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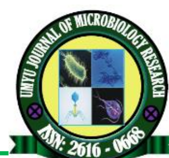

Amylase Producing Potentials of Bacteria Isolated from Selected Dumpsites in Benin Metropolis, Edo State, Nigeria

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Abstract

Amylase enzyme has extensive usage in different industries and can be obtained from microorganisms (e.g., bacteria) before purification and usage. In the current study, the amylase-producing potential of bacteria isolated from selected dumpsites in Benin City, Edo State, Nigeria, was investigated. A total of 54 soil samples were obtained from three different dumpsites (Ikhueniro, Eyaen, and Uhumarioge) between August and October 2023. The pour plate method was used to quantify the total number of heterotrophic bacteria, and starch hydrolysis agar was used to assess the organisms' starch-hydrolyzing capacity. The bacterial isolates' capacity to produce amylase was assessed qualitatively using starch agar and quantitatively using colorimetric (3,5-dinitrosalicyclic acid reagent) techniques, while optimal pH and temperature for amylase production were done by spectrophotometric technique. The bacterial isolates were further identified by their cultural, morphological, biochemical, and 16S rRNA characteristics. A total of 23 different bacterial isolates were identified from the soil samples, out of which 8 were found to hydrolyze starch. Achromobacter anxifer, Providencia alcalifaciens, and Proteus mirabilis were further reported as the highest producer of amylase enzyme, with optimum temperature and pH being 35°C and 6, respectively. The study of amylase-producing bacteria from the soil of waste dump sites reveals a complex and dynamic microbial ecosystem with profound ecological, industrial, and scientific significance. Efforts must, therefore, be made to obtain this enzyme in sufficient amounts and ensure its usage in different industries.

Keywords: Amylase; dumpsites; Achromobacter anxifer; Providencia alcalifaciens and Proteus mirabilis

INTRODUCTION

Amylase is an enzyme that hydrolyses carbohydrates (Daniel *et al.*, 2010) and has been described as an important group of industrial enzymes (Oyenado and Omoruyi, 2024), accounting for around thirty percent of worldwide enzyme production (Kandra, 2003). When soluble starch is used by amylases, the resultant byproducts, like glucose or maltose, are absorbed by the cells and used as an energy source (Sudharhsan *et al.*, 2007). Endoamylases (α-amylase) and exoamylases (β-amylases, glucoamylase, and α glucosidase) are the two main subgroups of the amylase superfamily. While endoamylases hydrolyze the inside of a glucose chain in starch to form oligosaccharides of varying lengths, exoamylases work at the non-reducing sections of polysaccharides to create low molecular weight metabolites such as glucose (Pandey *et al.*, 2000). Microbial enzymes are naturally occurring compounds that have been proven to have higher potency than synthetic enzymes with different characteristics. A primary merit of using microbes for large-scale industrial amylase synthesis is their relative simplicity in terms of control, which often has a positive financial impact (Sreelekshmi *et al.*, 2019). Enzymes generated from microbial sources are widely utilized in a range of industrial processes because of their reduced cost of production, productivity, stability, flexibility, as well as worldwide availability (Ahmad *et al.*, 2019; Mishra, 2008; Burhan *et al.*, 2003).

Amylases have been documented to have a broad range of applications, including starch polymer synthesis (Nielsen and Borchert, 2000), textiles (Montazer and Harifi, 2018), detergents (Nielsen and Borchert, 2000), beverages (Santos *et al.*, 2023), biofuels (De Castro *et al.*, 2011), food processing and medicals (Azzopardi *et al.*, 2016). Bacteria are the most capable microbiological source of amylase production when it comes to large-scale synthesis, most importantly, the thermostable *Bacillus* strain (Pandey *et al.*, 2000).

