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Evaluation of *Bacillus* Species with Antimicrobial Producing Potentials from Dump Sites in Abakaliki

[1]Oko Augustine Okpani, [1]Eluu Stanley Chijioke, [2]Ahamefule Augustus Kelechi, [3]Ngele Kalu Kalu and [1]Nweze Nwabueze Peter

[1]Department of Biotechnology, Ebonyi State University, Abakaliki, Nigeria
[2]Department of Biotechnology, Federal University of Technology, Owerri, Nigeria
[3]Biology Microbiology Biotechnology, Federal University, Ndufu-Alike, Ikwo, Abakaliki, Nigeria

Abstract: Thisstudy was carried out to evaluate the potentials of *Bacillus* species isolated from soil collected from different dumpsites in Abakaliki with antibiotic producing potentials. Thirty soil samples were collected from different waste dumpsites and *Bacillus* species was isolated by serial dilution technique and 0.5 mL of the 5th dilution inoculated on freshly prepared Bushnell Haas broth solidified using agar. Microbial colonies were counted after 48 hours and representative of each colony was purified using the same media. Each *Bacillus* isolate was identified using standard microbiology procedure. Pure cultures of each *Bacillus* isolate was then cultured in nutrient broth and allowed for 5 daysand thereafter, used for antimicrobial screening against selected human pathogens including *E. coli, S. aureus, C. albicans* and *Klebsiellapneumoniae*. The result showed that number of colonies ranged from 0 to 468 x10^5cfu/g. The morphology showed that all the isolates were Gram positive, rod shaped, catalase positive, oxidasepositive, indole negative and were motile. Out of 35*Bacillus* species isolated 30 (85.71%) were active against *E. coli* with inhibition zones ranging from 15-30 mm, 24 (69.57%) were active against *S. aureus* with inhibition diameter ranging from 12-30 mm, 23 (65.71) of the isolates showed antibacterial activity against *K. pneumonia* with zones of inhibition ranging from 10-30 mm but against *C. albicans,* 28 (80%) of the isolates showed antifungal activities with inhibition zones that ranged from 15-34. This study has shown that most of the *Bacillus* isolates showed antibacterial and antifungal activities. Hence, there is need to harness these isolates with antimicrobial producing potentials for the treatment of human diseases.

Key words: *Bacillus* Species · Antibiotics · Antimicrobial Resistance · Abakaliki · Ebonyi State · Waste Dump Site

INTRODUCTION

Bacillus species are Gram-positive aerobic or facultative anaerobic, sporulating rod shaped bacteria that are ideally spread in nature [1]. *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 disinfectants and have antagonistic effect on other microorganisms [2].

Antibiotic is a metabolic products of certain species of microorganisms which functions to ultimately inhibit the growth of other bacterial pathogens and sometimes, the secreting organism at high concentration. The *Bacillus* species are known for the synthesis of secondary metabolites with remarkable diversity both in structure and function [3]. For instance, cerecin 7, Tochicin, Thuricin 7, thuricin 439 and entomocidus 9 [4] and few may be ribosomal in origin including sublancin [5], subtilosin A [6], subtilin [7] and TasA [8] are highly active against variety of microorganisms [9]. Bacitracin is the most important cyclic polypeptide antibiotic produced by *Bacillus* species and is primarily active against the Gram-positive bacteria. The bacitracin is used as anti-infective agent in a variety of pharmaceutical preparations including aerosols, topical lotions, skin ointments and creams. The bactericidal activity of bacitracin is the result of cell wall inhibition [10].

Corresponding Author: Oko Augustine Okpani, Department of Biotechnology, Ebonyi State University, Abakaliki, Nigeria.

