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Research Article

Isolation of Bacterial Strains from Akra Soil of District Bannu and their Biodegradation Capabilities

Shakeeb Ullah*[1] | Naila Seemab[1]| Inam Ullah Khan[1]| Muhammad Saqib[1]| Anila Khan[2]| Sana Ullah[1]

[1]Faculty of Veterinary and Animal Sciences, Gomal University D.I. Khan
[2]Institute of Biological Sciences, Gomal University, D.I. Khan

Correspondence
Shakeeb Ullah
Email: Shakeebullah@gmail.com

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ABSTRACT:

Background: Different strategies are present which allow identification of bacteria in different environments. The current gold standard is to culture the bacteria and identify them based on their morphological and metabolic characteristics. **Objectives:** The present study was designed to isolate bacterial strains having environmental role to degrade various pollutants from Akra soil samples, District Bannu, KPK, Pakistan. Microbial biodegradation is important phenomenon and has attracted attention of the researcher. **Methods:** Total 6 bacterial strains designated IOM-40, IOM-41, IOM-42, IOM-43, IOM-44 and IOM-45 were isolated. The isolated organisms were identified based on morphological, physiological and genetic characteristics. The isolated organisms were able to grow on T5, TSA, and nutrient agar media. **Results:** Optimum growth was recorded at 37–45°C, NaCl concentrations (0-0.5%) and at pH 7.0–7.2. Temperature 45°C and medium T5 were best for isolation of bacteria from arid environment of Akra soil. Strains IOM-41 and IOM-45 were rod-coccus shaped and Gram-staining-positive. Bacteria IOM-40 and IOM-43 were Gram-staining-positive and rod shaped. Organisms IOM-42 and IOM-44 were Gram-staining-negative and rod shaped. **Conclusion:** Organisms IOM-41, IOM-44 and IOM-45 were found possessing biodegradation properties. Isolation of bacteria from such arid environment could be used to mitigate environmental pollution.

KEYWORDS:

Isolation, Bacterial Strains,

1 | INTRODUCTION

Different strategies are present which allow identification of bacteria in different environments. According to Pahlow et al [1] the current gold standard is to culture the bacteria and identify them based on their morphological and metabolic characteristics. The phenomenon of biodegradation involves the disintegration of organic substances into smaller compounds by living microbial organisms. Once biodegradation reaches completion, it stands referred to as "mineralization". Nevertheless, in the majority of instances, the term biodegradation is commonly employed to depict practically any naturally influenced modification in a substrate. Therefore, knowledge of the microbes involved in biodegradation is necessary to comprehend the process itself. The biodegradation process involves a variety of microorganisms, such as bacteria, yeasts, and fungus [2.] Microbes, such as fungi, bacteria, and actinomycetes produce a crucial part in the degradation of both natural and synthetic plastics [3] Although the methods of biodegradation differ widely, carbon dioxide remains usually the outcome of the breakdown process[4].

From various hydrocarbons and derivatives of petroleum, one can obtain organic polymers with high molecular weight. These polymers remain commonly referred to as plastic[5]". Plastikos," a Greek word meaning "able to be shaped into different forms," is where the word "plastic" originates. According to Kale *et al* [6]plastics are defined as polymers that become movable when heated and can be poured into molds. With the exception of biodegradable bioplastic, petrochemicals are the source of the majority of plastic materials [7]. Plastic is composed of nitrogen, silicon, hydrogen, oxygen, and chloride. Polyethylene, which has the general formula C_nH_{2n}, makes up 64% of plastic[8.]