The *Bacillus* spp. contains these industrially significant microbes because of their quick growth rates, which result in brief fermentation cycles, their ability to secrete proteins into extracellular media, and their overall safety during handling (Pandey *et al.,* 2000). Several bacteria have the potential to produce amylases including *B. licheniformis, Bacillus subtilis, B. megaterium* and *B. steriothermophilus* (Pokhrel *et al.,* 2013). The current study was designed to screen for bacterial isolates for amylase production and their characterization because the soil is a rich source of microorganisms that secrete amylase.

MATERIALS AND METHODS

Study Area and Sample Collection

A total of 54 soil samples were obtained from 3 dumpsites, 1 in each local government area (Uhunmwonde, Oredo, and Ikpoba-Okha) within Benin Metropolis, Edo State, Nigeria. During each sample collection, soil samples were obtained from the topsoil, 15cm as well as 30cm deep into the soil. For proper comparison, samples were also collected 100m from the dumpsites. With the use of a sterile spatula, collected samples were transferred to sterile plastic bags in aseptic conditions and transported to the Microbiology Laboratory at Benson Idahosa University for immediate analysis.

Serial dilution

One gram (1g) of soil sample was dissolved in 9 ml of peptone water in a test tube by shaking. This preparation was used as a stock solution that was used to make several fold dilutions (10^1 - 10^6) of the serial dilution (Omoruyi and Amadi, 2022).

Total heterotrophic bacterial count

Approximately 0.1 ml from the third dilution (x 10^3) was added to the nutrient agar using the spread plate method. The agar plates were incubated at 37^0C for 24 h.

Identification of bacterial isolates

Bacteria isolates were identified by their cultural, morphological, and biochemical (citrate, oxidase, coagulase, motility, indole, sugar fermentation) characteristics as described by Omoruyi *et al.* (2011).

DNA extraction

The total genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Inqaba, South Africa). The bacterial isolate was first grown on Luria Bertani media for 24 hours at 37˚C in an incubator. After incubation, 2 ml of the liquid culture was added to a 3 ml microcentrifuge tube (Omoruyi and Ojubiaja, 2022). Centrifugation of the tubes was carried out at 13500 rpm for 3 minutes to pellet the cells. The supernatant was discarded, and the pellet was re-suspended in 480 µl of 50 mM EDTA. The re-suspended cells were used to extract cellular DNA according to the protocol provided by Wizard® Genomic DNA Purification Kit (Inqaba, South Africa). The extracted DNA was stored at 4˚C for further work.

Sanger sequencing

Sequencing was done using the Big Dye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria, South Africa. The sequencing was done at a final volume of 10 µl, the components included 0.25 µl Big Dye® terminator v1.1/v3.1, 2.25 µl of 5 x Big Dye sequencing buffer, 10 µM Primer PCR primer, and 2-10 ng PCR template per 100bp. The sequencing conditions were as follows 32 cycles of 96˚C for 10s, 55˚C for 5 sec, and 60˚C for 4 min (Omoruyi *et al.*, 2023). Obtained sequences were edited using the bioinformatics algorithm Trace edit. Similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) database using BLAST (Basic Local Alignment Search Tool). These sequences were aligned using ClustalX. The evolutionary distances were computed using the Jukes-Cantor method (Omoruyi *et al.,* 2023). The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0.

Screening for amylase activity (starch iodine test)

The starch hydrolysis test on a starch agar plate was used to screen bacterial cultures for amylolytic activity (Burhan *et al.*, 2003). Starch served as the only carbon source and was streaked on starch agar plates for the pure isolated colonies. Each plate was inundated with 250 mg of Gram's iodine crystals mixed with 2.5 g of potassium iodide solution in 125 ml of water. The plates were after that incubated at 37°C for 48 h. The absence of blue colour in the degradation zone and the diameter of the zone of clearance were indications of amylase production (Gupta *et al.* 2003).

