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Biodegradation of phenol by a newly isolated *Pseudomonas* sp. strain B1 from tiger nuts

***Adetitun, David Olugbenga[1], Akinbowale, Azeez O[1]. & Dutta, Kunal[2].**

[1]Department of Microbiology, Faculty of Life Sciences, University of Ilorin, Nigeria [2]Microbiology and Immunology Lab., Department of Human Physiology with Community Health Vidyasagar University, Medinipur-721102, West Bengal, India.

Corresponding author: adetitun.do@unilorin.edu.ng

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***Abstract*:** The bacterial flora of tiger nuts sold in Nigeria, Benin, Togo and Ghana were compared using the pour plate method. The ability of one of the isolated bacteria identified as *Pseudomonas* sp. (accession no. MN227323) via molecular tools to utilize phenol was assessed using mineral salts medium (MSM) with phenol as the only carbon source. Duplicate tubes were set up with control lacking phenol. The concentration of phenol used was 0.2 mM, 0.4 mM, 0.6 mM, 0.8 mM and 1.0 mM. The absorbance was read at 365nm and 400nm at 3-days interval for 15 days. *Pseudomonas* sp was isolated from tiger nuts bought from Togo and Benin. It was absent in those bought from Nigeria and Ghana. *Pseudomonas* sp was selected because our previous isolations from Nigeria had shown its ability to degrade hydrocarbons in vitro. Phenol utilization by *Pseudomonas* sp. was highest at 0.2 mM and followed by 0.4 mM. The utilization ability decreased as the concentration of phenol increased. The study concludes that *Pseudomonas* sp. can degrade phenol.
Keywords: *Pseudomonas* sp., Biodegradation Phenol, tiger nuts and absorbance.

1.0 Introduction

Tiger nut (*Cyperus esculentus*) is a tuber that grows freely and is consumed widely in Nigeria and in various other parts of West and East Africa [1]. Tiger nut tubers contain pyritic acid as the main saturated acid and oleic acid as the predominant unsaturated acid [2]. Tiger nuts are consumed for their nutritional or medicinal values [3]. Most fruits and vegetables are important sources of nourishment but they also harbour

varied loads of microbial flora [4].

One of the major environmental problems today is hydrocarbon contamination due to accidental and operational releases of petroleum. This has led to concerted efforts at studying the possibility of isolating more oil-degrading microorganisms for degradation purposes [5]. Hydrocarbon components have been reported to belong to the family of carcinogens and neurotoxics. Bioremediation is the promising technology for the treatment of these contaminated sites since it is cost-effective and will lead to complete mineralization [6].

Several bacteria are even known to feed exclusively on hydrocarbons. Microbial degradation of petroleum hydrocarbons in a polluted tropical stream in Lagos, Nigeria [7]. Nine bacterial strains which could degrade crude oil were isolated from the polluted stream. Aromatic compounds are sub-grouped into two general classes. Mononuclear aromatics are used extensively in fuels and also as industrial solvents. Polynuclear aromatics are generally less volatile [8]. Aromatics occur naturally in the environment mainly in fossil fuels but it is the increased environmental presence of these compounds due to industrial 3 emissions that is the subject of recent concern [9]. The degradation of aromatic compounds proceeds through well-known catabolic pathways where they are converted to one of dihydroxy aromatic substrates, such as: catechol, gentisate and protocatechuate [10].

Phenol is the basic structural unit of a wide variety of synthetic organics [11]. It is a listed priority pollutant by the United States Environmental Protection Agency and Agency for Toxic Substances [12]. Phenolic pollutants are generated from several sources such as the partial degradation of phenoxy herbicides, the use of wood preservatives and the generation of wastes by petroleum-related industries such as petroleum refineries, gas and coke oven industries, pharmaceuticals, explosive manufacture, phenol-formaldehyde resin manufacture, plastic and varnish industries and related metallurgical operations and petroleum pollution [13]. Biodegradation of phenol using pure and mixed cultures of suspended bacteria had been studied. Nevertheless, owing to the inhibitory effects of phenol on microbes, biological treatment of phenol-containing effluent has been facing challenges [14]. In order to remove higher concentration of phenol from effluents, it is peculiarly significant to isolate appropriate microorganisms that can naturally adapt as well as effectively degrade phenol at proportionately high concentration. Phenol-degrading microbes have shown substrate inhibition at higher concentrations. The purpose of this study is to determine phenol utilization capability of a newly isolated *Pseudomonas* sp. (accession no. MN227323) isolated from non-oil *Cyperus esculentus*.

