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Screening of Bacillus Species with Potentials of Antibiotics Production

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Abstract: Sixteen soil samples were collected from different refuse dump sites in Minna, the capital Niger State, and analysed for the presence of Bacillus species. Physical-chemical analysis of the soil samples revealed the followings: PH value 6.89-8.47; moisture content 1.58 – 21.21% and temperature 27-28ºC. Using both pour plate and streak method of inoculation, total bacterial count in the soil samples ranged from 3.8×104 cfu/g 16.0×104 cfu/g. The identified Bacillus species included: *Bacillus cereus* (30.8%), *Bacillus brevis* (1.9%) *Bacillus polymyxa* (3.8%), *Bacillus lichenifomis* (13.5%), *Bacillus sphererícus* (7.7%), *Bacillus mycoides* (13.5%), *Bacillus pumilus* (7.7%), *Bacillus subtilis* (3.8%), *Bacillus alvei* (1.9%), *Bacillus laterosporous* (1.9%), *Bacillus firmus* (9.6%) and *Bacillus circulars* (3.8%). Antibiotic production tests indicated that nine Bacillus species out of twelve isolated in this study could be used to produce antibiotics that had effect on the test organisms. However, *Bacillus polymyxa*, *Bacillus sphaericus* and *Bacillus laterosporous* had little or no effect on the tested organisms. This study suggests that some Bacillus species have potential to produce high quality antibiotics that can be use to control microbial growth in future.

Keywords: Antibiotics; Broad spectrum; Bacillus species; Narrow spectrum.

Introduction

Bacillus species are gram-positive aerobic or facultative anaerobic, sporulating rod shaped bacteria that are widely spread in nature [1,2], being implicated in food poisoning [3,4]. Bacillus species exhibit a wide range of physiologic abilities that allow the organism to flourish in every environment and compete favorably with other organisms within the environment, due to its ability to form spores produce metabolites that are heat stable, cold, radiation, and desiccation disinfect ants and have antagonistic effect on other microorganisms [5].

Antibiotics have been recognized as the only means of effective microbial growth control [5], after the discovery of penicillin and other antimicrobial agents by Alexander Fleming in 1928 [6]. Since that time to date, there has been continues search for more effective antibiotics that can stand the emerging menace of drug resistance among microorganisms world wide [5,7,8].

In Nigeria, resistance to antibiotics has resulted in morbidity and mortality from treatment failures and increased health care costs [9]. The increases in antibiotics resistant have been attributed to inappropriate use, inadequacies on the part of the manufacturers [5,10,11] and leads to the steady decline of effective antibiotics annually worldwide [5]. Unfortunately, legislative is still not enough to curb the menace [9]. This situation has become a serious challenge to drug manufacturers, public health practitioners' world wide. Therefore, this study is an attempt to identify bacillus species with potential of antibiotic production that could be use to stem the scourge of drug resistance particularly in Nigeria.

Material and Method

Collection of Soil Samples

Top soil samples to a depth of 4 inches or/and 10 centimeters were obtained from 16 refuse dump sites in Minna: Chanchaga, Army barracks, Shango, Tunga, Mobilroundabout, Uguwan Daji, F-layout, Bosso, Tudun Fulani, Maikunkele, Bosso estate, Dutsen kura, Kpakungu, Barkin sale, Sabongari, Maitumbi. All the samples were collected in sterile containers between the hours of 7:00 am and 11:00 am in the morning and the samples were analyzed in the microbiology laboratory, Federal University of Technology, Minna, Niger State, Nigeria.

Analysis of the Soil Samples

The Methods section should include only information that was available at the time the plan or protocol for the study was written; all information obtained during the conduct of the study belongs in the Results section.

Weighing and drying. The percentage moisture content was determined by weighing the soil samples before and after air-drying at 30°C as expressed mathematically below:

$$\text{Moisture content (\%)} = \frac{\text{weight lost}}{\text{weight of sample taken}} \quad 100$$

Determination of PH and Temperature. The PH values were obtained using PH meter (SKR104 ENGLAND) and thermometer (Mecury Thermometer) respectively and the results were recorded (Twice).

Inoculation. Forty gram of soil samples was mixed with 100 ml of distilled water in each case; a serial dilution of 101-105 was conducted in each case. One milliliter (1 ml) of each soil samples was taken from 104 and 105 diluents and was inoculated onto nutrient agar using pour plate technique. The plates were incubated at 37°C for 24-48 hours. Total colony count was taken and the result recorded. Colonies with different morphological appearance were subculture using streak method of inoculation for the purpose of identification onto fresh nutrient agar (NA).

Media Preparation. Media such as nutrient agar, mannitol salt agar and egg-yolk agar were used for isolation and it was done according to manufacturers' specification [3].

Identification of the Bacillus Isolates. The isolates were identified using gram reactions, catalase, motility, starch hydrolysis, voges proskauer (vp), citrate utilizations tests, endospore staining, and hemolysis test. The isolates were subjected to confirmatory test that was achieved by plating the isolates on egg-yolk agar and mannitol salt agar respectively. The colonies that failed to grow were regarded as non bacillus species.

Antibiotic Production Test

Each isolated Bacillus was culture under aerobic condition in nutrient broth for 72 hours at 37°C. The broth containing the bacillus species in each case was reinoculated on solid nutrient agar media seeded with the clinical strains of the following test organisms; *Staphyloccous aureus* (strain SA 020), *Streptococcus pyogens* (strain SP 008), *Salmonella hyphi* (strain ST 15), *Pseudomonas aeruginosa* (strain PA 011), *Escherichia coli* (strain ES 002) and *Klebsiella species* (strain KP 012). The cultures were incubated at 37°C for 24 hours. Clear zones of inhibition were measured and recorded.