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As a result of the growing global population and technological advancements, plastics are used extensively in all spheres of life and business, leading to issues with waste management [9]. As plastics are manufactured at a rate exceeding 25 million tons annually, the accumulation of plastic waste worldwide, which accounts for 10-15% of all municipal solid waste, is becoming a greater ecological concern [10]

Figure 1: Need of biodegradation in the management of plastic waste

Polycarbonate (PC) is a polymer that is frequently utilized due to its superior mechanical, physical, and chemical characteristics. The three main methods (each with inherent limitations) for managing plastic waste are incineration, recycling, and landfilling. Additionally, various alternative methods have been proposed to address the plastic waste issue, for example using biodegradable plastic materials or biodegradation [11] Although numerous bacteria can metabolize organic pollutants, it is essential to note that a solitary bacterium does not possess the enzymatic capability to break down all or the majority of organic compounds in contaminated soil. Because it takes the genetic material of multiple organisms, to break down the intricate blends of organic substances discovered in polluted regions, the most significant latent for biodegradation lies in mixed microbial communities [12]. Certain microorganisms possess an incredible variety of catabolic activities of microbes that occur naturally which empower them to decompose, alter, or amass an enormous array of matters, including metals, polychlorinated biphenyls (PCBs), radionuclides, hydrocarbons (such as oil), and polyaromatic hydrocarbons (PAHs) [13] Enzymes released by bacteria or other microorganisms cause biodegradation, which is the procedure by which polymers break down and eventually decompose [14]. Exoenzymes released by bacteria produce a crucial role in the polymers depolymerization, facilitating the fragmentation of these entities into more manageable segments that possess the ability to diffuse through the lipid bilayer and serve as carbon and energy sources [15]The enzymes lipases, amylases, esterases, and serine hydrolases are in charge of this breakdown. Moreover, lipases have been shown to accelerate the breakdown of polymers [16]. The current study aimed to isolate bacterial strains from Akra soil samples of District Bannu, Khyber Pakhtunkhwa, Pakistan

2 | MATERIAL AND METHODS

2.1 | Samples Collection

Akra is the largest (80 hectares) archaeological site in the District Bannu (Figure 2). Akra site was chosen for our research study due to its historical importance and possession of arid environmental conditions. Soil samples were collected after excavation up to 3 inches and brought to Institute of Microbiology (FVAS) Gomal University, Dera Ismail Khan in sterile plastic bags for isolation of bacteria.

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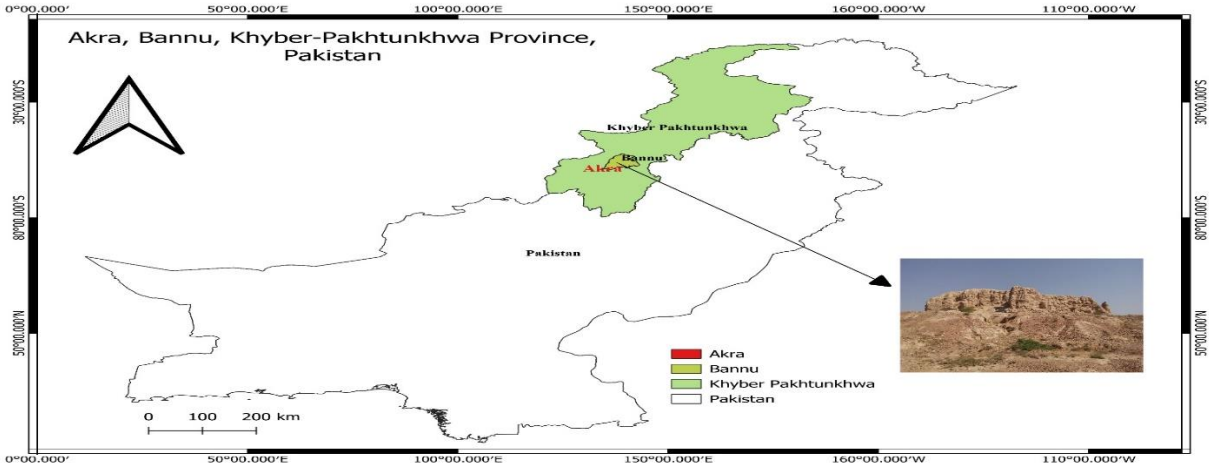

Figure 2: The Map showing the location of sampling sites

2.2 | Sample preparation

5g of soil sample was weighted and the 50mL of distilled water was taken in rounded bottom flask. The soil sample was added into the 50mL of distilled water. Mixture was put into shaking incubator overnight at 37°C. In this way, soil particles were dissolved.