2.0 Materials and Methods

2.1 Sample Collection

Cyperus esculentus was purchased from commercial retailers in Nigeria, Ghana, Togo and Benin Republic. [15] and [16] were consulted for bacteriological isolation, characterization and analysis.

Isolation of Bacteria from Collected Sample

Exactly fifty gram (50g) of Cyperus

esculentus nut was placed in sterile distilled water in a 250 mL conical flask near Bunsen flame. Pour plate technique was used to isolate bacteria from the flask. The plates were inoculated in duplicates using standard microbiological procedures. Developed colonies were counted using a colony counter and reported. Bacterial colony selected based on colonial morphology were sub-cultured unto sterile solidified nutrient agar using the streak plate method. This was repeated until pure axenic cultures were obtained.

2.2 Identification of the Isolated Bacterial Strain

Bacterium isolate obtained was characterized using colonial, cellular morphology and molecular tools involving DNA extraction, Polymerase Chain Reaction (PCR), sequencing and analysis. Amplified 16S rRNA gene sequences were analyzed using the National Centre for Biotechnology Information (NCBI) database-MEGA blast, 16S rRNA data repository was chosen as a preferred search parameter. The organism was identified based on the percentage similarities of the sequences with those in the NCBI database. The 5 molecular tests were carried out at the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria.

2.3 Phenol Utilization Test

A set of three (3) 50mL dispenser bottles) were labeled as T1, T2 and C respectively for each concentration of phenol tested. Where T2 is the duplicate of test T1 and both consisting of sterile MSM + phenol + *Pseudomonas* sp. C was control consisting of sterile MSM + Phenol. Exactly 30ml of already prepared sterile MSM was aseptically poured into these dispenser bottles for each experimental set up in one litre MSM (g/L) described by [17]: 0.5 KH2PO4; 1.4 Na2HPO4; 0.2 MgSO4.7H2O; 0.3 KNO3; 1 (NH4)2SO4. Precisely 0.5 mL of 0.2 mM of phenol was added except in control C. Exactly 0.5 mL of 10-3 dilution of *Pseudomonas* sp. MN227323 was also added aseptically to the test set ups. This process was then repeated for 0.4 mM, 0.6 mM, 0.8 mM and 1.0 mM of phenol. The absorbance was read at 365nm and 400nm using the spectrophotometer. Utilization study of phenol was determined using a procedure based on the UV absorbance method to measure the concentration of phenol on a 3-day interval after initial reading for day zero for 15 days.

2.4 Phenol Determinant

For the determination of phenol content, some amount of supernatant liquid (80% cuvette fill) was poured into the cuvette and placed into Genesys 20, monochromator in spectrophotometer. Then the absorbance was measured against distilled water blank.

3.0 Results

Experimental culture 6 The degradation of 0.2 mM of phenol at the start of the experiment (which was day 0) was determined to give an absorbance mean of 0.053 and was reduced to 0.012, to give a difference of 0.041 at the end of day 15. The control remained constant from the first day of the experiment to the last day (Figure 1). The degradation of 0.4 mM of phenol at the start of the experiment (which was day 0) was determined to give an absorbance mean of 0.0535 and it was reduced to