Amylase extraction

After transferring a loop full of bacterial culture from starch-nutrient agar slants to starch-nutrient broth at pH 7 for activation, the mixture was shaken for 24 h at 40°C and 120 rpm. Soluble starch (10 g/L), peptone (5 g/L), $(NH_4)2SO_4$ (2 g/L), KH_2PO_4 (1 g/L), K_2HPO_4 (2 g/L), $MgCl_2$, (0.01 g/L) at pH 7 were used to make the fermentation medium. After inoculating the fermentation medium with 20% v/v activated culture, it was shaken and kept at 37°C for 24 h.

The crude extract, which was used as an enzyme source, was obtained by centrifuging the culture medium for 15 min at 5000 rpm after the fermentation process was completed.

Measurement of absorbance

With a few changes, the assay for amylase activity was conducted as previously described (Oyeleke and Oduwole, 2009). In summary, 0.5 ml of diluted enzyme and 1.5 ml of 1% starch in 2 ml of 0.1M phosphate buffer (pH 6.5) were incubated for 15 min at room temperature (37°C). After adding 1 milliliter of the 3,5-dinitrosalicyclic acid (DNS) reagent, the reaction was stopped, allowed to boil for 10 minutes, and then diluted with 8 ml of distilled water. The amount of enzyme that releases 1µm of reducing sugar (maltose equivalents) per minute under test conditions was defined as one unit of α-amylase activity. Every experiment was run in triplicate.

Determination of optimum pH for amylase activity

One percent 1% starch was used as a substrate. Substrate solution was prepared in sodium phosphate buffer at pH 6, 7, and 8 in different test tubes. 0.5 ml each of diluted crude enzyme solution was added into buffer tubes. Then, the mixture was incubated at room temperature for 15 min. Reactions were terminated by adding 1 ml DNS reagent, and the mixture was incubated in boiling water for 10 min. After cooling at room temperature, the final volume was made to 12 ml with distilled water, and the activity of enzymes was determined by taking the absorbance at 600 nm.

Determination of optimum temperature for amylase activity

An aliquot amount (1.5 ml) of substrate was taken into six different test tubes, and 2 ml of phosphate buffer pH 7 was added to each test tube. Tubes were marked with different temperatures (at 30, 35, 40, 45, 50, 55°C). 0.5 ml of diluted enzyme solution was added to each tube. Then, tubes were incubated at a specific temperature for 10 minutes. Reactions were terminated by adding 1 ml DNS reagent, and the mixture was incubated in boiling water for 10 min. After cooling at room temperature, the final volume was made to 12 ml with distilled water, and the activity of enzymes was determined by taking the absorbance at 600 nm.

Statistical analysis

One-way analysis of Variance (One-way ANOVA) was used with Duncan Multiple Range's Test on SPSS Statistics version 17.0 to determine the difference between heterotrophic counts obtained from dumpsites, control sites, and the different soil layers. Results were expressed as means ± SD with statistical difference when $p<0.05$.

RESULTS

The results of the current study show that heterotrophic bacteria are present in waste dumpsites in the Benin metropolis, Edo State, Nigeria (Tables 1, 2, and 3). The heterotrophic bacterial count ranged from 52.00 ± 11.37 – 55.09 ± 9.64 x 10^3, 27.00 ± 6.00 – 53.00 ± 9.17 x 10^3, and 24.00 ± 7.77 – 35.00 ± 6.57 x 10^3 in topsoil, middle layer and bottom layer respectively from Ikhueniro dumpsite (Table 1). There was also no significant difference between the heterotrophic bacterial counts obtained from the Ikhueniro dumpsite and control site (100 m from the dumpsite) except for the middle layer in September sampling and the bottom layer in October sampling. Meanwhile, heterotrophic bacteria from topsoil obtained from the Eyaen dumpsite in August 2023 showed a significant difference from the equivalent control sample (Table 2), while no significant difference was recorded in the heterotrophic bacterial counts obtained from both the different layers and control samples from Uhumarioge waste dumpsite (Table 3). A total of 23 bacterial isolates were identified from all soil samples during the study period, with only 8 showing amylase production potential (plates 1, 2, 3, & 4; Table 4).