Results

Table 1 presented the physiochemical analysis of the 16 soil samples which include PH 6.89-8.47, percentage moisture content (1.58-21.21), total colony count as indicated in table 2 was 3.8-16.0× 104 CFUlg and the percentage frequencies of occurrence was 1.9 – 30.8%. The total bacteria count according to the sample is presented in Table 2.

Table 1. Moisture content, temperature and PH of the soil samples

Sample code*	WBD (g)	WAD (g)	WL (g)	%MC	Temp. (°C)	pH
Cg	278.40	274.00	4.40	1.58	28	6.89
Ab	145.00	125.20	19.80	13.66	28	7.60
Sg	121.80	109.80	12.00	9.85	27	7.73
Tg	118.20	106.00	12.20	10.32	24	8.36
Mba	146.20	125.20	21.00	14.36	29	7.28
Ud	182.70	166.50	16.20	8.86	25	7.26
Fl	166.00	155.20	10.80	6.50	25	7.44
Bs	128.20	101.00	27.20	21.21	28	8.13
Tf	183.00	159.70	23.30	12.73	29	7.47
Mk	179.20	145.90	33.3	18.58	24	7.87
Be	153.30	120.90	32.40	21.13	25	8.37
Dk	175.80	143.40	32.40	18.43	26	8.47
Kg	259.80	215.70	44.10	16.97	28	7.19
Brs	197.30	164.20	33.10	16.77	26	7.54
Sbg	209.50	187.70	21.80	10.40	29	7.05
Mb	159.70	127.80	31.90	19.97	27	7.49

WBD (g) / WAD (g) = Weight before and after the samples were dried in grams;
WL (g) = Weight loss in grams;% MC = Percentage moisture content of the soil samples;
Temp. (°C) = Temperature of the soil samples; PH = values obtained from PH meter;
* sample codes were derived from the names of the sampling sites

Table 2. Total bacteria count per sample

Sample code	Total bacteria count (10^4 CFUlg)
Cg	10.8
Ab	12.0
Sg	12.4
Tg	10.8
Mba	3.8
Ud	8.8
Fl	9.6
Bs	9.2
Tf	12.8
Mk	5.6
Be	15.6
Dk	6.4
Kg	8.4
Brs	16.0
Sbg	9.6
Mb	10.6

The type of Bacillus identified in the investigated samples and the relative frequency of occurrences are presented in Table 3.

The results of the antibiotics production test of the isolates are presented in Table 4.

Table 3. Isolated Bacillus and associated frequency of occurrence

Organisms	Frequency of occurrence (%)
Bacillus cereus	30.8
B. brevis	1.9
B. polymyxa	3.8
B. licheniformis	13.5
B. sphaericus	7.7
B. mycoides	13.5
B. pumilus	7.7
B. subtilis	3.8
B.alvei	1.9
B.laterosporous	1.9
B. firmus	9.6
B. circulans	3.8

Table4. Antibiotics production tests of the isolates

Isolates	Test organisms (zones of inhibition in mm)					
	S. aureus	***S. pyogens***	***P. aeruginosa***	***E. coli***	***Klebsiella spp***	***S. typhi***
B. cereus	n.a.	16	n.a.	11	12	14
B. brevis	7	11	n.a.	7	16	n.a.
B. polymyxa	n.a.	n.a.	n.a.	n.a.	15	n.a.
B. licheniformis	5	11	n.a.	n.a.	26	16
B. sphaericus	n.a.	n.a.	10	n.a.	23	29
B. mycoides	n.a.	26	n.a.	n.a.	10	n.a.
B. pumilus	5	13	n.a.	n.a.	6	16
B. subtilis	19	27	n.a.	n.a.	17	26
B. alvei	n.a.	24	19	n.a.	9	19
B. laterosporous	n.a.	n.a.	n.a.	n.a.	n.a.	7
B. firmus	15	16	n.a.	9	n.a.	n.a.
B. circulans	n.a.	13	n.a.	n.a.	9	11

n.a. = not available

Discussion

Bacillus species are known to inhabit soil, because the organisms are documented to withstand both high and low temperature condition. This special attribute exhibited by bacillus species makes the organisms most successful among other bacteria species known [3]. The ability of the organisms to survive more under dry environmental condition as revealed in this study is a clear indication that bacillus species can be considered xerophytes. Similarly, the ability of isolating bacillus species from situation of both slightly acidic and alkaline environment as revealed in Table 1 makes suggests that Bacillus species are basidiophytes. The peculiarity of Bacillus species in an environment with elevated nutrient is an indication that the organisms have better competitive ability compare to other bacteria species present in the environment under study. It follows also that Bacillus cereus have more adaptive features compare to other bacillus isolates, follow by *Bacillus mycoides*, *Bacillus licheniformis*, *Bacillus firmu*, *Bacillus pumilus* etc. (Table 3).

Antibiotic production in this study, revealed that all the twelve (12) isolates of bacillus species produced very little inhibitory effect as noticed on *Pseudomonas aeruginosa*, and *E. coli*. However, the inhibitory effect was more on *Klebsiella* species (83%), *Streptococcus pyogen* (75%), *Salmonella typhi* (66.7%), *Staphylococcus aureus* (41.7%), *E. coli* (25%) and *P. aeruginosa* (16.7%).

This investigation has revealed the potentials of Bacillus species especially on antibiotic production, it is therefore necessary for isolation and purification of the chemical substances (Metabolites) for detail chemical studies in order to determine their composition and structures.

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