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Isolation and Enzymatic Activity of Thermo-tolerant Bacteria from Waste Dumpsites in Umudikeand Environs

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The research studied the isolation of thermo-tolerant bacteria with enzymatic activity from waste dumpsites. 30 soil samples were collected using sterile spatular into sterile universal bottles, labelled and taken to the laboratory analyses. The soil samples were serially diluted and inoculated by spread plate method on different media and incubated at 30^{o}C for 48 hrs. The isolates were observed for colonial morphologies and later sub-cultured to get pure isolates which were stored in agar slants and kept in the refrigerator for further use. The isolates were Gram stained and also subjected to biochemical and sugar fermentation tests. The isolates were then cultured at thermophilic temperature and those with positive results were screened for the production of amylase, protease and lipase activity. The effect of temperature, pH and nitrogen sources on enzyme activity of the thermophilic bacterial isolates was also assessed. Results show that *Bacillus subtilis* (70.0%), *Bacillus licheniformis* (43.3%), *Bacillus cereus* (56.7%), *Pseudomonas aeruginosa* (56.7%), *Streptomyces* species (36.7%), *Bacillus brevis* (43.3%)and *Norcadia* species (30%) were recovered from the soil samples. All the bacterial isolates tolerated 55^{o}C of temperature while only *Bacillus cereus, Bacillus brevis* and *Norcadia* species were isolated at 60^{o}C. All the isolates except *Pseudomonas aeruginosa* gave amylase activity while all the isolates gave protease activity. All the isolates except *Bacillus licheniformis* and *Nocardia species*

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had lipase activity. Temperature and pH had varied effects on the enzyme activity of the isolates. The potentials of these bacterial isolates as good sources of enzymes at elevated temperatures, varied pH and nitrogenous sources for commercial uses was recognized.

Keywords: Activity; bacteria; enzyme; pH; temperature; thermo-tolerant.

1. INTRODUCTION

Thermophilic bacteria are microbes that mostly inhabit hot environments, live and survive in temperatures above 70°C [1]. They have been less explored due to difficulties in isolation and maintenance of pure culture. Therefore, their diversity and biotechnological potential remains to be explored from majority of the thermal habitats. The adaptation to these harsh habitats explains the high genomic and metabolic flexibility of microbial communities in these ecosystems and makes thermophiles and their thermostable proteins very suitable for some industrial and biotechnological applications [1]. As a consequence of growth at high temperatures and unique macromolecular properties, thermophiles can possess high metabolism, physically and chemically stable enzymes and lower growth but higher end product yields than similar mesophilic species [2].

Waste dump can be defined as waste type that includes predominantly household waste (domestic waste) with sometimes the addition of commercial waste collected by a municipality and deposited within a given area [3].

Thermostable proteases are of greater advantage in applications because they not only do not usually denature at high temperatures, but they also remain active at such temperatures. Several workers have reported thermophilic bacteria from diverse environmental habitats such as geothermal sites and hot springs. Protease constitute a large and complex group of hydrolytic enzymes with important application in medical, pharmaceutical, biotechnology, leather, detergents and food industries [4].

Microorganisms present a remarkable potential for proteolytic enzymes productiondue to their extensive biochemical diversity and susceptibility to genetic manipulation [5].

Amylases are enzymes that break down starch or glycogen. Recent discoveries of starch degrading enzymes have led to increased application of amylases in various industrial processes. Thermostable amylase not only solves stability issues, but also accelerates reaction rate, reduces possible contaminations and lowers viscosity of medium, thus directly benefitting the starch-processing industries under high temperatures [6].

Lipase are serine hydrolase that catalyze both hydrolysis and occupy a place of prominence among biocatalysts owing to their ability to catalyze a wide range of reactions and are an important group of biotechnologically relevant enzymes and they find a massive applications [7].

Many industries need thermophiles for the production of thermostable enzymes that have wide applications, hence there is a need for isolation of specific bacteria that will be of economic importance.

This research aimed at isolation and identification of thermophilic bacteria from soil waste dump site samples with ability to produce industrial enzymes.

2. MATERIALS AND METHODS

2.1 Sample Collection

Thirty (30) soil samples were collected from 6 different sites within Umudike metropolis; Ahiaeke, Government College, Timber, NDDC GEJ Hostels of Michael Okpara University of Agriculture as well as Living Rock Church Junction respectively insterile universal bottles using a sterile soil spatula and immediately taken to the laboratory for analysis.

2.2 Cultivation and Enumeration of Total Bacterial Population

One gram of each soil sample was diluted in 9ml of sterile peptone water in 250ml conical flask and placed in an orbital shaker at 150 rpm for 15 minutes to get a homogenized soil suspension. 0.1 ml of the diluted soil sample was aseptically inoculated onto sterile Petri dishes containing Nutrient Agar (HIMEDIA, India). To inhibit contaminating fungi in triplicates and incubated in Southern Biological Incubatorat 37°C for 24 hours. After incubation, the number of colonies on each plate was counted using a colony

counter and the average count determined [8]. The isolates were sub-cultured to get pure isolates which were later Gram Stained, subjected to biochemical and sugar fermentations tests.

2.3 Determination of Bacterial Thermo-Tolerance

Pure cultures of 24 hour old of the bacterialisolates weretested for their thermo-tolerant ability by inoculating them into 5 ml of Nutrient broth medium (HIMEDIA) in test tubes. The tubes were incubated at 45^{o}C for 12hours after which each broth culture was streaked onto freshly prepared Nutrient Agar medium and incubated at 37^{o}C for 24 hrs to determine their viability [8].