2.3 | Serial dilution

9mL of distilled water was measured and taken in five glass tubes. 1mL from the mixed sample was put into each tube one by one by micropipette for serial dilution.

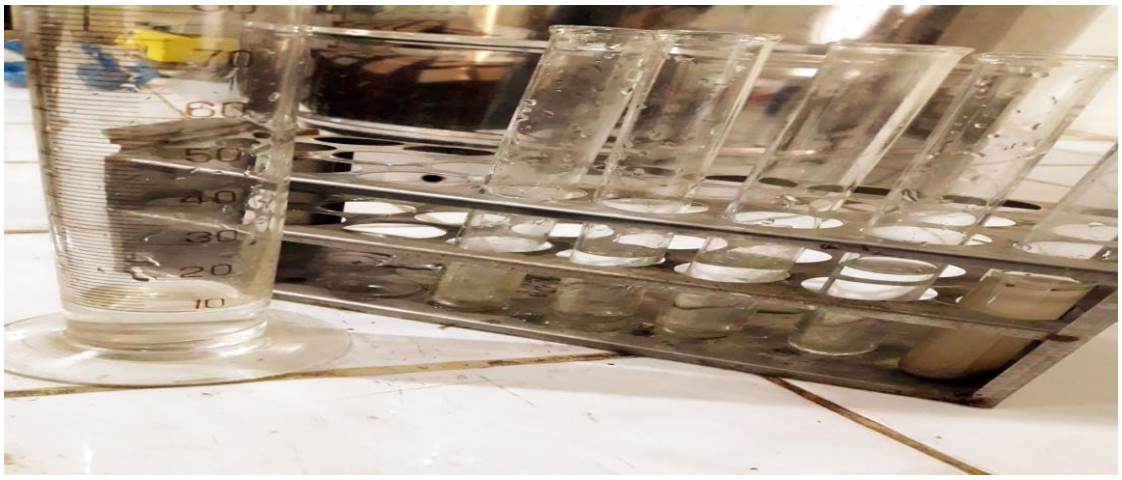

Figure 3: Serial dilution performed in microbiology laboratory

2.4 | Culture Media

T5 agar (*Thermus* 5 agar), NA (Nutrient Agar), and TSA (Trypticase Soy Agar) media were prepared and poured into petri dishes.

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Figure 4: Culture Media Preparation

2.5 | Thermus 5 (T5)

Thermus 5 (T5) agar medium was prepared with the composition yeast extract, 2g; Glucose, 1g; Lotus extract, 1g; Peptone, 0.5g; $CaCO_3$, 1g; solution of salt in trace amount,1 mL; Agar, 18g; Distilled water, 1L; pH was 7.0-7.2.

2.6 | Nutrient Agar (NA)

Nutrient Agar medium was composed of peptone, 5g; Beef extract, 3.0g; Agar, 18g; Distilled water, 1000mL; pH, 7.0 - 7.2.

2.7 | Tryptic Soy Agar (TSA)

TSA medium with the following composition was also used for isolation of bacterial strains. Casein digest, 15g; Soyabean digest, 5g; Sodium chloride, 5g; Agar, 15g; Distilled water, 1L; pH, 7.0-7.2.

2.8 | Incubation

Then, with the help of micropipette, 100µl of diluted sample was taken and spread on each petri plate. At the following temperatures; 30°C, 37°C, and 45°C prepared petri plates were incubated. Bacterial growth was checked at different temperatures after 5 days. Different colonies appeared on each plate, were re-streaked and purified strains were obtained, which were stored in 20% (w/v) glycerol at -40 °C.

