Isolation, Molecular Characterization and Screening of Atrazine-Degrading Bacteria from Some Agricultural Soil of Jere Nigeria

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Abstract— Atrazine, a major herbicide considered an endocrine disruptor and a potential carcinogen, is widely used in Nigeria; its widespread and long-term use results in high soil residue levels, thus necessitating a continuous search for atrazine biodegradation microorganisms. Microbial degradation of herbicides represents a time- and cost-effective way of eco-restoration. This research aimed to isolate and characterize bacteria capable of degrading and utilizing atrazine as a sole carbon source. Soil samples were collected from four locations, namely Alau, Dusuman, Unimaid Campus, and Zabarmari farmlands of the Jere local government area of Borno State, in November 2022 after the rainy season. The samples were collected and analyzed for pH, organic matter, organic carbon, electrical conductivity, potassium, phosphorus, and nitrogen. Statistical analysis was done to determine the significant difference in the soil parameters. There were no significant ($p > 0.05$) differences in the parameters of all the soils tested. Serial dilution was followed by the isolation of bacteria in a mineral salt medium (MSM) containing atrazine as the carbon source in the media. Three (3) isolates belonging to three genera (*Pseudomonas, Bacillus,* and *Paenibacillus*) exhibited remarkable stimulation in their growth and were considered acclimatized and highly Atrazine-resistant, therefore, they were selected for Atrazine-biodegradation. *Pseudomonas aeruginosa* had the highest degradation percentage of 93.7%. *Bacillus subtilis* also had a higher percentage of degradation (89.4%), and *Paenibacillus validus* had a percentage degradation of 82.2% on atrazine. The isolate with the highest atrazine degradation was isolated from Zabarmari farmland and was identified morphologically, biochemically, and molecularly characterized as *Pseudomonas aeruginosa* based on a 16S rRNA gene sequence, and a phylogenetic tree was constructed using the NCBI database. *Pseudomonas aeruginosa* could be a suitable candidate for the biodegradation of atrazine-polluted sites.

Keywords— Atrazine, Heavy Metal, Bacteria, Agricultural Soil.

I. INTRODUCTION

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1, 3, 5-triazine), an s-triazine herbicide with a molecular formula of $C_8H_{14}ClN_5$ [1], is a commonly used herbicide that is nonpolar, minimally retained by the polar soil colloids, and powdery white. It is unstable at high temperatures. It has a boiling temperature of 200°C and a melting point of 175°C. It is extremely soluble in organic solvents and has a solubility of 33 mg/L in water at 20°C [2]. Due to atrazine's lengthy half-life, which ranges from 13 to 261 days, it is typically found in the earth's surface water and groundwater systems [3]. Because of this, it may wash out of the root zone and into groundwater resources, especially if administered before a period of heavy rainfall or irrigation [4]. It is still one of the most commonly used herbicides in the world, despite being banned in the European Union in 2004 [5]. Broadleaf weeds, some grass weeds, and other permanent weeds that hinder crop growth can all be controlled and eliminated by atrazine [1]. Major crops are also treated with atrazine to prevent pre- and post-emergence grassland and broadleaf weeds. The substance is both efficient and affordable, making it a good fit for manufacturing systems with extremely slim profit margins, as is frequently the case with maize. With its biggest market in maize production, particularly in corn, sorghum, and sugarcane production, atrazine is used on grassland, conifers, macadamia nuts, pineapples, chemical fallows, and grasslands. It is also used to control weeds in industrial settings [6]. Atrazine is dangerous when inhaled, comes into contact with the skin, or is ingested [7]. It has been implicated as a possible human carcinogen with long-term reproductive and endocrine-disrupting effects, as well as an epidemiological link to reduced sperm counts in men [5]. It might be harmful, with potential effects on birth abnormalities, low birth weights, and menstruation irregularities in humans [8]. Findings from studies showed that atrazine can raise health hazards even at low levels [4]. Atrazine is less hazardous to humans than other chlorinated herbicides, but its existence in soils and discharge into surface and groundwater resulted in serious environmental issues [9]. Both biotic and abiotic mechanisms can remove or degrade atrazine from soil, sediments, and water environments; however, biodegradation is more efficient and still a very promising strategy [10]. The organisms that decompose atrazine that are better understood are those of the bacterial species *Pseudomonas* and *Bacillus spp.* [11, 12]. The two options for accelerating atrazine biodegradation are bioaugmentation and biostimulation [13].

II. MATERIALS AND METHOD

A. Study Area

Jere Local Government, one of Borno State's twenty-seven (27) Local Government Areas, is the area of the study. In 1996, Jere was carved out of Maiduguri Metropolitan Council (M.M.C.) [14]. It has an area of 160 square kilometers and is located between latitudes 11°40 and 12°00N and longitudes 13°50 and 12°020 1E. [15]. Its borders with Mafa Local Government Area, Maiduguri Metropolitan Council, and Konduga Local Government Area all fall inside the state. Dry and hot seasons, with low temperatures between 15-20°C and maximum temperatures of 37°C - 45°C, define the region's climatic condition. The annual rainfall varies from 500 to 700 millimeters [16]. The typical rainy season runs from May to October with short wet seasons and low relative humidity. The soil is mainly sandy loam and clay loam with short grasses and prickly bushes, and the geography is typically lowland plain. Jere Local Government Area is expected to have a total population of 211,204 people, with a 2.8 percent annual growth rate. [17]. Most of the population works as farmers, traders, or government employees. The two largest ethnic groupings are Shuwa-Arab and Kanuri and Hausa; Babur Bura, Fulani, and numerous immigrant settlers from both within and outside of Nigeria are among the other ethnic groups [18]. Four (4) wards were selected out of the twelve (12) wards in the area. These include Alau, Dusuman, Unimaid Campus, and Zabbarmari wards.