0.0163, to give a difference of 0.0372 at the end of the experiment on day 15. The control that was taken for 0.4 mM remained constant at the end of the experiment (Figure 2). The degradation of 0.6 mM of phenol at the start of the experiment (that is day 0) was determined to give an absorbance mean of 0.0545 and it was reduced to 0.021, to give a difference of 0.0335 at the end of the experiment on day 15. The control remained unaltered at the end of the experiment (Figure 3). The degradation of 0.8 mM phenol at the start of the experiment (i.e. day 0) was determined to give an absorbance mean of 0.057and it was reduced to 0.032, to give a difference of 0.025 at the end of the experiment on day 15. The control remained unchanged all through (Figure 4). The degradation of 1.0 mM of phenol at the start of the experiment (i.e. day 0) was determined to give an absorbance mean of 0.058 and it was reduced to 0.046, to give a difference of 0.012 at the end of the experiment on day 15. The control remained constant at the end of the experiment (Figure 5).

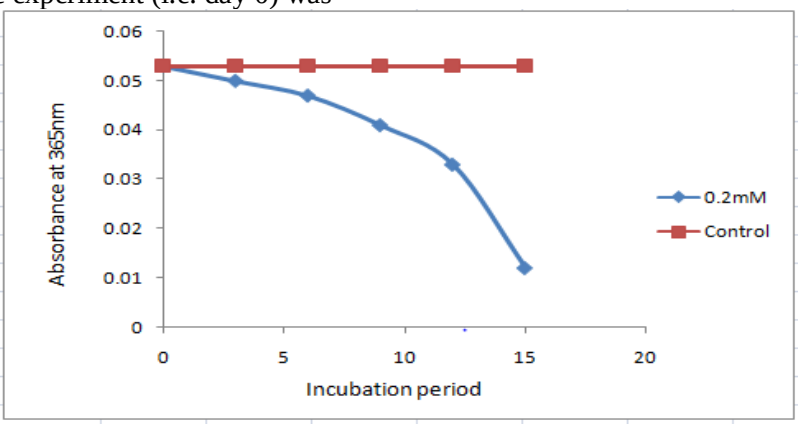

Figure 1: Utilization of 0.2 mM of phenol by *Pseudomonas* sp. **MN227323**

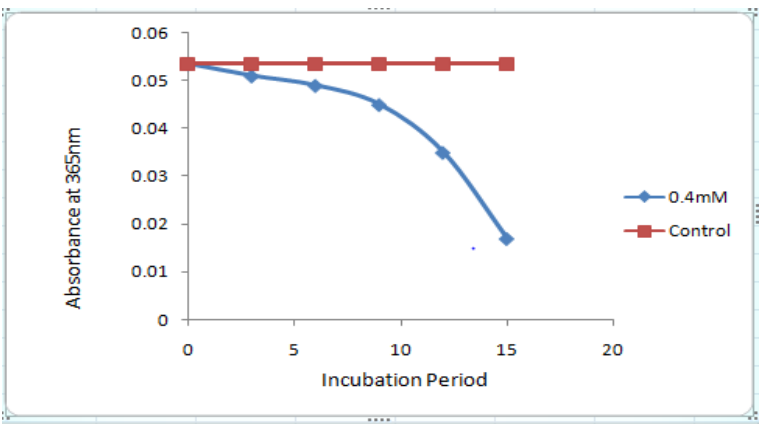

Figure 2**:** Utilization of 0.4mM of phenol by *Pseudomonas* sp. **MN227323**

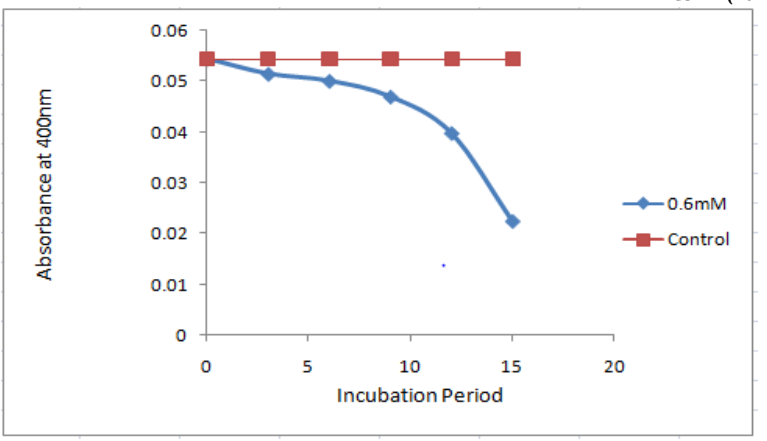

