European Journal of Applied Sciences 4 (5): 211-215, 2012
ISSN 2079-2077

DOI: 10.5829/idosi.ejas.2012.4.5.2011

Screening and Isolation of the Soil Bacteria for Ability to Produce Antibiotics

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Abstract: Antibiotic is one of the most important commercially exploited secondary metabolites produced by bacteria and employed in a wide range. Most of the antibiotics used today are from the microbes. Bacteria are easy to isolate, culture, maintain and to improve their strain. Bacillus species being the predominant soil bacteria because of their resistant endospore formation and production of vital antibiotic like bacitracin etc. are always found inhibiting the growth of the other organisms. In the present research study, soil bacteria with the antibiotic activity was screened and isolated. The media used in this research was nutrient agar medium. 1g of the soil samples were dissolved in 10ml of sterile water to make soil suspensions. Portion of the suspensions were inoculated on the nutrient agar by streaking and were incubated at 37°C for 24 hours. After which colonies with a clear zone of inhibition were observed. The bacteria isolated were; *Bacillus lentus, Micrococcus roseus, Bacillus alvei, Enterobcteraerogene and Bacillus pumillus*. The inhibitory activities of the isolated microorganisms were checked against some of the important opportunistic microflora like *Staphylococcus aureus and Pseudomonas* species.

Key words: Bacteria · Inhibition · Heterogeneous · Soil · Antibiotic · Endospore

INTRODUCTION

The term soil refers to the outer loose material of the earth crust. It may be regarded as a three phase's system composes of solids, liquids and gases, dispersed to form a heterogeneous matrix. On the whole the soil is composed of five major components, these include; Mineral matter, Water, Organic matter, Air and living Organisms. The various component of the soil environment constantly changed and the quantity of these constituents are not the same in all soil but vary with locality. Living portion of the soil body includes small animals and microorganisms but it is generally considered that its microorganisms that plays the most important role in the release of nutrient and carbondioxide for plant growth. The bacteria are the most abundant group usually more numerous than the four combined. Soil bacteria can be rod, (bacilli) cocci (spherical) spirilla (spirals), of these, bacillus are more numerous than the others. They are one of the major groups of soil bacteria population and are very widely distributed [1]. The number and type of bacteria present in a particular soil would be greatly influenced by geographical location such as soil temperature, soil type, soil pH, organic matters contents, cultivation, aeration and moisture content [2].

The term antibiotic means against life. In our every day usage however, we use the word to describe a set of chemical that inhibit or kill bacteria. Antibiotics are one of the most important commercially exploited secondary metabolites produced by the bacteria and employed in a wide range. The British scientist Alexander Fleming is credited with being first to notice that another organism could inhibit bacteria growth in 1928. He noticed that growth of bacterium *Staphylococcus aureus* was inhibited by a mold (fungus) that contaminated his plate. The mold was later identified as *Penicillin notatum* and the antibiotic isolated a short time later was named penicillin [3]. There are numbers of bacteria having potential to produce antibiotic example of which is bacillus species which produce antibiotic like bacitracin, pumulin and gramicidin which are active against Gram positive bacteria such as *Staphylococci, Streptococci, Corynebacter, Streptomyces* species which produce antibiotic like

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tetracycline, cloramphenicol, vancomycin, gentamycin which are active against Gram negative bacteria and lactobacillus species which antibiotic like nisin which is produce by *Lactobacillus lactis* [4].

Some Bacteria That Have Ability to Produce Antibiotics: *Bacillus* species of the family *Bacillaceae* is the largest in the order. The genus contain gram positive, endospore forming, chemotherotrophic rod that are usually motile with peritrichious flagella, it is aerobic and catalase positive. Many species of *Bacillus* are of considerable importance because they produced the antibiotic [4]. *Bacillus species* produces many kinds of antibiotics which share full range antimicrobial activity such as bacitracin which is produce by *Bacillus licheniformis* is a mixture of at least 5 polypeptides. These antibiotics consist of 3 separate compounds, bacitracin A, B and C. Bacitracin A is the chief constituent. It is active against many Gram positive organisms such as *Stapylococci, Streptococci,* anaerobic cocci, *Cornyebacter* and *Clostridia* but not against most other Gram negative bacteria [5].

Gramicidin which is produced by *Bacillus brevis*, a linear polypeptide antibiotic mixture of gramicidin A, B, C and D. Gramicidin D a channel forming ionophore that flip-flop slowly across the membrane and surprisingly was found to inhibit phosphate group ATPase other several species for example *B. thuringiensis* and *B. sphaericus* term a solid protein crystal, the paraporal body next to their endospores during spore formation. The *B. thuringiensis* parasporal body contain protein toxin that kill over species of moths by dissolving in the alkaline gut of caterpillars and destroying the epithelium. The *B. sphaericus* parasporal body contains protein toxic for mosquito larvae and may be useful in controlling the mosquito that carry macana [4].

