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Degradative and antimicrobial potentials of *Bacillus* species isolated from water samples and contaminated soil in Ado-Ekiti metropolis, Nigeria

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ABSTRACT

This study investigated the ability of *Bacillus* species isolated from soil and water samples to degrade some petroleum products and pesticides as well as its ability antimicrobial potency against selected pathogens. *Bacillus* species were isolated from soil collected from dumping site and water samples from Ado-Ekiti, Nigeria on Luria Bertani agar. The biodegradative and antimicrobial activities of the isolates were conducted using minimal nutrient medium and agar well diffusion methods respectively. Commercial antibiotics were used as positive control. All the *Bacillus* isolates showed varied degree of degradation on petroleum products and pesticides. Only *B. polymyxa* showed antimicrobial activity against *Staphylococcus aureus* with zone of inhibition of 18.00mm. The commercial antibiotics were most inhibitory to most of the test pathogens in varying degrees.The isolates can be used in mitigating pollution caused by oil spillage and overuse or abusive use of herbicides and fungicides. However, these isolates are not good candidate as antibacterial agents for the selected test pathogens.

INTRODUCTION

The release of contaminant to the environment, including petroleum and petroleum-derived products, is one of the main causes of global contamination (Rahman *et al.*, 2002) which also constitutes risk to human and animal health because of their toxigenic and carcinogenic properties. Mechanical and chemical methods for remediation of hydrocarbon-polluted environment are expensive, technologically complex and lack public acceptance. The biodegradation of hydrocarbons has a high ecological significance, as it constitutes the major process for remediation of contaminated areas (Prabhu and Phale, 2003). Thus, bioremediation remains the method of choice for effective removal of hydrocarbon pollutants in the environment. Hydrocarbon degrading bacteria and fungi are mainly responsible for the mineralization of oil pollutants and are distributed in diverse ecosystem (Alloway and Ayres 1993). The microbial degradation of hydrocarbon seems to be a promising tool in the control of pollution and is the subject of a large number of current researches on the biochemistry and the genetics involved in activity. Bacteria, yeast and filamentous fungi have been reported as transforming agents (Hester and Mendelssohn, 2000).

Screening for new antibiotics from natural sources is becoming increasingly important for the pharmaceutical industry (Schmidt, 2004) as pathogenic bacteria are quickly becoming resistant to commonly used therapeutic agents. Secondary metabolites such as bacitracin, gramycidin, polymyxin and tyrotricidin are produced only by some species of a genus *Bacillus* (Stachelhaus *et al*., 1995; Drablos *et al*., 1999) which are more effective against Gram positive than Gram negative bacteria. Lipopeptides produced by *Bacillus* also demonstrate anti-fungal, anti-viral, anti-ameobocytic and anti-mycoplasma activities ((Milner *et al*., 1995; Peypoux *et al*., 1999; Steller *et al*., 1999).The chemical and physical diversity of peptide antibiotics makes them ideal candidates not only for therapeutic applications but also in other areas, especially the agri-food industry (Mukherjee *et al*., 2004). The present investigation therefore attempts to investigate the biodegradative and antimicrobial potency of *Bacillus* species isolated from soil and water samples.

MATERIALS AND METHODS

Collection of sample

Surface soil sample was collected from a dumping site while water sample were collected in air tight bottles from different regions of Ado-Ekiti, metropolis, Ekiti State, Nigeria. All the samples were brought into the laboratory for further analysis.

Test organisms

Streptococcus pyogenes, Serratia marcescens, Klebsiella pneumoniae, Staphylococcus aureus and *Proteus mirabilis* were collected from the Microbiology Laboratory, State Teaching Hospital, Akure.

Isolation and identification of microbial isolates

Serial dilution technique was used in isolating *Bacillus* species from soil and water samples using Luria Bertani Agar (LBA). The bacterial isolates obtained were characterized using various morphological and biochemical tests described by Fawole and Oso (2001) and identification was carried out using Cowan and Steel (1993) and Holt *et al.* (2000).

Antimicrobial study

Bacillus species isolated from the different samples were tested against some clinical bacteria isolates such as *Streptococcus pyogenes, Serratia marcescens, Klebsiella pneumoniae, Staphylococcus aureus* and *Proteus mirabilis* using agar well diffusion method. The *Bacillus* cultures were centrifuged at 6000g for 15mins to remove cell debris. After centrifugation, 0.5ml of the supernatant were transferred carefully into the labelled holes while sterile distilled water was filled into one hole serving as the control and the antibiotic disc served as positive control. The inoculated plates were incubated for 24hrs at 37˚C and the diameter of the zone of inhibition was measured (Schillinger and Lucke, 1989)..

