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BIODEGRADATIVE AND ANTIMICROBIAL ACTIVITIES OF *PSEUDOMONAS* ISOLATED FROM SOIL AND WATER SAMPLES IN ADO-EKITI METROPOLIS, NIGERIA

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Abstract

Context: The release of petroleum hydrocarbon and pesticides into the environment is a major concern because of their toxigenic and carcinogenic potentials. Microorganisms which are biodegradable and most often less toxic have been found to use these materials as carbon sources. Also, the development of resistance of microbial pathogens to commercial antibiotics has necessitated an alternative to commercial antibiotics.
Objectives: This study investigated the ability of *Pseudomonas* species isolated from soil and water samples to degrade some petroleum products and pesticides as well as its antimicrobial potency against selected pathogens.
Materials and methods: *Pseudomonas* species were isolated from soil samples from mechanic workshop, dump sites and stream water. The isolates were tested for the ability to degrade petroleum products (diesel, engine oil and kerosene) and some pesticides. These isolates were also tested for antimicrobial activities against some pathogenic bacteria and fungi.
Results: Three *Pseudomonas* were obtained from the samples; *Pseudomonas fluorescens* (DS) was isolated from a dumping site and two *Pseudomonas aeruginosa*, MW and SW were isolated from mechanic workshop and stream water. The three isolates showed varying ability to degrade petroleum products and pesticides. *Escherichia coli*, *Proteus mirabillis* and *Serratia marcescens* were susceptible to the three *Pseudomonas* isolates in varying degree while *Staphylococus aureus* and *Klebsiella pneumoniae* were resistant to the isolates. All the fungi except *Candida albicans* were susceptible to the *Pseudomonas* spp**.**

Key words: *Pseudomonas,* soil and water sample of Nigeria, Bio-degradative and antimicrobial activity.

Introduction

Pseudomonas is a genus of gram-negative aerobic gamma proteobacteria belonging to family pseudomonadaceae containing 191 validly described species (Euzeby 1997). All *Pseudomonas* have a functional tricarboxylic acid cycle and can oxidize substrates to carbon dioxide ($C0_2$), they are chemoorganotropic with a strictly respirating metabolism using oxygen and in some cases nitrates as terminal electron acceptor (Palleroni and Norberto 2010). Many can degrade exceptionally wide variety of organic molecules through a process called mineralization applied in sewage treatment (Abdel-el-Haleem 2003). Microorganisms with the ability to degrade many hydrocarbons have been described and their mechanisms of action have been studied. Hydrocarbons contain benzene, cyclopentadiene dicylopentadiene, stryene, toluene and xylene as major components and many other hydrocarbons as minor components (Santhini *et al.* 2009). Environmental pollution with hydrocarbon products is a major concern in

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many countries. This has led to a concerted effort in studying the feasibility of detoxifying hydrocarbon contaminants using oil-degrading microorganisms (Umanu *et al.* 2013). Pollution control protocols involving physico-chemical methods have often increased the problem rather than eliminated it. Biodegradation is an attractive method for the remediation of contaminated sites because of its economic viability and environmental soundness (Walker and Crawford 1997, Dinkla *et al*. 2001).

The importance of bacteria and fungi as sources of valuable bioactive metabolites is very well established (Alanis 2005). These bioactive metabolites have been shown to be antifungal, antibacterial and antiviral in action. The use of microorganisms in biological control of diseases has been necessitated owing to the problem of pathogen resistance to synthetic antibiotics (Rekha *et al*., 2010). Moreover, microorganisms have been shown to be biocompatible, biodegradable and non toxic in nature. *Pseudomonas fluorescens* is an antibiotic producer and has a wide spectrum of antimicrobial activity *against Salmonella typhimurium* (Laine *et al.* 1996), *Bacillus subtilis, Proteus vulgaris, Candida albicans* (Trujillo *et al.* 2007). These characteristics have made them good candidates for use as seed inoculants, root dips for biological control of soil-borne plant pathogen and also as antibacterial agents. It has also shown to have antimicrobial activity against *Staphylococcus aureus, Escherichia coli* and *Aeromonas hydrophila* (Vachee *et al.* 1997, Czerwonka *et al.* 1997). The present investigation sought to find out the biodegradative and antimicrobial properties of *Pseudomonas* isolated from soil and water samples.