Table 1: Total heterotrophic bacterial count obtained from Ikhueniro dump site in Ikpoba-Okha LGA of Edo State.

Month	Sample A (Cfu/g x10^3)			Sample B: 100 m from dumpsite (cfu/g x10^3)		
	T-layer	M-layer	B-layer	T-layer	M-layer	B-layer
August	53.00±6.55^a	27.00±6.00^b	35.00±6.57^b	55.00±6.51^a	27.00±7.78^a	43.00±3.61^a
September	55.00±9.64^a	53.00±9.17^a	24.00±7.77^b	54.00±14.84^a	25.00±10.01^A	29.00±7.02^a
October	52.00±11.37^a	46.00±6.51^a	41.00±5.00^a	46.00±6.51^a	45.00±5.57^a	26.00±8.02^A

KEY: T-layer: soil sample obtained from the soil top layer; M-layer: soil sample obtained at 15cm depth; B-layer: soil sample obtained at 30cm depth. Different alphabets indicate significant differences, while capital letters indicate significant differences from the control

Isolate A2 had the highest amylase production potential with a zone of clearance of 27 mm, followed by isolate A3 (24 mm) and isolate A1 (21 mm), while isolate A9X had the least amylase-producing potential with a zone of clearance of 3 mm (Table 4). This was also confirmed by the quantification analysis of amylase production, with isolate A2 and A9X having the highest (0.018 ± 0.002) and least amylase production (0.018 ± 0.002), respectively (Table 4).

Table 2: Total Heterotrophic bacterial count obtained from soil samples in the Eyaen dump site at Uhunwonde LGA of Edo State.

Month	Sample A (cfu/g x10^3)			Sample B: 100 m from dumpsite (cfu/g x10^3)		
	T-layer	M-layer	B-layer	T-layer	M-layer	B-layer
August	26.00±9.64^a	31.00±4.51^a	30.00±10.02^a	50.00±7.77^{Ab}	25.00±7.21^a	43.00±7.02^b
September	29.00±3.06^a	32.00±9.54^a	30.00±2.65^a	35.00±10.21^a	22.00±5.60^a	23.00±5.03^a
October	23.00±5.90^a	33.00±7.64^a	30.00±5.29^a	27.00±10.10^a	34.00±6.11^a	37.00±4.60^a

KEY: T-layer: soil sample obtained from the soil top layer; M-layer: soil sample obtained at 15cm depth; B-layer: soil sample obtained at 30cm depth. Different alphabets indicate significant differences, while capital letters indicate significant differences from the control

Table 3: Heterotrophic bacterial count obtained from soil samples in the Uhumarioge dump site at Oredo LGA of Edo State.

Month	Sample A (cfu/g x10^3)			Sample B: 100 m from dumpsite (cfu/g x10^3)		
	T-layer	M-layer	B-layer	T-layer	M-layer	B-layer
August	40.00±7.10^a	39.00±8.33^a	37.00±7.02^a	42.00±6.03^a	26.00±4.20^a	30.00±9.10^a
September	37.00±7.51^a	38.00±2.65^a	26.00±2.00^a	28.00±5.86^a	28.00±2.00^a	26.00±4.04^a
October	45.00±5.29^a	46.00±6.51^a	43.00±3.61^a	31.00±2.21^a	53.00±9.17^a	35.00±8.74^a

KEY: T-layer: soil sample obtained from the soil top layer; M-layer: soil sample obtained at 15cm depth; B-layer: soil sample obtained at 30cm depth. Different alphabets indicate significant differences, while capital letters indicate significant differences from the control.