For several pathogenic bacteria such as Staphylococcus aureus, Pseudomonas aeruginosa and Mycobacterium tuberculosis, the emergence of multi drug resistant (MDR) strains has been reported, which make infections with these strains increasingly difficult to treat with currently available antibiotics [11, 12]. Antimicrobial resistance has become an emerging threatglobally and decreases the chances for prevention and treatment of infectious diseases caused by viruses, bacteria, parasites and fungi [13, 14]. A report by the World Health Organization (WHO, 2014[23]) indicated that there is an increase of morbidity and mortality of infectious diseases due to antimicrobial resistance, which have been estimated to lead to a world-wide economic loss of up to 100 trillion US dollars (USD) in 2050 as the result of a 2%-3% reduction in the gross domestic product (GDP) [15]. A conservative estimation is that AMR now annually attributes to 700, 000 deaths globally, with a potential leap to 10 million in 2050 [16]. Antibiotics resistance is a response of microorganisms against antimicrobial compounds, which can arise via several mechanisms such as chromosomal mutations, binding site modifications or horizontal transfer of genes conferring resistance [17], [18], [19]. Since antibiotics are synthesized by specific microbial species including some *Bacillus* speciesto inhibit the growth of other microorganisms during the course of natural competition to dominate an environment, it is therefore, most likely that some dominant *Bacillus* species in our local waste dumpsites may have the potentials to synthesize powerful antibioticsubstances that can be the used in the treatment of infections hence the need to harness them. Hence, thisstudy was aimed at evaluating the antimicrobial production potentials of *Bacillus*speciesisolated from different dumpsites in Abakaliki, Ebonyi State, Nigeria.

MATERIALS AND METHODS

Test Pathogens: The test pathogens including laboratory strains of *E. coli, Staphylococcus aureus, Klebsiella pneumonia* and *Candida albicans*were obtained from microbiology sample collection centre, Microbiology laboratory, Ebonyi State University, Abakaliki, Nigeria.

Collection and Preparation of Soil Samples: Soil samples were collected from 35 different waste dump sites in Abakaliki, Ebonyi State, Nigeria. The samples (approximately 5 g each) were collected using sterile spoon and packaged in clean, dry and sterile polythene sample bag. All the samples were transferred to microbiology laboratory, Ebonyi State University, Abakaliki for further studies.

Isolation of *Bacillus* spp.: *Bacillus* specieswere isolated from the soil samples by the serial dilution method. One gram (1g) of each soil sample was weighed out and dissolved in 9 mL of sterile water and vortexed to mix well making up to 10 mL volume in a test tube. From the first tube, 1 mL of the aliquot was transferred to the next tube, this was done until the 5th test tube. Then, 0.5 mL of each aliquot from the 5th dilution was transferred to freshly prepared *Bacillus* isolation (Busna Hans broth with agar agar) agar mediumand allowed to grow 48 hours. Individual colonies were then sub-cultured in freshly prepared nutrient agar plate by streaking and allowed to grow for 24 hours to obtain pure isolates.

Determination of Number of Colonies Formed: The number of colony formed per plate of each soil sample was determined by manually counting the number of distinct colonies on the plate and multiplying it by the dilution factor. This was recorded and expresses as colony forming units (x10^5*cfu*).

Identification of *Bacillus* Species Isolated: The plates were examined by their colony characteristics and suspected colonies were identified using standard microbiology procedure [20]. The isolates were identified by their Gram staining, morphological and biochemical characteristics.Suspected isolates by Gram-positive and rod-shaped were selected for additional identification tests. Subsequent identification tests including citrate hydrolysis, motility, Indole and catalase tests were performed. A total of 35 isolates were identified to be *Bacillus* species by collecting like terms across samples.

Extraction of Antimicrobial Compound from *Bacillus* Spp.: Each isolate was cultured in nutrient broth medium and incubated at 30°C for 5 hours. After incubation, a part of the crude culture medium was used for antimicrobial screening.

Screening for the Antimicrobial Activity of *Bacillus* Isolates: The antimicrobial activity of each isolate was tested against pathogenic bacteria including *Staphylococcus aureus*, *Escherichia coli* and methicillin resistant *Klebsiella pneumoniae* as well as *Candida albicans* using disc diffusion method. The microbial

suspensions of freshly grown cultures were prepared in sterile water and adjusted to a McFarland's standard concentration. Mueller Hinton Agar plate, (MHA) was inoculated by dipping a sterile cotton swab into the cell suspension and streaking it across the agar surface in three directions. The plates were dried at ambient temperature for 15 minutes. Five (5) holes were bored on the Mueller Hinton plate using sterile 6 mm cork borer. The bacterial extract was used to impregnate the discs. The plates were incubated for 24 hours at 37°C as described by Stein [21]. The antimicrobial activities of the aliquots were recorded in mm.