Antibiotic sensitivity pattern of the test bacteria

Antibiotic sensitivity test was carried out on *Bacillus* sp. and test pathogens using conventional antibiotic disc. This was done placing the antibiotic disc on solidified Mueller Hinton agar plate already seeded with the organisms and incubated at 30°C for 24hrs. The antibiotic used contained the following commercial antibiotics namely; Pefloxacin (PEF), Gentamycin (CN), Ampiclox (APX), Zinnacef (Z), Amoxacillin (AM), Rocephin (R), Ciprofloxacin (CPX), Streptomycin (S), Septrin (SXT), Erythromycin (E), Chloramphenicol (CH), Augmentin (AU), Tarivid (OFX), Sparfloxacin (SP).

Degradation study

Degradation study was carried out using Minimal salt broth (MSB) (Yin et al., 1998). This was sterilized at 121^{o}C for 15 minutes in the autoclave. About 100ml of minimal salt broth was measured into each Erlenmeyer flask and was supplemented with 0.5ml of the petroleum products (crude oil,

petrol, diesel, engine oil and kerosene) and pesticides (herbicides and fungicides). Each Erlenmeyer flask was inoculated with the different *Bacillus* species isolated. All the Erlenmeyer flasks were incubated at 37^{o}C for 5 days; the turbidity was recorded on each day using a spectrophotometer at 540nm optical density.

Statistical analysis

All the experiment carried out was done in triplicates and statistical analyses of data obtained were carried out using analysis of variance (ANOVA) and Duncan's Multiple Range Test for the estimation of means (SPSS windows 16 version).

RESULTS

Biodegradative ability of *Bacillus* species on petroleum hydrocarbons and selected herbicides

Four *Bacillus* species were isolated from contaminated soil and water and they were: *Bacillus subtilis, B. megaterium, B. cereus* and *B. polymyxa.* The degradative abilities of *Bacillus* species on selected petroleum hydrocarbon is shown in Figure 1. All the *Bacillus* species tested were able to degrade the hydrocarbon to different extent. *Bacillus cereus* had the highest degradative ability on crude oil, engine oil, kerosene and petrol by having the highest turbidity when compared to the other *Bacillus* species. This shows the ability of the organism to use these hydrocarbons as a carbon source. *Bacillus megatarium* had the highest degradative ability on diesel. Table 1 shows the degradative abilities of *Bacillus* species on selected herbicides. *Bacillus subtilis* had the highest degradative ability on premextra with turbidity increase from 2.50-6.12 as compared to *Bacillus megatarium* which showed the least degradative ability on premextra with turbidity ranging from 0.56-1.59. *Bacillus cereus* had the highest degradative ability on glyphosphate as turbidity increased from 0.76 on day1 to 8.90 on day 5 as compared to *Bacillus polymyxa*, whose turbidity decreased from 4.90 on day2 to 1.91 on day5.

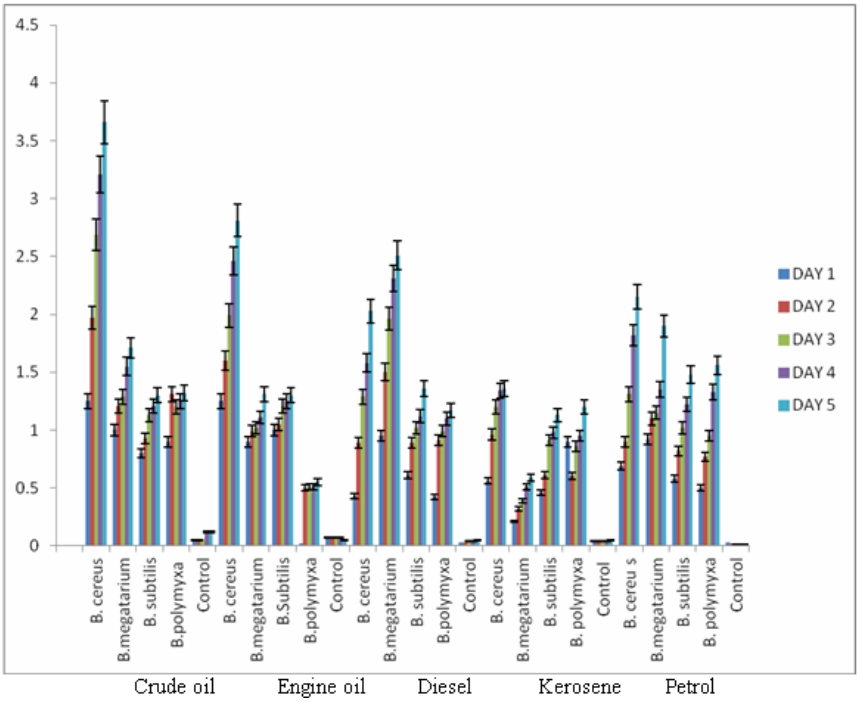

Fig. 1: Degradative abilities of *Bacillus* species on selected petroleum hydrocarbon