Table 4: Phenotypic and quantitative amylase production by bacteria isolated from selected dumpsites in Uhunmwonde, Oredo, and Ikpoba-Okha LGAs of Benin Metropolis, Edo State

Isolate codes	Zones of clearance (mm)	Absorbance (600 nm) Mean ± Std. Deviation
A1	21	0.025 ± 0.001
A2	27	0.116 ± 0.002
A3	24	0.110 ± 0.001
B2X	6	0.067 ± 0.002
A4X	12	0.051 ± 0.002
A9X	3	0.018 ± 0.002
A3X	13	0.039 ± 0.002
B1X	6	0.075 ± 0.002

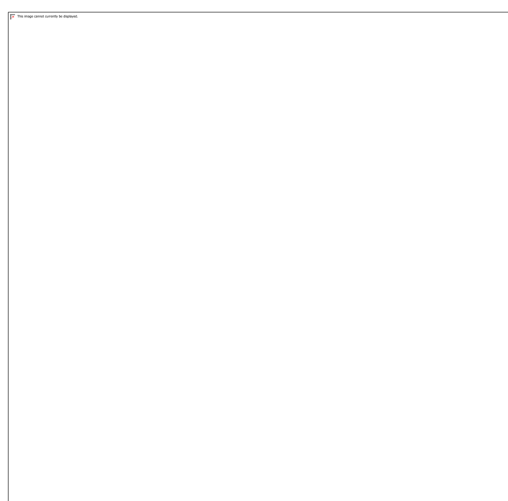

Plate 1: Isolate A1 showing positive starch hydrolysis test

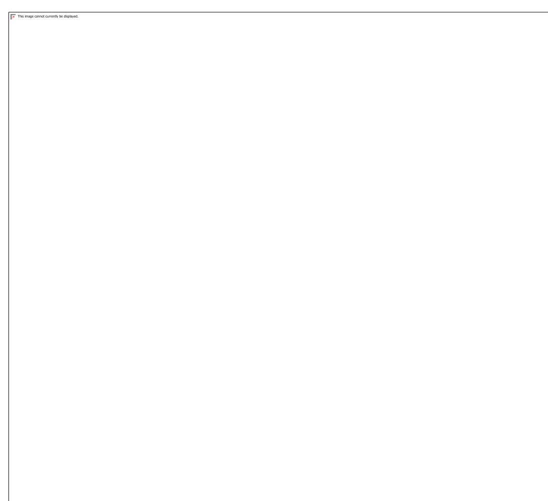

Plate 2: Isolate A2 showing positive starch hydrolysis result

Plate 3: Isolate A2 showing Clear Zone on Starch Agar Plate by Crude Enzyme Extract