Materials and Methods

Sample Collection

Water samples from two streams and two wells (ground water) were collected in air tight bottles, labelled as SW1, SW2 and GW1, GW2 respectively. Also soil samples were collected from dumping site and mechanic workshop. All the samples were obtained from Ado–Ekiti, Nigeria and brought into the laboratory for further analysis.

Test organisms

Escherichia coli, *Proteus mirabillis*, *Serratia marcescens, Staphylococus aureus* and *Klebsiella pneumoniae* were collected from the State Specialist Hospital, Akure, Ondo State, Nigeria while *Fusarium solani, Candida albicans, Fusarium oxysporum, Selerotinia selerotorum* and *Rhizoctonia solani* were collected from the Department of Microbiology, Federal University of Technology, Akure (FUTA), Nigeria.

Isolation and identification of *Pseudomonas* from the samples

Serial dilution technique was used in isolating *Pseudomonas* from the water samples using King's B medium. The same procedure was repeated for the soil samples. 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).

Degradation

Degradation study was carried out using Minimal salt broth (MSB) (Yin *et al.* 1998). This was sterilized at 121^0C for 15 min in the autoclave. About 100 ml of minimal salt broth was measured into each Erlenmeyer flask and was supplemented with 0.5 ml of the petroleum products (petrol, diesel, engine oil and kerosene) and pesticides.

Antimicrobial study

Agar well diffusion method was used to determine the antimicrobial activities of *Pseudomonas* spp. isolated from the different samples against some clinical bacterial isolates; *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Serratia marcescens* and *Proteus mirabilis* and five fungi; *Fusarium solani, Candida albicans, Fusarium oxysporum, Selerotinia selerotorum* and *Rhizoctonia solani* on nutrient agar and potato

dextrose agar for bacteria and fungi respectively. All these were done in triplicates. The plates were observed for zone of inhibition which was measured with the aid of a meter rule. Antibiotics discs were used as positive control.

Results

Three *Pseudomonas* were obtained from the samples; *Pseudomonas fluorescens* (DS) was from a dumping site and two *Pseudomonas aeruginosa* MW and SW from mechanic workshop.

Biodegradative ability of *Pseudomonas* isolates

The degradation ability of *Pseudomonas* of different hydrocarbon i.e. petrol, diesel, engine oil and kerosene are shown in Table 1. *P. aeruginosa* MW had the highest degradation on petrol, kerosene and engine oil followed by *P. fluorescens* (DS) which had highest degradation on diesel. *Pseudomonas aeruginosa* (SW) had lower degradation on the petroleum products. Table 2 shows the degradative ability of the *Pseudomonas* spp. on different herbicides. *Pseudomonas aeruginosa* (SW) had the greatest ability to degrade Primextra herbicide while *P. fluorescens* (DS) had greatest ability on Glyphosate herbicides. *Pseudomonas aeruginosa* (MW) had the least degradative ability on primextra herbicides. Table 3 shows the degradative ability of *Pseudomonas* on two fungicides; Benlate and Mancozeb. *Pseudomonas fluorescens* (DS) had the highest degradation on both benlate and mancozeb, followed by *P.aeruginosa* (SW) while *P. aeruginosa* (MW) recorded the least degradative ability on both fungicides.

Table 1. Biodegradative ability of *Pseudomonas* isolates on petroleum products measured by optical density (OD) after 5 days of incubation.

Isolates	Diesel	Petrol	Kerosene	Engine oil
P. aeruginosa (MW)	0.41±0.02	7.14±0.00	0.46±0.01	3.19±0.01
P. aeruginosa (SW)	0.22±0.00	1.21±0.01	0.31±0.01	1.72±0.02
P. fluorescens (DS)	1.02±0.02	1.35±0.02	0.25±0.01	0.35±0.01

Table 2. Degradative ability of *Pseudomonas* spp. on selected herbicides measured by optical density (OD) of MSB after 1 to 4 days.