B. Sampling Sites

According to the map below, the study's sampling areas were the Alau, Dusuman, Unimaid Campus, and Zabbarmari wards of the Jere Local Government Area, Borno State, Nigeria.

C. Sample Collection

Soil samples were collected from selected farmlands in the Jere Local Government Area of Borno State that have long been exposed to herbicide contamination in November 2022, immediately after the rainy season. Four (4) composite soil samples were collected from each location (Ward) from Alau (11.43’57oN, 13.15’55oE), Dusuman (11.56’02oN, 13.14’52oE), Unimaid farm (11.49’10oN, 13.12’11oE), and Zabbarmari (11.56’06oN, 13.14’34oE) farmlands (4 random samples from each farmland), and a standard microbiological method of soil sample collection was used. The soil samples were collected by using an auger up to a depth of 20 cm. The samples were wrapped in aluminum foil twice and wrapped in two plastic bags to minimize the possibility of contamination. The samples were clearly labeled and stored in containers appropriate for the analysis being undertaken. All samples were transported to the laboratory within 24 hours of collection.

D. Sample Preparation for Physicochemical Analysis

The soil samples were air-dried for one week, ground with a clean porcelain mortar and pestle, and passed through a 2.0 mm sieve. The soil samples were kept in polythene bags for further analysis. Soil samples were analyzed for the following physicochemical parameters

E. Enumeration and Isolation of Bacteria from the Soil Samples

Serial dilution was carried out in 9 test tubes containing 9 ml of distilled water, and 1 g of a soil sample that had been weighed using a weighing scale was added and suspended in the 9 ml of distilled water. It was appropriately mixed and serial dilutions of [10-1, 10-2, 10-3, 10-4, 10-5, 10-6, 10-7, 10-8, and 10-9]. The diluted samples from 10-7, 10-8, and 10-9 were plated on Petri dishes containing prepared media for solidification. For this isolation, agar-agar and nutritional agar were used. Agar-agar was used to solidify the mineral salt media [MSM] for bacterial growth and extract the total number of bacteria that can resist the media with atrazine as the sole carbon source, whereas nutrient agar was used to isolate the total number of microorganisms present in the soil sample for counting. When using mineral salt medium [MSM], there are [in grams per liter]: $[NH_4]_2SO_4$ 0.1 g, K_2HPO_4 0.1 g, $CaSO_4$ 0.05 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, and $FeSO_4 \cdot 7H_2O$ 0.01 g [19], and distilled water 1.0 liter, pH 7.0. The 1000 ml of distilled water was mixed with 28 g of the agar-agar powder to create the mineral salt media. The isolation medium was made up of mineral salt media with 50 mg/liter of atrazine [as the final concentration of the media] serving as the only carbon source. Another 1000 ml of distilled water was mixed with 20 g of nutrient agar to make the nutrient agar culture plate. Each of the nutrient agar and mineral salt media was dissolved in one liter of distilled water before being heated to dissolve them and sterilize them in an autoclave for 15 minutes at 121°C [20]. Before putting it into sterile petri dishes, let it cool and allow it to solidify before adding 1 ml of each sample to the plates. The inoculated Petri dishes were kept in an incubator for 48 hours at a temperature of 37°C [21] for the total number of aerobic heterotrophic bacteria count. For both the mineral salt media and the nutrient agar media, colonies were seen on the plates, enumerated, and recorded as colony-forming units per gram of soil [cfu/g].

F. Isolation of Atrazine-Degrading Bacteria

Atrazine-degrading bacteria were isolated from the samples by choosing the bacteria that thrived on the mineral salt agar plates and repeatedly subculturing them until pure cultures were obtained. For further research, the pure cultures were kept in an atrazine minerals salt agar slant at 4°C.

G. Identification of the Bacterial Isolates

The atrazine-grown bacterial isolates were put through physiological and biochemical tests. Gram staining, the catalase test, the citrate utilization test, the oxidase test, the indole production test, the motility test, the sugar fermentation test, the methyl-red test, the Voges-Proskauer test, the mannitol test, and [22]. Manual of Determinative Bacteriology tests were used for confirmation.

III. EFFECT OF ATRAZINE CONCENTRATION ON THE GROWTH OF BACTERIA

The inoculum used for all the experiments was prepared by growing the bacterial isolates in separate bijou bottles containing 10 ml of mineral salt medium [MSM]. Then 1 ml of the 48-hour culture was used as inoculum. This was used to inoculate bijou bottles containing 10 ml MSM, and the concentration of atrazine in mineral salt medium was adjusted to 100 mg/L, 200 mg/L, 300 mg/L, 500 mg/L, 1000 mg/L, and 1500 mg/L. The uninoculated bottles were used as a control. The bottles were incubated in a rotary shaker at 120 rpm and 30℃ for 21 days. Degradation was measured by checking cell growth through optical density [OD600] [23] at 3-day intervals. The physical appearance of the isolates was observed to see the changes in the turbidity of the media at 3-day intervals. Turbidity in a liquid medium is an indication of cell growth. The bacterial cell culture was placed in a transparent cuvette, and the absorbance was recorded at a wavelength of 600 nm along with a control to check for biodegradation of atrazine by the bacterial isolates using the spectrophotometer [model PD 303 UVVIS]. The bacterial cell culture was then subcultured again on nutrient agar plates for confirmation of bacterial growth to evaluate the tolerance of bacteria to atrazine and obtain pure culture.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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