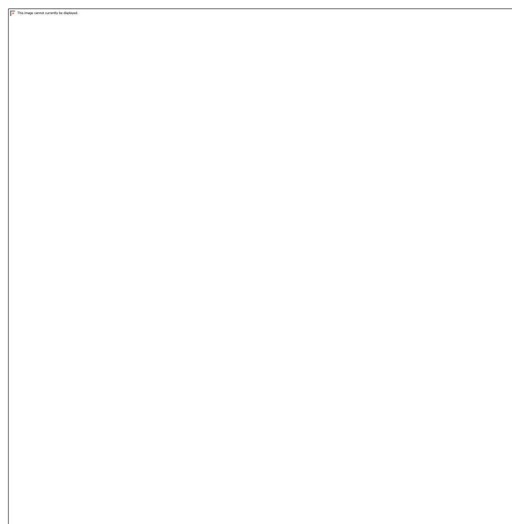

Plate 4: Isolate A3 showing positive starch hydrolysis result

The effects of pH on amylase production by the bacterial isolates are presented in Table 5. At a neutral pH of 7, the lowest amylase production activity (0.007 ± 0.004) was observed in isolate A2, while the highest amylase producer was in isolate A9X (0.107 ± 0.006). At pH 6, the order of amylase production was A3X (0.081 ± 0.003) > A1 (0.048 ± 0.003) > A4X (0.046 ± 0.004) > B1X (0.042 ± 0.006) > B2X (0.036 ± 0.007) > A3 (0.034 ± 0.006) > A2 (0.029 ± 0.005) > A9X (0.019 ± 0.003). At a pH of 8.0, isolate B2X (0.081 ± 0.004) was the highest producer of amylase, followed by isolate A9X (0.071 ± 0.003), while isolate A1 was the least producer of amylase (0.016 ± 0.006). The effect of temperature on amylase production is shown in Table 6. At 40°C, isolate A2 had the lowest amylase production activity, 0.033 ± 0.002 u/ml, while at this same temperature, isolate A3 elicited maximal amylase production activity, 0.095 ± 0.003 u/ml (Table 6). At 55^{0}C, the order of amylase production was A9X (0.133 ± 0.004) > A2 (0.097 ± 0.003) > B1X (0.071 ± 0.004) > B2X (0.070 ± 0.003) > A3X (0.065 ± 0.005) > A4X (0.065 ± 0.004) > A3 (0.058 ± 0.003) > A1 (0.051 ± 0.001). Meanwhile, amylase producers isolated in this study had an optimum temperature range between 35 and 55°C.

The best 3 producers of the amylase enzyme were further identified using molecular techniques. BLAST analysis revealed isolate A1 to be *Achromobacter anxifer* strain BIULMG 26857, isolate A2 as *Providencia alcalifaciens* strain BIUFDAARGOS_408 and isolate A3 as *Proteus mirabilis* BIUHI4320 (Table 7). The gene sequences have been deposited in the NCBI database, and the accession number has been obtained.

Table 5: Effect of pH on the amylase produced by different bacteria isolated from dumpsite soil

	Amylase activity (u/ml)		
Isolates/pH	**6**	**7**	**8**
A1	0.048 ± 0.003	0.093 ± 0.007	0.016 ± 0.006
A2	0.029 ± 0.005	0.007 ± 0.004	0.023 ± 0.004
A3	0.034 ± 0.006	0.096 ± 0.007	0.050 ± 0.008
B2X	0.036 ± 0.007	0.033 ± 0.004	0.081 ± 0.004
A4X	0.046 ± 0.004	0.061 ± 0.003	0.050 ± 0.003
A9X	0.019 ± 0.003	0.107 ± 0.006	0.071 ± 0.003
A3X	0.081 ± 0.003	0.041 ± 0.003	0.053 ± 0.004
B1X	0.042 ± 0.006	0.070 ± 0.002	0.038 ± 0.000

Table 6: Effect of temperature on the amylase produced by bacteria isolated from different dumpsites soil

	Amylase activity (u/ml)					
Isolates/Temperature	30	35	40	45	50	55
A1	0.065 ± 0.001	0.059 ± 0.002	0.071 ± 0.001	0.097 ± 0.002	0.070 ± 0.001	0.051 ± 0.001
A2	0.066 ± 0.002	0.155 ± 0.002	0.033 ± 0.002	0.058 ± 0.002	0.112 ± 0.003	0.097 ± 0.003
A3	0.063 ± 0.002	0.079 ± 0.002	0.095 ± 0.003	0.065 ± 0.002	0.074 ± 0.003	0.058 ± 0.003
B2X	0.064 ± 0.003	0.098 ± 0.002	0.094 ± 0.003	0.093 ± 0.003	0.111 ± 0.002	0.070 ± 0.003
A4X	0.061 ± 0.003	0.072 ± 0.003	0.082 ± 0.003	0.120 ± 0.003	0.091 ± 0.002	0.065 ± 0.004
A9X	0.070 ± 0.003	0.106 ± 0.002	0.084 ± 0.003	0.077 ± 0.003	0.071 ± 0.003	0.133 ± 0.004
A3X	0.075 ± 0.003	0.062 ± 0.003	0.083 ± 0.004	0.090 ± 0.003	0.073 ± 0.004	0.065 ± 0.005
B1X	0.073 ± 0.004	0.083 ± 0.003	0.064 ± 0.002	0.090 ± 0.002	0.122 ± 0.004	0.071 ± 0.004

Table 7: Sequence Analysis of 16S rRNA analysis of selected amylase-producing soil-borne bacterial isolates