Isolates	Day 1		Day 2		Day 3		Day 4		Day 5	
	Primextra	Glyphosate	Primextra	Glyphosate	Primextra	Glyphosate	Primextra	Glyphosate	Primextra	Glyphosate
A	0.07±0.01	0.37±0.00	0.19±0.00	0.51±0.01	0.61±0.01	0.74±0.01	0.88±0.01	1.11±0.01	0.87±0.01	1.62±0.01
B	0.73±0.00	0.28±0.00	1.17±0.01	0.30±0.01	3.33±0.02	0.48±0.02	6.11±0.03	0.52±0.02	6.46±0.03	0.61±0.01
C	0.69±0.00	1.36±0.00	0.92±0.00	5.21±0.01	1.60±0.00	9.02±0.02	5.44±0.01	14.60±0.00	5.63±0.01	19.40±1.00

Table 3. Degradative ability of *Pseudomonas* sp on selected fungicides measured by optical density (OD) of MSB after 1 to 4 days.

Isolates	Day 1		Day 2		Day 3		Day 4		Day 5	
	Benlate	Mancozeb	Benlate	Mancozeb	Benlate	Mancozeb	Benlate	Mancozeb	Benlate	Mancozeb
A	0.003±0.01	0.01±0.00	0.21±0.01	0.04±0.00	0.28±0.01	0.04±0.00	0.28±0.02	0.05±0.02	0.27±0.01	0.07±0.00
B	0.15±0.02	0.11±0.00	0.37±0.00	0.38±0.01	1.12±0.00	1.41±0.00	0.29±0.02	1.69±0.03	3.03±0.03	1.77±0.02
C	0.18±0.00	0.23±0.00	0.42±0.00	0.55±0.02	1.86±0.02	1.73±0.02	2.04±0.02	2.02±0.01	4.61±0.01	2.21±0.01

Antimicrobial activities of *Pseudomonas* spp. on selected pathogenic microorganisms

Escherichia coli, *Proteus mirabillis* and *Serratia marcescens* were susceptible to *Pseudomonas* isolates while *Staphylococus aureus* was resistant to the isolates. *Klebsiella pneumoniae* was resistant to *P. aeruginosa* (MW) but showed fair resistance to *P. aeruginosa* (SW) and *P. fluorescens* (DS) (Table 4). The susceptibility pattern of the fungal isolates to *Pseudomonas* is shown in Table 5. All the fungi except *Candida albicans* were susceptible to *Pseudomonas* spp**.**

Antibiogram of selected pathogenic bacteria to commercial antibiotics

Klebsiella pneumoniae had a high sensitivity to streptomycin while *S. aureus* was sensitive to ofloxacin with a zone of inhibition of 32.00mm. *P. mirabillis* was resistant to amoxacillin and streptomycin. *S. aureus* was resistant to sparfloxacin while *K. pneumoniae* and *E. coli* were resistant to chloramphenicol and amoxacillin respectively (Table 6).

Table 4. Susceptibility pattern of some pathogenic bacteria to different *Pseudomonas* spp.

Isolates	Diameter of zones of inhibition (mm)				
	Proteus mirabilis	*Serratia marcescens*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Escherichia coli*
P. aeruginosa MW)	11.0±1.00	10.0±2.00	0.00±0.00	0.00±0.00	8.00±1.33
P. aeruginosa (SW)	11.0±0.00	13.0±2.00	0.00±0.00	5.00±0.00	9.00±1.00
P. aeruginosa (DS)	13.0±0.00	15.0±1.00	0.00±0.00	4.00±1.00	6.00±1.00
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table 5. Susceptibility pattern of some fungal isolates to different *Pseudomonas* spp.