Figure 3: Utilization of 0.6 mM of phenol by *Pseudomonas* sp. **MN227323**

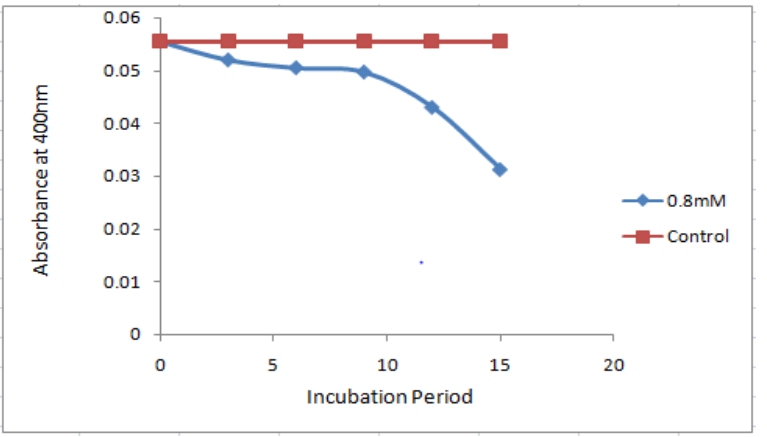

Figure 4: Utilization of 0.8 mM of phenol by *Pseudomonas* sp. **MN227323**

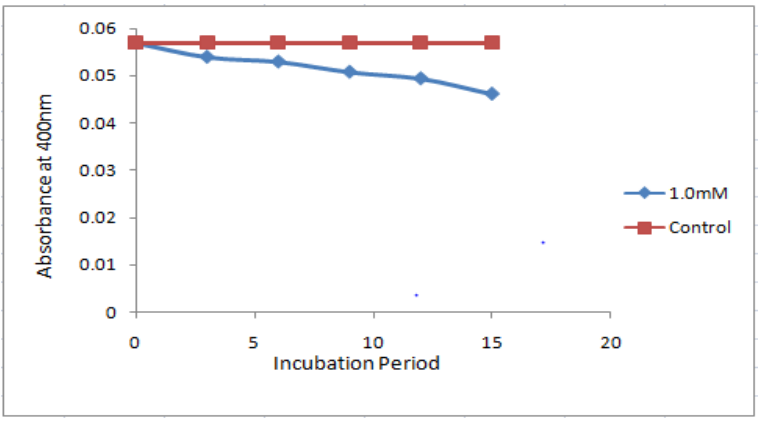

Figure 5: Utilization of 1.0 mM of phenol by *Pseudomonas* sp. **MN227323**

Table 1 show the morphological, cellular characteristics and identity of

bacterial isolates from *C. esculentus* nuts using molecular tools. The bacterium selected for biodegradation of phenol was rod shaped. Molecular identity showed that the isolate was *Pseudomonas* sp. In Table 2 is shown the isolated and identified bacterium, closest relative, phylum, sequence similarity and sequence length (megablast). The occurrence of *Pseudomonas* sp from *C. esculentus* nuts by country (Table 3) indicated *Pseudomonas* sp was isolated from *C. esculentus* nuts of Benin Republic and Togo but absent in those of Nigeria and Ghana

. Table 1: Morphological, Cellular and Identity of Bacterial Isolate from *C. esculentus* Nuts using Molecular Tools.

S/n	Shape	Colour	Edge	Optical Character	Elevation	Surface Texture	Cellular shape	Gram reaction	Identity using Molecular Tools
1	Round	Whitish	Entire	Transluscent	Raised	Smooth	Rod	-	*Pseudomonas* sp

Table 2. Isolated and identified bacterium, closest relative, phylum, sequence similarity and sequence length (nt).