Isolate	Closet match from Gene bank database	Query cover (%)	Percentage identity	E-value	Accession No.
A1	*Achromobacter anxifer* strain BIULMG 26857	85	85.81	0.0	PQ032333
A2	*Providencia alcalifaciens* strain BIUFDAARGOS_408	98	82.85	0.0	PQ032334
A3	*Proteus mirabilis* BIUHI4320	98	93.64	0.0	PQ032335

DISCUSSION

The outcome of this study revealed the abundance of heterotrophic bacteria in waste dumpsites from selected local government areas in Benin City, Edo State, Nigeria. Meanwhile, no significant difference was observed between the bacteria population from the waste dumpsite and control sites (100 m distance from the dumpsites), as well as the different strata of the soil around the dumpsite area. Soil, including dumpsites soil, remains a good reservoir for heterotrophic bacteria (Bello *et al*., 2021; Oyeleke and Oduwole, 2009); however, the abundance, nature, and dominant bacteria species are dependent on the activities in and around the soil environment. Several studies from different soil and dumpsites have also revealed the presence and abundance of amylase-producing bacteria. Bello *et al*. (2021), in a recent study, reported the presence of amylase-producing bacteria from non-dumpsite soil from 3 different local government areas in Sokoto State, Nigeria.

Similarly, an abundant ecosystem of amylase-producing bacteria from soil samples obtained from both local yam peel dumpsite (Oyeleke *et al*. 2010) and cassava waste (Oyeleke and Oduwole, 2009) were reported in Minna, Niger State, Nigeria. Although there was no clear distinction reported between the best amylase-producing bacteria from both cassava peels and cassava peels soil dumpsite, Madika *et al*. (2017) positioned that dumpsite soil microbes are better amylase producers when compared to microbes isolated from other matrix. The position asserted by Madika *et al*. (2017) is in agreement with the result of our current study, as dumpsite soil microbes had the highest zone of starch hydrolysis. As a mark of clear distinction between samples obtained from the dumpsites and the control samples taken 100 m away from the dumpsites, it is worthy of note that the three most active amylase producers isolated in this study were isolated from soil samples taken from the top layers of the dumpsites. Of particular interest were isolated A1, A2, and A3, which had zones of starch hydrolysis of 21 mm, 27 mm, and 24 mm, respectively. These results are comparable with the results of previous studies and fall under the same range as high amylase-producing bacteria. Madika *et al*. (2017) found a similar range from bacteria isolated from dumpsites, with the highest zone of starch hydrolysis reported to be 29 mm. However, the report of other studies found relatively low zones of amylase activities from microorganisms isolated from different waste dumpsites; Oyeleke and Oduwole (2009) [0.3 mm – 3.1 mm), Nimisha *et al*. (2019) [0.11 mm - 2.96 mm] and Verma *et al*. (2011) [0.3 mm

– 2.8 mm]. The definitive identity of the genus *Achromobacter*, being one of the bacterial amylase producers documented in this study, agreed with the report made by Mahalakshmi and Jayalakshmi (2016), who revealed the identity of multiple enzymes (amylase, cellulase, and xylase) producing bacteria from the marine environment. Also, in another study, *Achromobacter iophagus* was isolated as an amylase-producing bacteria from a cassava peel dumpsite using submerged fermentation (Arekemase *et al.*, 2020).

The isolate *Providencia alcalifaciens* strain BIUFDAARGOS_408 isolated in this study produced amylase with optimum enzyme activity at a temperature of 40^{0}C and pH 6, which is in agreement with the work of Vaidya *et al.* (2015). Anwer et al. (2020) reported that an acidophilic, detergent-stable amylase-producing strain of *Providencia rettgeri* had an optimum enzyme activity of 40^{0}C. The third amylase-producing bacteria identified to be *Proteus mirabilis* BIUHI4320 in the present study also showed the highest enzyme activity at a temperature of 40^{0}C and neutral pH.