Isolates	Diameter of zones of inhibition (mm)				
	Fusarium solani	*Fusarium oxysporum*	*Sclerotinia selerotonum*	*Rhizoctonia solani*	*Candida albicans*
P. aeruginosa (MW)	22.00±1.00	19.00±0.00	25.00±2.00	2.00±1.00	0.00±0.00
P. aeruginosa (SW)	24.00±2.00	19.00±1.00	26.00±2.00	5.00±0.00	0.00±0.00
P. aeruginosa (DS)	27.00±2.00	20.00±1.00	24.00±3.00	7.00±0.00	0.00±0.00
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table 6. Susceptibility pattern of used pathogenic bacteria to commercial antibiotic discs.

Antibiotics	Diameter of zone of inhibition (mm)				
	Proteus mirabilis	*Serratia marcesens*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Escherichia coli*
Amoxacillin	0.00	19.00	17.00	35.00	0.00
Augmentin	22.00	23.00	23.00	24.00	21.00
Chloramphenicol	17.00	15.00	21.00	0.00	20.00
Ciprofloxacin	27.00	29.00	30.00	30.00	27.00
Gentamicine	18.00	19.00	23.00	26.00	18.00
Pefloxacin	20.00	20.00	26.00	27.00	20.00
Co-trimoxazole	21.00	20.00	19.00	04.00	13.00
Sparfloxacin	16.00	20.00	0.00	26.00	21.00
Streptomycin	0.00	19.00	17.00	38.00	24.00
Ofloxacin	19.00	21.00	32.00	18.00	20.00

Discussion

The present study confirms the presence of *Pseudomonas* in soil and water as a proof to their ubiquitous nature (Euzeby 1997, Palleroni and Norberto, 2010. With the increasing uses of petroleum hydrocarbon products, contamination of soil and water by these products is becoming one of the major environmental problems (Barathi and Vasudevan 2001; Van Hamme *et.al* 2003). The bioremediation provides effective and efficient strategy to speedup the clean up processes. *P.fluorescens* DS exhibited the highest degradative ability against diesel, followed by *P. aeruginosa* MW and *P. aeruginosa* SW. However, in case of petrol, *P. aeruginosa* MW exhibited the highest degradative ability, followed by *P. fluorescens* DS and *P. aeruginosa* SW. Degradative abilitiues against kerosene was highest in *P. aeruginosa* MW followed by *P. aeruginosa* SW and *P. fluorescens* DS, with engine oil, *P aeruginosa* MW had the highest degradative ability, followed by *P. aeruginosa* SW and *P.fluorescens* DS. This was in conformity with a study carried out by Obayori *et al* (2009) in which *P.aeruginosa* had fair degradative ability on crude oil and engine oil but exhibited weak degradative ability on kerosene and diesel oil. Wongsa *et.al* (2004) have showed that *Serratia marcescens* had high efficiency to degrade gasoline, diesel kerosene and lubricating oil while *P. aeruginosa* showed less degradative ability on kerosene, diesel and gasoline.

All the isolates had degradative potentials though MW had the highest degradative abilities against petrol, kerosene and engine oil. This agrees with the reports given by Rusansky *et.al (*1987), Kiyohara *et.al (*1992), Johnson *et.al* (1996), Barathi and Vasudevan (2001) and Pokethityook *et.al* (2003), that *Pseudomonas* species are the most common bacteria hydrocarbon degrader.

All the isolates exhibited degaradative abilities on the used herbicides and fungicides but *Pseudomonas* sp DS showed the highest degradative ability against Benlate and Mancozeb followed by SW and MW. Herbicides and fungicides can be used convienently alongside with these *Pseudomonas* sp as biocontrol. This is in accordance with previous findings of Abdel-el- Haleem (2003) who reported that *Pseudomonas* sp can be used in both environmental (sewage treatement) and biotechnological applications.

Conclusion

Since *Pseudomonas* spp are non toxic and abundant in nature, they can be used in bioremediation of highly hydrocarbon contaminated area. In the same way, *Pseudomonas* spp can also be used for biocontrol purposes.

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