RESULTS

Result of Colony Counts of *Bacillus* Spp. In Soil Samples (CFU): The result of the colonies counts showed that soil samples 14 and 8 collected from Presco campus dumpsite has the highest number of colonies (4.68 $x10^7$ cfu/g and 456 $x10^5$cfu/g), followed by sample 10 (352 $x10^5$cfu/g) collected from Ahia Ofu dumpsite and sample 23 collected from CAS campus waste dumpsite recorded (328 $x10^5$cfu/g). Meanwhile, soil samples 2, 4, 21 and 30 recorded low colony counts (36, 28, 16 and 33 $x10^5$cfu/g). However, the soil samples 7 and 28 collected from Mechanic site and Tipper garage respectively recorded no colony as shown in Fig. 1.

Biochemical Identification of the *Bacillus* Isolates: The results of the biochemical identification of representative isolates of *Bacillus* species is presented in Table 1 below.

Antimicrobial Activities: The result of the antimicrobial activities of the *Bacillus* species isolated from different dump sites showed that out of 35*Bacillus* species isolated 30 (85.71%) were active against *E. coli* with inhibition zones ranging from 15-30 mm, 24 (69.57%) were active against *S. aureus* with inhibition diameter ranging from 12-30 mm, 23 (65.71) of the isolates showed antibacterial activity against *K. pneumonia* with zones of inhibition ranging from 10-30 mm but against *C. albicans,* 28 (80%) of the isolates showed antifungal activities with inhibition zones that ranged from 15-34 (Fig. 2).

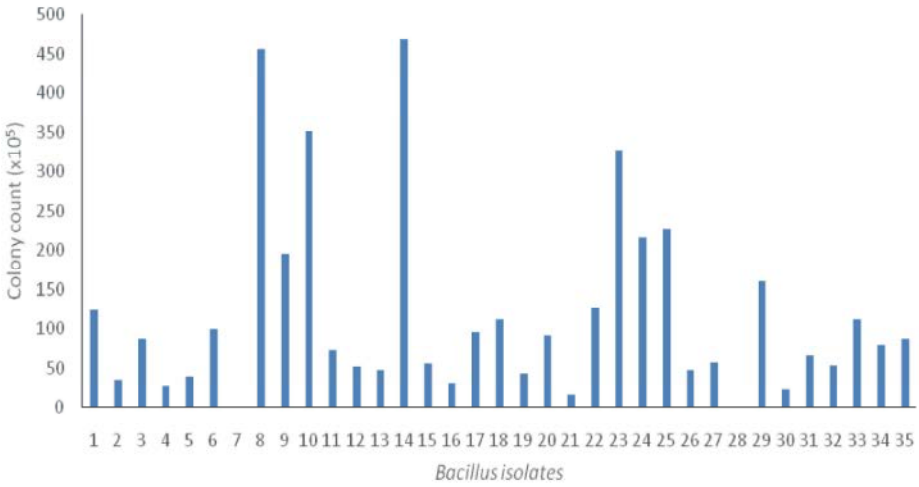

Fig. 1: Number of bacteria colonies formed per soil samples

Table 1: Morphological and Biochemical Identification of the Isolates

	Test					
Isolate	Gram staining	Catalase	Oxidase	Motility	Indole	Morphology
1	+	+	+	+	-	Rod
2	+	+	+	+	-	Rod
3	+	+	+	+	-	Rod
4	+	+	+	+	-	Rod
5	+	+	+	+	-	Rod
6	+	+	+	+	-	Rod
7	+	+	+	+	-	Rod
8	+	+	+	+	-	Rod
9	+	+	+	+	-	Rod
10	+	+	+	+	-	Rod