2.9 | Results

Soil samples were collected from Akra soil of district Bannu, Khyber Pakhtunkhwa, Pakistan. As discussed in methodology, after sampling, serial dilution was performed in Microbiology laboratory, Gomal University, Dera Ismail Khan. Different colonies were purified by streaking method after culturing of samples in incubators at 30°C, 37°C, and 45°C. Morphological identification was performed with the help of microscopy. The detailed information of 16S rRNA gene similarities and phenotypic features of isolated organism are given in Table 1 & 2. The 16S rRNA gene was amplified through PCR. Samples of the present study were sent for sequencing to Novo gene (China). FASTA sequences were evaluated. Phylogenetic trees were built which are given in Figures 8 and 9. Isolated strains were also checked for their biodegradation capabilities.

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2.9.1 | Isolation, identification and characterization of isolated strains

Different samples were collected from Akra soil of district Bannu, Khyber Pakhtunkhwa, Pakistan. Strains were isolated from the collected samples. Isolated strains were IOM-40, IOM-41, IOM-42, IOM-43, IOM-44, and IOM-45. IOM-40 showed 99.09% sequence similarity with *Nocardioides cavernae* YIM A1136^T. IOM-41 showed 99.58% similarity homology with *Arthrobacter nitrophenolicus* SJCon^T. IOM-42 showed 97.65% similarity with *Caldimonas caldifontis* YIM 78140^T. IOM-43 showed 99.22% similarity with *Pseudarthrobacter niigatensis* LC4^T. IOM-44 showed 99.17% similarity with *Microvirga arsenatis* 3D203^T and organism IOM-45 showed 99.72% similarity with *Arthrobacter nitrophenolicus* SJCon^T (Table 1). Strains IOM-41, IOM-44 and IOM-45 were found possessing biodegradation capabilities.

Table 1: Taxonomic affiliation of all isolated organisms of the present study

Strain Ids	Top-hit taxon	Top-hit strain	16S rRNA gene Similarity (%)	Accession Numbers
IOM-40	YIM A1136T	Nocardioides cavernae	99.09%	PP922761
IOM-41	SJConT	Arthrobacter nitrophenolicus	99.58%	PP922764
IOM-42	YIM 78140T	Caldimonas caldifontis	97.65%	PP922767
IOM-43	LC4T	Pseudarthrobacter niigatensis	99.22%	PP922771
IOM-44	3D203T	Microvirga arsenatis	99.17%	PP922773
IOM-45	SJConT	Arthrobacter nitrophenolicus	99.72%	PP922779

Table 2: Morphological and phenotypic properties of all isolates

Strain Ids	Shape	Gram stain	Catalase	Oxidase
IOM-40	Rod	+	+	_
IOM-41	Rod-coccus	+	+	_
IOM-42	Rod	_	+	+
IOM-43	Rod	+	+	_
IOM-44	Rod	_	+	_
IOM-45	Rod-coccus	+	+	_

The details of the taxonomic classification of organisms of the present study are tabulated in the given Table 3. All the species along with their genus, family, order, class, and phylum are presented in the table below (Table 3):

Table 3. Hierarchy / Taxonomic classification of organisms of the present study

Phylum	Class	Order	Family	Genus	species
Actinomycetota	**Actinomycetes**	**Propionibacteriales**	Nocardioidaceae	*Nocardioides*	*N. cavernae* (IOM-40)
Pseudomonadota	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Caldimonas*	*C. caldifontis* (IOM-42)
Actinomycetota	Actinomycetes	Micrococcales	Micrococcaceae	*Arthrobacter*	*A. nitrophenolicus* (IOM-41) *A. nitrophenolicus* (IOM-45)
Actinomycetota	Actinomycetes	Micrococcales	Micrococcaceae	*Pseudarthrobacter*	*P. niigatensis* (IOM-43)
Pseudomonadota	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	*Microvirga*	*M. arsenatis* (IOM-44)

Total six bacterial strains belonging to five genera; namely *Nocardioides, Caldimonas, Arthrobacter, Pseudarthrobacter and Microvirga* were isolated and identified based on phenotypic characteristics and genetic level (Figure 5).