The varied impacts of pH and temperature on expressed amylase activity by the 8 bacterial amylase producers were also documented in this study. The effects of these two parameters on bacterial amylase production activity have also been reported by several researchers (Luang-In *et al.*, 2019; Panneerselvam and Elavarasi, 2015; Naidu and Saranraj, 2013; Oyeleke *et al.*, 2010). It was observed that *Achromobacter anxifer* strain BIULMG 26857 and *Proteus mirabilis* BIUHI4320 had maximal amylase activity when grown at neutral pH. This trend was similar to an observation reported by Luang-In *et al.* (2019) with respect to an amylase producer, *Bacillus* sp. 3.5AL2, which the author indicated exhibited maximal enzyme activity when cultured under neutral pH conditions. Also, this trend was in agreement with a report by Oyeleke *et al.* (2010), which indicated the beneficial effects of neutral pH conditions on the amylase activity of *B. megaterium*. From this study, it was observed that exposure to an ambient temperature value of 35ºC led to maximal amylase activity by the isolate identified as *Providencia alcalifacien* strain FDAARGOS_408. This result is in keeping with the study made by Mahalakshmi and Jayalakshmi (2016), which reported the optimum temperature for amylase activity by *A. xylosoxidan* to be 35ºC.

It is not uncommon for amylase to work best at a moderately high temperature and neutral pH, as reported in the current study, which is also supported by the results of other studies, such as Vaigya *et al.* (2015). They reported that *Bacillus* sp. had a maximum enzyme activity at 40^{0}C. In order to be effective in industrial processes, it is necessary for an enzyme to work at high temperatures (Sivaramakrishnan *et al.*, 2006). Temperature and pH have been described as two key factors that can directly affect amylase activities by both prokaryotes and eukaryotes, and a temperature range of 50 and 75°C has been advocated as the best range wherein optimal amylase activities can be observed (Oyeleke *et al.*, 2010). However, based on the results of this study, it can be postulated that this suggested range might not apply to all prokaryotic amylase producers, as the optimum temperature range was 35 - 55°C.

The study of amylase-producing bacteria from the soil of waste dumpsites reveals a complex and dynamic microbial ecosystem with profound potential ecological, industrial, and scientific significance. The significance of amylase lies not only in the breakdown of complex carbohydrates but also in its application across diverse industries, including food and beverages, detergents, pharmaceuticals, and biotechnologies.

From an ecological perspective, amylase-producing bacteria are essential contributors to carbon cycling and nutrient recycling in natural environments. They facilitate the decomposition of organic matter, release carbon into the soil, and influence soil health and ecosystem functionality. Gupta and Kapoor (2017) reported that the microbial communities in waste dump sites are unique, reflecting the diverse array of substrates present and the complex interactions among microorganisms. In waste dump sites, these bacteria thrive in diverse environments influenced by waste composition, environmental conditions, and microbial interactions. Different types of waste materials, such as municipal solid waste, agricultural residues, and industrial byproducts, provide distinct niches for amylase producers. This diversity, shaped by the complexity of waste materials, pH, temperature, moisture, and microbial interactions, offers opportunities for biotechnological applications. Amylase-producing bacteria can, therefore, be harnessed for enzyme production and employed in bioremediation efforts to reduce environmental contamination, especially in developing countries like Nigeria, where waste management is still a serious challenge (Tchobanoglous *et al.*, 2013).

CONCLUSION

In this study, eight bacteria were identified to produce amylase with *Achromobacter anxifer* strain LMG 26857, *Providencia alcalifaciens* strain BIUFDAARGOS_408, and *Proteus mirabilis* BIUHI4320 as the best producers of the enzyme.

The optimum pH as well as temperature of the produced amylase was found to be pH of 6 and 35ºC

Recommendation

Waste dumpsites in the Benin metropolis are a reservoir for amylase-producing bacteria. More studies on the production and industrial application of this enzyme are therefore warranted.

Conflict of interest

The authors declare that there are no potential conflicts of interest

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