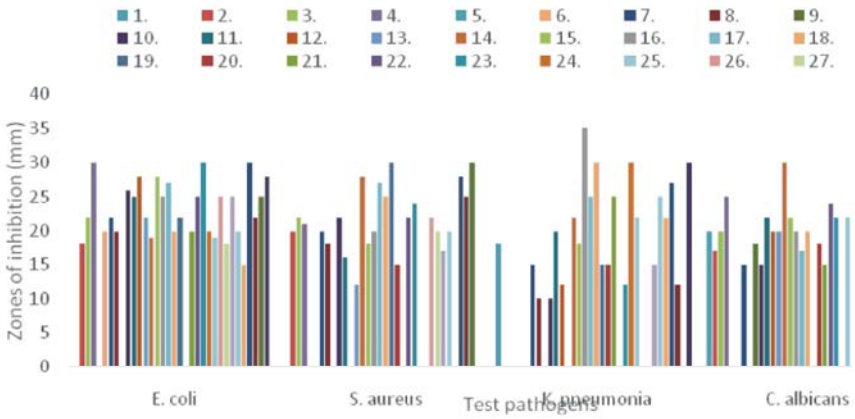

Fig. 2: Antimicrobial activities of *Bacillus* species isolated from soil samples collected from different dump site in Abakaliki

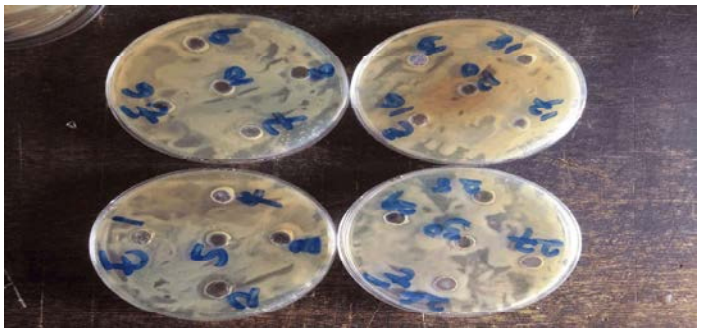

Fig. 3: Antimicrobial activities of the *Bacillus* isolates against *E. coli*

Fig. 4: Antimicrobial activities of the *Bacillus* isolates against *C. albicans*

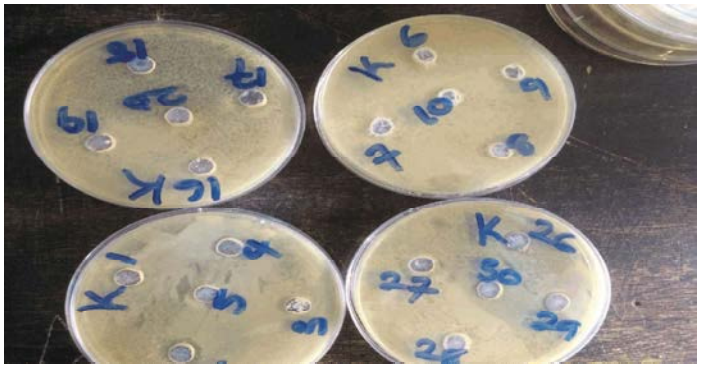

Fig. 5: Antimicrobial activities of the *Bacillus* isolates against *K. pneumoniae*

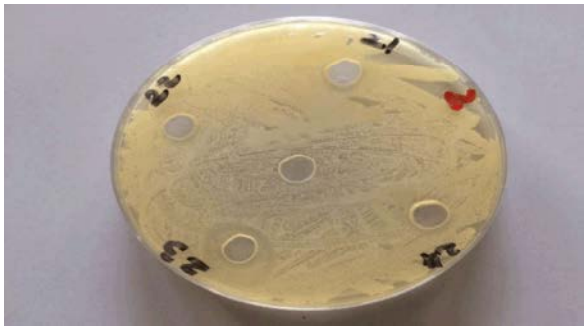

Fig. 6: Antimicrobial activities of the *Bacillus* isolates against *S.aureus*

DISCUSSION

The present study was carried out to evaluate *Bacillus* species with antimicrobial producing potentials from soil sample collected from different waste dumpsites in Abakaliki, Ebonyi State. This study showed that majority of the *Bacillus* isolates has antimicrobial production potentials some of which were broad spectrum in activity.

