
Bacillus polymyxa	-	+	+	+
Bacillus subtilis	+	+	+	+
Bacillus lincheniformis	+	+	+	+
Pseudomonas aeruginosa	-	-	-	-

Key: + Inhibition; No Inhibition.

DISCUSSION

The total viable bacterial counts of the soil samples screened during the course of this research reveals that, the bacterial load for each soil sample varies from

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$2.11x10^5$-$4.45x10^5$ (TABLE 1), this might be due to the soil type and composition, and also to environmental factors such as pH, temperature and moisture content. This is in agreement with[12,13] who reported that variance in bacterial load of soil might be due to extreme pH, high temperature and moisture content. The result of this study also reveals that *Bacillus* species with antibiotic properties are present in the soil samples screened. This is also in agreement with[11,14]. Who reported that, Bacilli are the predominant soil bacteria because of their resistant endospore formation and their ability to produce antibiotics of medical importance.

The *Bacillus* species were identified as *Bacillus subtilis, Bacillus lichenforrnis* and *Bacillus polymyxa* with both *Bacillus subtilis* and *Bacillus polymyxa* having the same frequency of occurrence of 30% while *Bacillus lichenforrnis* and *Pseudomonas aeruginosa* has 20%. The predominance of *Bacillus subtilis, Bacillus licheniformis, Bacillus polymyxa* in soil samples from soil was also reported by[1,11,13] also reported that in a competitive environment, inhibition zones are developed by both organic acid producers and antibiotic producers. The organic acid producers are eliminated by growing them on calcium carbonate medium because they develop clear zones around them on calcium carbonate ($CaCO_3$) medium. Organic acids react with $CaCO_3$ and dissolved to calcium oxide (CaO) and carbon dioxide (CO_2). On $CaCO_3$ medium only organic acid producers develop a clear zone. As shown in (TABLE 3), the bacteria isolates were not organic acid producer but are antibiotic producers[14,15]. reported that *Bacillus subtilis, Bacillus licheniformis* and *Bacillus polyinyxa* have the ability to produce substances with antibiotic properties in a competitive environment. The result of this research also reveals moderate inhibition of the growth of Gram positive (*Staphylococcus auereus*) and Gram negative (*Escherichia coli, Salmonella* sp. and *Pseudomonas aeruginosa*) bacteria by the isolates. This is in agreement with[15] who reported the inhibition of Gram positive and Gram negative bacteria by bacillus species.

CONCLUSION

The study revealed that, bacteria with the potential to produce antibiotic are present in the soil. Though a large list of antibiotics are commercially available, the search for the most effective one is still on, and this work may contribute in providing information on the antibiotic producing microorganisms.

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