S/n	Bacterium	Closest Relative	Phylum	Sequence similarity (%)	Sequence length (nt)
1	*Pseudomonas* sp	*Pseudomonas cichorii*	Proteobacteria	98	854

Table 3: Occurrence of *Pseudomonas* sp. **MN227323** from *C. esculentus* Nuts by Country

Country	*Pseudomonas* sp. **MN227323**
Benin	Present
Ghana	Absent
Nigeria	Absent
Togo	Present

4.0 Discussion of Findings

The sequenced *Pseudomonas* sp was found to have unique sequences and given a unique Genbank accession number of NCBI. The 16S rRNA gene sequence was further analyzed to identify the phylogenetic position of the isolated strain (Figure 6) using MEGA 2018 as described previously [18].

The choice of *Pseudomonas* sp for biodegradation of phenol in this study is due to the fact that many works in Nigeria have used phenol isolated from Nigerian soil for such tests. The organism used in this work was not isolated from Nigeria. [19] reported that during removal of crude oil from soil, *Pseudomonas* aeruginosa and Bacillus megaterium were used. In another report by [20], it was discovered that *Pseudomonas* aeruginosa could degrade most of crude oil with indirect or direct addition of rhamnolipid surfactant. [21] reported that *Pseudomonas desmolyticum* NCIM 2112 (Pd 2112) demonstrated an ability to degrade diesel and kerosene. [22] reported that many strains of *Pseudomonas* can grow on inorganic salts with an organic carbon source.

The obtained results showed that the lover the phenol concentration the higher the rate of the biodegradation or utilization of the phenol (figure 1 to figure 5). This is in concord with the report on *Pseudomonas putida* by [23]. In these experiments, the best degradation took place by day 15. [24],

reported that phenol was completely degraded after 22 hours in their experiments. [25] reported that *Pseudomonas putida* degraded phenol after 23 days with the aid of a laboratory bioreactor and a feed process.

5.0 Conclusion

In conclusion, phenol can be easily degraded by *Pseudomonas* sp and this has the potential to assist with the bioremediation of phenol from the environment.

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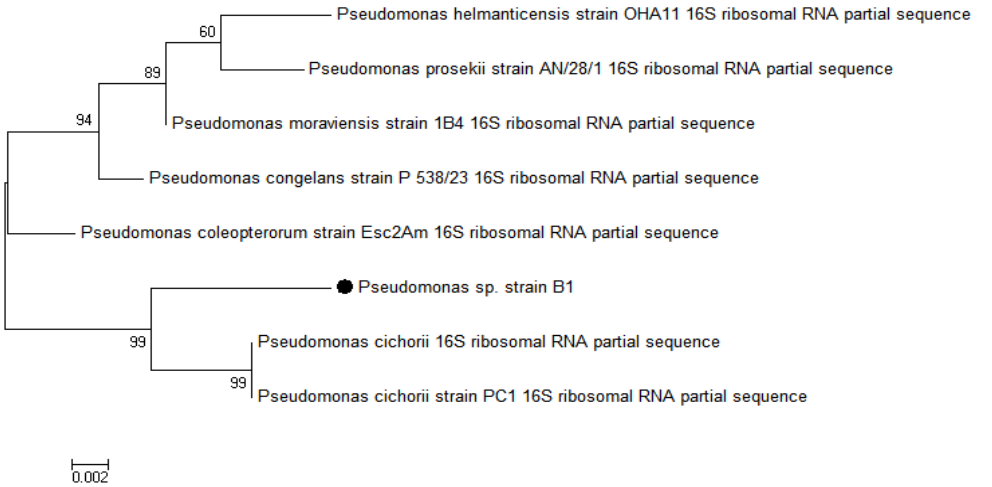

Figure 6: Phylogenetic position of the isolated *Pseudomonas* sp. (accession no. **MN227323).** The numeric value on the top of the branches indicates bootstrap replicates values. The phylogenetic tree was constructed using 1000 bootstrap replicates.

6.0 References

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