Table 1: Degradative abilities of *Bacillus* species on selected herbicides

Isolates	Premextra					Glyphosphate				
	Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5
B.cereus	1.80 ±0.00	1.95 ±0.10	2.10 ±0.00	2.80 ±0.03	3.80 ±0.00	0.76 ±0.00	2.55 ±0.05	5.20 ±0.00	6.30 ±0.00	8.90 ±0.00
B. megatarium	0.56 ±0.00	0.72 ±0.03	1.25 ±0.05	1.32 ±0.00	1.59 ±0.01	2.35 ±0.05	2.90 ±0.00	3.12 ±0.00	3.51 ±0.01	3.80 ±0.00
B. subtilis	2.50 ±0.05	3.17 ±0.03	4.60 ±0.00	5.21 ±0.00	6.12 ±0.00	3.00 ±0.00	4.03 ±0.00	4.91 ±0.01	5.05 ±0.05	5.55 ±0.00
B. polymyxa	1.20 ±0.00	1.60 ±0.00	1.98 ±0.00	1.98 ±0.00	2.40 ±0.00	4.10 ±0.00	4.90 ±0.00	2.00 ±0.00	2.00 ±0.00	1.91 ±0.09
Control	0.60	0.60	0.60	0.60	0.63	0.66	0.67	0.65	0.66	0.67

Also, B. *subtilis* had the highest degradative ability on benlate and mancozeb as its turbidity increased from 4.50 on day1 to 5.40 on day 5and from 6.54 on day 1 to 11.54 on day 5 on benlate and mancozeb respectively. Decrease in turbidity was seen in both *B. megatarium* and *B. polymyxa* with turbidity reducing 2.50 on day1 to 1.90 on day 5 and 3.22 on day1 to 1.90 on day 5 respectively. It was observed that *B. cereus* could not grow well on mancozeb as its turbidity decreased from 2.09 on day1 to 1.41 on day 5 (Table 2).

Table 2: Degradative abilities of *Bacillus* species on selected fungicides

Isolates	Benlate					Mancozeb				
	Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5
B.cereus	1.90 ±0.00	2.10 ±0.10	2.40 ±0.00	2.57 ±0.03	3.80 ±0.03	02.09 ±0.01	2.00 ±0.00	1.91 ±0.03	1.63 ±0.00	1.41 ±0.01
B. megatarium	2.50 ±0.00	2.51 ±0.03	2.70 ±0.00	2.40 ±0.00	1.90 ±0.00	1.00 ±0.00	1.00 ±0.00	0.91 ±0.01	0.83 ±0.01	0.69 ±0.01
B. subtilis	4.50 ±0.00	4.91 ±0.03	4.99 ±0.01	5.20 ±0.00	5.40 ±0.00	6.54 ±0.02	9.61 ±0.01	9.50 ±0.00	10.01 ±0.05	11.54 ±0.04
B. polymyxa	3.22 ±0.01	3.52 ±0.00	3.00 ±0.00	2.10 ±0.00	1.90 ±0.00	4.63 ±0.01	5.21 ±0.01	6.69 ±0.00	7.00 ±0.00	7.68 ±0.00
Control	0.70	0.70	0.70	0.73	0.72	0.70	0.70	0.70	0.70	0.70

Antibacterial activities of *Bacillus* species on selected pathogenic bacteria

None of these bacteria was susceptible to *Bacillus* species except *S. aureus* which was susceptible to *B. polymyxa* with zone of inhibition of 18.00mm (Table 3). However, the susceptibility pattern of the test pathogens to commercial antibiotics showed that *Proteus mirabilis* was sensitive to Amoxacillin and *Staphylococcus aureus* was sensitive to Zinnacef while *Streptococcus pyogenes* was very sensitive to Ampiclox, Zinnacef and Amoxacillin while all other isolates were susceptible to the antibiotics in varying degrees as shown in Tables 4 and 5.