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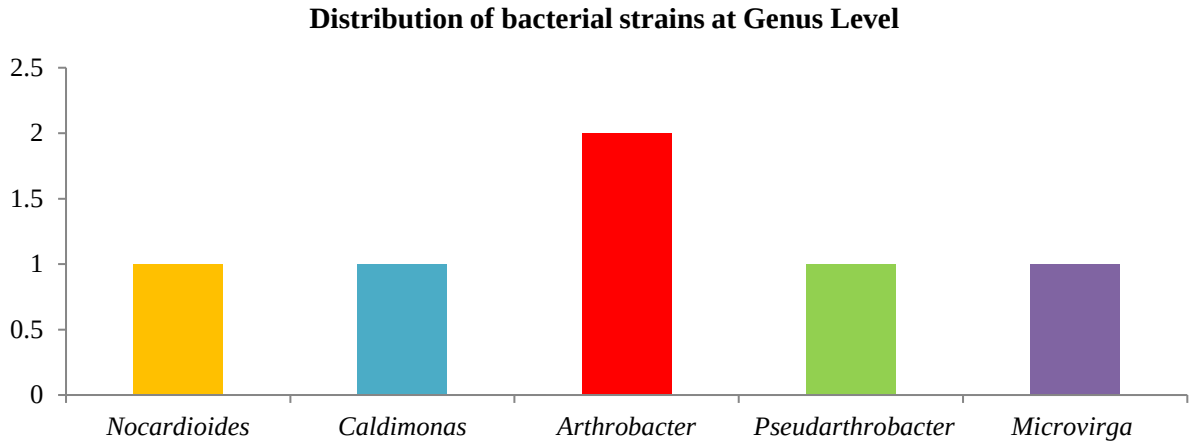

Figure 5: Isolated bacterial strains at genus level

Two isolated strains of the present study belonged to the Arthrobacter genus while other four isolated strains belonged to the genus Nocardioides, Caldimonas, Pseudarthrobacter, and Microvirga.

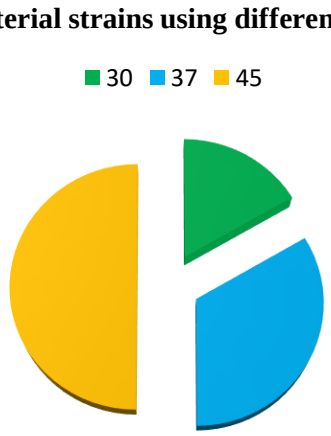

Figure 6: Distribution of bacterial strains using different culture media

In the present research study, the media used for the culturing of different species were Nutrient Agar (NA), *Thermus* 5 agar (T5 agar), and Trypticase Soy Agar (TSA). Four strains were recovered using T5 agar medium while one strain each was isolated on NA and TSA media (Figure 6). All the isolated organisms were able to grow on the above mentioned media. T5 was the best culture medium because most of the strains were isolated using this medium.

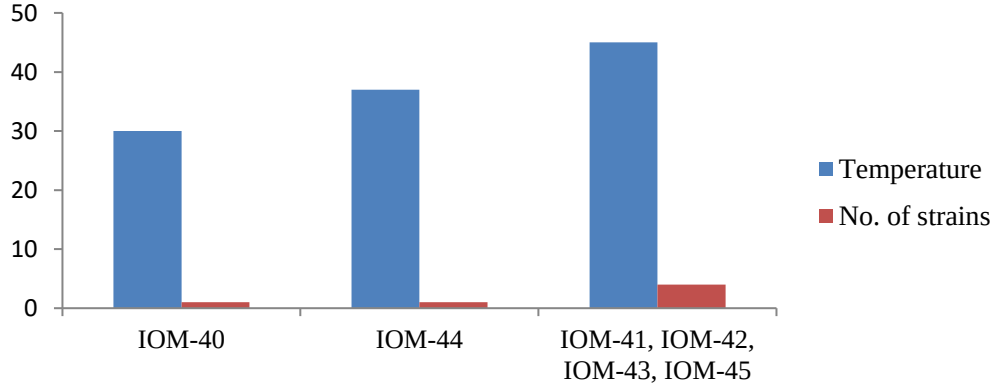

Figure 7: Effect of incubation temperature on bacterial diversity

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Different temperatures (30°C, 37°C and 45°C) were used for isolation of bacteria. The effect of 45°C was good because four strains out of six were isolated at this temperature (Figure 7).