Streptomyces is the largest genus, there are around 150 species member of the genus that is strict aerobes, have cell wall type I and form chain of non-motile. *Streptomyces species* are determined by the means of a mixture of morphological and physiological characteristic and are involve in antibiotic production. *Streptomyces* is very important medically the natural habitat of most *Streptomyces*is the soil, where they may be constitute from 1to 20% of the culturable population. *Streptomyces* are best known for the synthesis of a vast of antibiotic [6]. Stanley Waksman's discovery that *S. griseus* produces Streptomycin was an enormously important contribution to science and public health. Streptomycin drug was the first drug to effectively combat tuberculosis and in 1952 Waksman earned the Nobel Prize. In addition this discovery set up a massive search resulting in the isolation of new streptomyces species that produce other compound of medicinal importance. In fact, since that time, the streptomycetes have been found to produce over 10,000 bioactive compounds. Hundreds of these natural products are now used in medicine and industry about two thirds of the antimicrobial agent used in human and veterinary medicine are derived from the Streptomycetes. Example includes amphoteracin B, chloramphenicol, erythromycin, neomycin, nystatin and tetracycline. Some *Streptomyces* species *produce* more than one antibiotic [6]. *Lactobacilli are* bacteria containing non sporing rods and sometimes coccobacilli that lack catalase and cytochromes, some usually facultative anaerobic or microaerophilic produce lactic acid as their main product and have complex nutritional requirement. *Lactobacillus* species produce many kind of antibiotic which share a full range of antimicrobial activity, example of which is nisin which is produce by *Lactobacillus lactis* and active against many gram positive organism such as *Corynebacter, Clostridia,* anaerobic cocci [4].

MATERIALS AND METHODS

Collection and Preparation of Soil Sample: In systematic screening program for isolation of bacteria 10 soil samples were collected at different location within Birnin Kebbi metropolis were collected from upper layer where most of the microbial activity take place and thus where most of the bacteria population is concentrated. Soil samples (approximately 5g) were collected using some clean dry and sterile polythene bag along with sterile spatula. 1g of the soil samples were dissolved in 10ml of water to make soil suspensions.

Isolation of Bacteria: The media used in this research was nutrient of agar medium. 28g of nutrient agar powder was weighed and dissolved in 1000ml and of distilled water. It was stirred vigorously and dissolved using hot plate after which was sterilized in autoclave for 15 minutes at 121°C. It was then allowed to cool after which it was dispensed in Petri dishes and allowed to solidify.

Sample Inoculation: Portions of the suspension were inoculated on the nutrient agar by streaking and were incubated at 37°C for 24hours. After which colonies with a clear zone of inhibition were observed.

Gram's Staining: Colonies that were grow on nutrient agar where gram stained in accordance with standard gram staining procedure described by Todar *et al*. [7].

Sub Culturing: Bacteria isolates having shown a cleared zone of inhibition on nutrient agar plates were sub cultured into nutrient agar slants for short time preservation and to purify the isolates. The bacteria were inoculated in nutrient agar slant using a sterile wire loop and inculcated at 37°C for 24hours. The slant bottles containing the bacteria were kept in refrigerator at 4°C for short time storage before biochemical tests were ran on the isolates for identification.

Biochemical Tests

Indole Test: One percent tryptophan broth in a test tube was inoculated with bacteria colony. After incubation period of 37°c for 48hours, then one militre (1ml) of chloroform was added to the broth. The test tube was shaken gently, then 2.1 of Kovac's reagent were added and this was also shaken gently and allowed to stand for twenty (20) minutes. The formation of red colouration at the top layer indicated positive and yellow colouration indicates negative.

Catalase Test: This was carried out by putting a drop of hydrogen peroxide on a clean slide. With the edge of another slide, a colony of the organism was picked and allowed to be in contact with the hydrogen peroxide. Presence of bubbles indicates positive reaction while absence of bubble indicates negative reaction.

Citrate Utilization Test: This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and this was inoculated for 24 hours to 72 hours. The development of deep blue colour after incubation indicates a positive result.

MR-VP Test: Five milliliters (5ml) or MR VP broth was inoculated with the test organism an incubated for 48-72hours at 37°c after which, one milliliter (1ml) of the broth was transferred into a small tube. Some small quantity (2.3 drops) of methyl red test was added. A red colour one the addition of the indicator signified a positive methyl red test while yellow colour signified a negative test. To the rest of the broth in the original tube some drops (five) of 4% potassium hydroxide (KHO) were added followed by some (fifteen) drops of 5% ∝-naphtol in ethanol. The test tube (sealed with cotton plug) was shaken and place in a sloping position. The development of a red colour starting from the liquid-0air interface within 1 hour indicated a VP positive test while no colour change indicated a VP negative test.