The result showed that out of 35*Bacillus* species isolated, 30 (85.71 %) showed antimicrobial activities against *E. coli*with inhibition diameter ranging from 18 mm to 30 mm while only 19 (54.29 %) showed inhibition against *Staphylococcus aureus* with inhibition zone ranging from 12 mm to 30 mm which is significantly lower than the standard drug chloramphenicol (36 mm).*S. aureus* is known to be the leading cause of nosocomial infections [22].*Staphylococcus aureus* is the prevalent cause of blood stream infections, skin and soft tissue infection and pneumonia [23]. The ability of some of these isolates to inhibit the growth of *S. aureus* showsthat they possess the ability of producing effective antibiotics against the pathogens.

Meanwhile 23 (65.721 %) of the isolates showed antimicrobial activity against *Klebsiella pneumonia* with inhibition zones ranging in diameter from 10 to 35 mm but 28 (80 %) of the *Bacillus* isolates showed antimicrobial properties against *Candida albicans*with inhibition zone ranging in size from 12 to 34 mm (Fig. 2). The *Bacillus* species has been identified as the most popular for producing peptide antibiotic compounds such as polymyxin, colistin and circulin [24].

Meanwhile, in all, 88.57 % (31/35) of the *Bacillus* isolates showed antimicrobial activities. This is greater than the report of WHO [25] who stated that out of 35 various soil samples only five bacterial isolates were found to produce inhibitory zones around their colonies on plates pre-inoculated by the pathogenic bacterial and fungal strains.

A study done in Brazil where *E. coli,* six *Candida* species and 5 dermatophytes were tested including *T. mentagrophyte* and *M. gypseum* showed antibiotic activity of *Bacillus* isolates only on the *E. coli* and two *Candida* species [24].This suggest that there may be more abundance of antimicrobial producing strains of *Bacillus* species in waste dump sites in Abakaliki, Nigeria.

Many antibiotics in use have been isolated from *Bacillus* species and are known to be the main source of lead compounds for antimicrobial drugs [25]. In line with the result of this study, Mannanov and Satarova [15] reported isolation and characterization of abacteriocin produced by a newly isolate of *Bacillussubtilis*. The structures and biosynthetic pathways ofribosomal antibiotics like subtilin, ericin and sublancinand non-ribozomal antibiotics such as iturin and fengycin were reviewed by Diekema *et al.*[8]. In addition to peptide derivatives, lipopeptide antibiotics like surfactinhave been also isolated and characterized from this earn born genus [11]. Biologically active inhibitory agents with hydrophobic properties were characterized by Stover and Driks [21] after extracting of broth culture of *Bacillus* sp. by chloroform. Bacilysocin, a novel and broad spectrum phospholipidantibiotic, was purified and characterized from butanol extract fraction of *Bacillussubtilis* 168 [17]. These two latest reports indicated that *Bacillus* species can produce agents with chemically differentproperties from peptide group.

CONCLUSIONS

The development of resistance and lesser safety margins provoked the scientists to search for antimicrobial producing *Bacillus* species with maximum higher antimicrobial activity. With this background, the present study was conducted to assess the antibiotic production potentials of *Bacillus* species isolated from soil samples collected from different waste dump site in Abakaliki, Ebonyi State, Nigeria. The findings of this study show that soil samples from different waste

dumpsites in Abakaliki, Nigeria harbor *Bacillus* species with the potential of producing novel antibiotics. Also the isolates that showed good zones of inhibitions against all pathogens tested could be potential sources of broad spectrum antimicrobial agents. There is need to harness these organisms in the production of powerful antimicrobial agents to be used in disease prevention and treatment.

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