4 | DISCUSSION

In the present research performed we succeeded in isolating and identifying six bacterial strains from Akra soil of District Bannu, in which three strains (IOM-41, IOM-44 and IOM-45) had the abilities of biodegradation and thus played tremendous role in cleaning the environmental pollution. The six isolated strains were IOM-40, IOM-41, IOM-42, IOM-43, IOM-44, and IOM-45. All of the following isolated strains were showing similarity of 99.09%, 99.58%, 97.65%, 99.22%, 99.17%, 99.72% with class of type strain *Nocardioides cavernae* YIM A1136^{T}, *Arthrobacter nitrophenolicus* SJCon^{T}, *Caldimonas caldifontis* YIM 78140^{T}, *Pseudarthrobacter niigatensis* LC4^{T}, *Microvirga arsenatis* 3D203^{T}, *Arthrobacter nitrophenolicus* SJCon^{T} respectively.

The current study also focuses on the detection of such microorganisms that play important role in the biodegradation of variety of materials. Soil bacteria are stable and effective; they are regarded as an essential component of biodegradation [17] The target of the recent study is to find out novel bacterial strains from the soil source. For this purpose, Akra soil (located in the District Bannu, Khyber Pakhtunkhwa, Pakistan) was observed and then selected for isolating such bacterial strains that will be advantageous for the environment. This soil is famous because it is the most significant of all the early historical sites in Bannu and the most physically noticeable (arid nature) of all the ancient locations in the Bannu region.

In the recent study, it was investigated that *Actinomycetes* are playing pivotal role in biodegradation. Out of the six isolated strains, the two isolated organism were IOM-41 and IOM-45. Both of these isolated strains resemble *Arthrobacter nitrophenolicus* SJCon^{T}. IOM-41 strain showed 99.58% homology to *Arthrobacter nitrophenolicus* SJCon^{T}. IOM-45 strain showed 99.72% resemblance to *Arthrobacter nitrophenolicus* SJCon^{T}. In an earlier study, strain SJCon^{T} belonging to genus *Arthrobacter* has been found to degrade 2-chloro-4-nitrophenol [17.] It had observed in the previous study that strain *Arthrobacter nitrophenolicus* ND6, fermented from tobacco waste was used in nicotine breakdown of compost fertilizer. With native Nicotine Degrading Bacteria (NDB), uninoculated compost was served as a control. In the recent study, six strains are isolated from Akra soil of district Bannu, Khyber Pakhtunkhwa, Pakistan. Out of the six isolated strains, one isolated strain is "IOM-44". This isolated strain matches with *Microvirga arsenatis* 3D203^{T}. They were 99.17% similar to each other. There are many species of *Microvirga* genus known and published. One of the species isolated, identified, and discussed in the present study is *Microvirga arsenatis*[19].

4 | CONCLUSION

Total 6 bacterial strains designated IOM-40, IOM-41, IOM-42, IOM-43, IOM-44 and IOM-45 belonging to five genera (*Nocardioides*, *Caldimonas*, *Arthrobacter*, *Pseudarthrobacter* and *Microvirga*) were isolated from an arid environment of Akra soil, District Bannu, KPK, Pakistan. In the present study all the isolates were identified based on molecular technique (16S rRNA gene) and their ancestry was addressed using phylogenetic analysis. Most of the isolated were belonging to phylum Actinobacteria. Actinomycetes are famous for their secondary metabolites (natural products) and their vast applications in medicine, agriculture and in control of environmental pollution. Discovery of novel bacterial isolates and probing of their novel biosynthetic gene clusters will further enhance the applications of bacterial strains for cleaning of environments. Furthermore, plastic is a major pollutant particularly in aquatic environment harming aquatic organisms such as fish. So, isolation and characterization of bacteria from an arid environment which degrade plastic will be important for our country Pakistan to ameliorate plastic pollution injurious effects, which we are facing now days.

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