Triple Sugar Iron Agar Test (TSI): The medium contains three (3) sugars namely: glucose, lactose and sucrose. The pH indicator is phenol red and detection system for hydrogen sulphide (H_2S) is included. This medium was prepared as agar slope and the test organism was inoculated by stabbing the medium with the aid of sterilized straight wire loop and the surface of slope is inoculated by streaking and then incubated at 37°C for 24hours, after which observation was made. Gas production was determined by cracking of the medium, formation of H_2S was determine by the blackening of the whole buffer or a streak of ring of blackening at the slant butt junction, glucose fermentation was determine by the yellowing of the butt. The fermentation of lactose or sucrose or both was determined by the yellowing of both the butt and the slant and the motility was determined by observing the line inoculation; sharply defined line of inoculation indicating positive motility [8].

Test Bacteria: The test bacteria used in this study were obtained from Department of Science Laboratory Technology, Waziri Umaru Federal Polytechnic, Birnin Kebbi and they include the followings: *Pseudomonas aeruginosa, Staphylococus aureus, Shigella spp and Proteus Spp.*

Confirmation of Antibacterial Activity: Bacteria isolates having potential of producing antibiotics were tested against the test organisms in order to confirm the antibacterial activity. This was done by using agar well diffusion method as described by Manga and Oyeleke, [8].

RESULTS

Biochemical characterization for bacterial isolates from the soil samples was presented in Table 1. The bacteria were identified as *Bacillus lentus, Micrococcus roseus, Bacillus alvei, Enterobacter aerogene and Bacillus pumillus.* Table 2 presented the zone inhibition in millimeter (mm) of bacteria isolates against test bacteria.

DISCUSSION

In searching for new antibiotics, relatively simple and rapid methods have been developed for screening microorganisms for antibiotic producing ability.

Table 1: Morphology and biochemical characterization of the bacterial isolates

	BAC1	BAC 2	BAC 3	BAC 4	BAC 5
Gram reaction	+	+	-	+	+
Shape	Bacilli	Cocci	Bacilli	Bacilli	Bacilli
Arrangement	Chain	Chain	Single	Chain	Chain
Endospore test	+	-	-	+	+
Motility	+	-	+	+	+
Oxygen relationship	Facultative anaerobe	Facultative anaerobe	Facultative anaerobe	Facultative anaerobe	Facultative anaerobe
Catalase	+	+	+	+	+
Coagulase	-	-	-	-	-
Citrate utilization	-	+	+	+	-
Indole test	-	-	-	-	-
Methyl red	+	+	+	-	-
Starch hydrolysis	+	-	-	-	-
Glucose	+	-	+	+	+
Lactose	-	-	+	-	-
Sucrose	+	+	+	+	+
Mannitol	-	-	-	-	-
Probable identify	*Bacillus lentus*	*Micrococcus roseus*	*Entrerobacter aerogene*	*Bacillus pumillus*	*Bacillus alvei*

Key: + (Positive) ; - (negative)

Table 2: Zone inhibition in millimeter (mm) of bacteria isolate against test bacteria (*Bacteria isolate (having potential of antibiotics production*)

Test organisms	*Bacillus lentus*	*Micrococcusroseus*	*Enterobacter aerogene*	*Bacillus pumillus*	*Bacillus alvei*
Shigella spp	Nil	12mm	Nil	Nil	Nil
E. coil	Nil	Nil	Nil	Nil	Nil
S. aureus	15mm	Nil	Nil	Nil	15mm
Pseudomonas spp	Nil	Nil	17mm	Nil	12mm
Proteus spp	Nil	Nil	Nil	12mm	Nil

Soil sample are commonly employed in the antibiotic producing organism. Bacillus species are ubiquitous in nature. Antibiotic isolated may be bactericidal or bacteriostatic in nature. Production of antibiotic by microorganisms from soil is affected by many factors including nitrogen and carbon source. Temperature variation also affects the synthesis of antibiotic. Antibiotic is synthesis during the idophase stage of the growth and there is no correlation between the bacteria sporulation and antibiotic production [9]. The soil bacteria isolated shows antibiotic activity under normal growth condition and was found inhibiting some gram positive and some gram negative organism both *Bacillus lentus* and *Bacillus alvei* shows antibacterial activity against *Staphylococcus aureus. Bacillus pumillus* only show slight zone of inhibition on *proteus spp* while it is inactive against others. *Micrococcus roseus* was also proved to have antibacterial activity only against *Shigella spp. Enterobacter aerogene* and *Bacillus alvei* shows clear zone of inhibition against *Pseudomonas spp* but the former is higher than the later. *Escherichia coli* proved to be resistance to all the isolates that is there is no clear zone of inhibition observed against the bacteria.

CONCLUSION

It is concluded that *Bacillus lentus, Micrococcus roseus, Enterobacter. Aerogene, Bacillus pumillus and Bacillus alvei* isolated during the course of this research from soil samples in Birnin Kebbi metropolis have potential of produce antibiotics.

Recommendation: I recommended that the strain improvement by the mutagenic agents may still enhance the activity. Extraction and purification method can be employed for the pure antibiotic production.

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