Table 3: Antimicrobial activities of *Bacillus* species against some bacteria pathogens

Isolates	Diameter of zones of inhibition (mm)				
	Proteus mirabilis	*Serratia marcesens*	*Staphylococcus aureus*	*Klebsiella pneumonia*	*Streptococcus Pyogenes*
1	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
2	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
3	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00
4	00.00±0.00	00.00±0.00	18.00±0.00	00.00±0.00	00.00±0.00

Key: 1 – *B. subtilis*
2 – *B. cereus*
3 – *B. megaterium*
4 – *B. polymyxa*

Table 4: Antibiotics susceptibility of Gram negative test pathogens

Test pathogen	Zones of inhibition (mm) S	SXT	CH	SP	CPX	AM	AU	PEF	OFX	CN
Proteus mirabilis	22.00 ±0.60	21.00 ±1.00	17.00 ±0.33	16.00 ±0.00	27.00 ±0.33	00.00 ±0.00	22.00 ±0.03	20.00 ±0.20	19.00 ±0.63	18.00 ±0.67
Serratia marcesens	19.00 ±0.67	20.00 ±0.33	15.00 ±0.00	20.00 ±0.00	29.00 ±0.00	19.00 ±1.20	23.00 ±0.67	20.00 ±0.93	21.00 ±0.67	19.00 ±0.33
Klebsiella pneumoniae	20.00 ±0.00	19.00 ±0.33	16.00 ±1.10	18.00 ±1.33	26.00 ±0.33	16.00 ±0.67	20.00 ±1.00	18.00 ±0.00	20.00.00 ±0.00	17.00 ±0.33

Table 5: Antibiotics susceptibility of Gram positive test pathogens

Test pathogen	Zones of inhibition (mm) R	CPX	S	SXT	E	PEF	CN	APX	Z	AM
Staphylococcus aureus	20.00 ±0.33	20.00 ±0.67	18.00 ±0.67	21.00 ±0.33	18.00 ±1.60	17.00 ±1.00	20.00 ±0.67	16.00 ±0.67	00.00 ±0.00	17.00 ±1.67
Streptococcus pyogenes	18.00 ±0.00	17.00 ±0.10	19.00 ±0.67	20.00 ±0.33	16.00 ±0.33	17.00 ±1.10	18.00 ±1.00	00.00 ±0.00	00.00 ±0.00	00.00 ±0.00

DISCUSSION AND CONCLUSION

Soil is considered as one of the most suitable environments for microbial growth (Cavalcanti *et al*., 2006). Four *Bacillus* species namely *Bacillus subtilis, B. megatarium, B. cereus* and *B. polymyxa* were isolated from contaminated soil and water. The present work shows the degradation of petroleum hydrocarbon, fungicides and herbicides. Most of these *Bacillus* species live in the soil, multiply and produce enzymes that help in bioremediation (Adesemoye *et al*., 2006). Generally, the *Bacillus* species used for this work were all able to degrade all petroleum hydrocarbons. This might be attributed to their ability to produce spores which may shield them from the hydrocarbon (Adesemoye *et al*., 2006). However, their ability to degrade the selected fungicides was low which may be due to the fact that fungicides contain a little amount of antimicrobial agent (Alloway and Ayres, 1993). The high spectrophotic value obtained in herbicides especially glyphosphate might be as a result of the ability of the organisms either to break or multiply in the presence of the chemical. Increase in spectrophotometer reading indicates that *Bacillus* species to efficiently utilize the petroleum hydrocarbons, fungicides and herbicides for their growth and establishment (Prabhu and Phale, 2003).

The antibacterial activities of *Bacillus* species against selected test pathogens show that only *B. polymyxa* could inhibit the growth of *S. aureus* Yilmaz *et al*. (2006) also observed that *S. aureus* was resistant to *B. cereus* and *B. megaterium* although Perez *et al*. (1993) showed the sensitivity of *S. aureus* to various strains of *Bacillus* species. *Oscariz et al*. (1999) isolated and identified a bacteriocin-producing strain of *B. cereus* from a soil sample which was active against most Gram-positive but not Gram-negative bacteria. The findings of the present study indicate that *Bacillus* cereus had no antimicrobial effects on both Gram-positive and Gram-negative bacteria. All the commercial antibiotics were more inhibitory to the test pathogens than *Bacillus* species except zinnacef which was not inhibitory to *S. aureus* and *S. pneumoniae* and amoxacillin which was inhibitory to *S. pyogenes* and *P. mirabilis*. Also, *S. pyogenes* was not sensitive to ampiclox.

CONCLUSION

The results obtained in this study have shown that *Bacillus* species used in this work have the ability to degrade the hydrocarbons, fungicides and herbicides. It can therefore be employed in mitigating pollution caused by oil spillage and overuse or abusive use of herbicides and fungicides. However, these isolates are not good candidate as antibacterial agents for the selected test pathogens.

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