2.4 Determination of Enzyme Activity

2.4.1 Amylase activity

Starch hydrolysis method was used to identify the amylolytic properties of the thermotolerant isolates. The isolates were streaked on the starch agar plates and incubated at 37^{0}C for 24 hours. After incubation, 1% iodine solution was flooded on the starch agar plate. The presence of blue color around the growth indicated negative results [9].

2.4.2 Protease activity

Protease activity was detected on Muller-Hinton Agar (HIMEDIA) containing3% Bovine Serum Albumen (BSA). The plates were streaked with test isolates and incubated at 37^{0}C for 24hrs. The presence of a transparent zone around the colonies indicatedcaseinase activity [10].

2.4.3 Lipase activity

Lipase activity was determined on tributyrineagar streaked with bacterial isolates and incubatedimmediately at 37^{o}C for 24-48 hours. Plateswas observed for clear zone around the bacterial growth [11].

2.5 Effect of Temperature on Enzymeactivities

The isolates were streaked on the starch agar plates and incubated at 30^{o}C, 35^{o}C, 40^{o}C, 45^{o}C, 50^{o}C, 55^{o}C and 60^{o}C respectively for 24 hours. After incubation, 1% iodine solution was flooded on the starch agar plate. The presence of brown color around the growth indicated negative results while blue-black colour showed positive result (Cowan, 2016).

2.6 Effect of pH on Enzyme Activities

The medium for each enzymes was prepared and six buffer solutions with pH of 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 were labeled in six beakers in order to differentiate the six different buffers. The pH for each enzyme were initially testedusing the pH meter and adjusted by adding 5ml of appropriate buffer into the respective beakers using the graduated pipette. After the appropriate pH was determined, the test organism was added and incubated at 37^{o}C for 24 hours [12].

3. RESULTS

Table 1 shows the bacterial isolates from the waste dumpsites which are *Bacillus subtilis, B. cereus, B. brevis, B. licheniformis, Pseudomonas aeruginosa*, *Nocardia* species and *Streptomyces* species.

Fig. 1 shows the occurrence of bacteria in the soil samples. *Bacillus subtilis* had the highest percentage occurrence (70) while *Nocardia* species had the least (30%).

Table 2 shows the thermo-tolerance result. All the bacteria had profuse growth at 30^{o}C, but moderate growth at 45^{o}C. At 50^{o}C and 55^{o}C respectively, all the bacteria had scanty growth, but 60^{o}C, only *B. cereus, B. brevis*and *Nocardia* species grew thought scantily.

Table 3 shows that all the bacterial isolates except *Pseudomonas aeruginosa* had amylase activity while all the isolates had protease activity. All the isolates had lipase activity except *B. licheniformis* and *Nocardia* species.

Fig. 2 shows the effect of temperature on bacterial enzyme activity. The activity was optimum at 30^{o}C, but gradually decreased with increase in temperature up to 60^{o}C. The highest activity at 60^{o}C was from *B. licheniformis* while the least activity was from *Streptomyces* species.

Effect of pH on the bacterial enzyme activity is shown on Fig. 3. Generally, there was an increase in activity from pH 3.0 to 7.0 with best activity recorded at pH 7 for all the isolates except *Norcadia* species whose best activity was at pH 6. There was a general decrease in activity beyond pH 7.0.

Table 1. Morphological and biochemical properties of the bacterial isolates

Colony	Grams reaction	Spore	Flagella	Motility	Cell shape	Catalase	Indole	Oxidase	Mannitol	Sucucrose	Fructose	Lactose	Suspected organism
Circular, rough, white	+	+	+	+	Short chains	+	-	+	A/-	A/-	-	-	*B. subtilis*
White, raised	+	+	+	+	Irregular	+	-	+	A/-	A/-	+	A/-	*B. cereus*
Pale yellow	+	+	+	+	Rods	+	-	+		A/-	-	-	*B. brevis*
Rough	+	+	+	+	Convex irregular	+	-	-	+	+	+	+	*B. lincheniformis*
Capsulated	-	Non spore	Single	+	Rod	+	-	+	+	-	-	-	*P. aeruginosa*
Non capsulated	+	-	-	-	Rod	+	-	+	-	-	-	-	*Nocardia* species
Filamentous	+	+	+	-	chain	+	-	+	-	-	+	-	*Streptomyces* species

Key: + = Positive - = negative, A = acid present

Table 2. Thermo-tolerance of bacterial isolates

Bacterial isolates	30^{o}C	35^{o}C	40^{o}C	45^{o}C	50^{o}C	55^{o}C	60^{o}C
Bacillus subtilis	+++	+++	+++	++	+	+	-
Bacillus cereus	+++	+++	+++	++	+	+	+
Bacillus lincheniformis	+++	+++	+++	++	+	+	-
Pseudomonas aeruginosa	+++	+++	+++	++	+	+	-
Streptomyces species	+++	+++	+++	++	+	+	-
Nocardia species	+++	+++	+++	++	+	+	+
Bacillus brevis	+++	+++	+++	++	+	+	++

Key: +++ = Profuse positive growth, ++ = Moderate positive growth, + = Scanty growth, - = No growth

Table 3. Enzymatic activity of the bacterial isolates

Bacterial isolates	Amylase	Protease	Lipase
Bacillus subtilis	+	+	+
Bacillus brevis	+	+	+
Bacillus cereus	+	+	+
Bacillus licheniformis	+	+	-
Pseudomonas aeruginosa	-	+	+
Nocardia species	+	+	-
Streptomyces species	+	+	+

Key: + = Positive, - = Negative

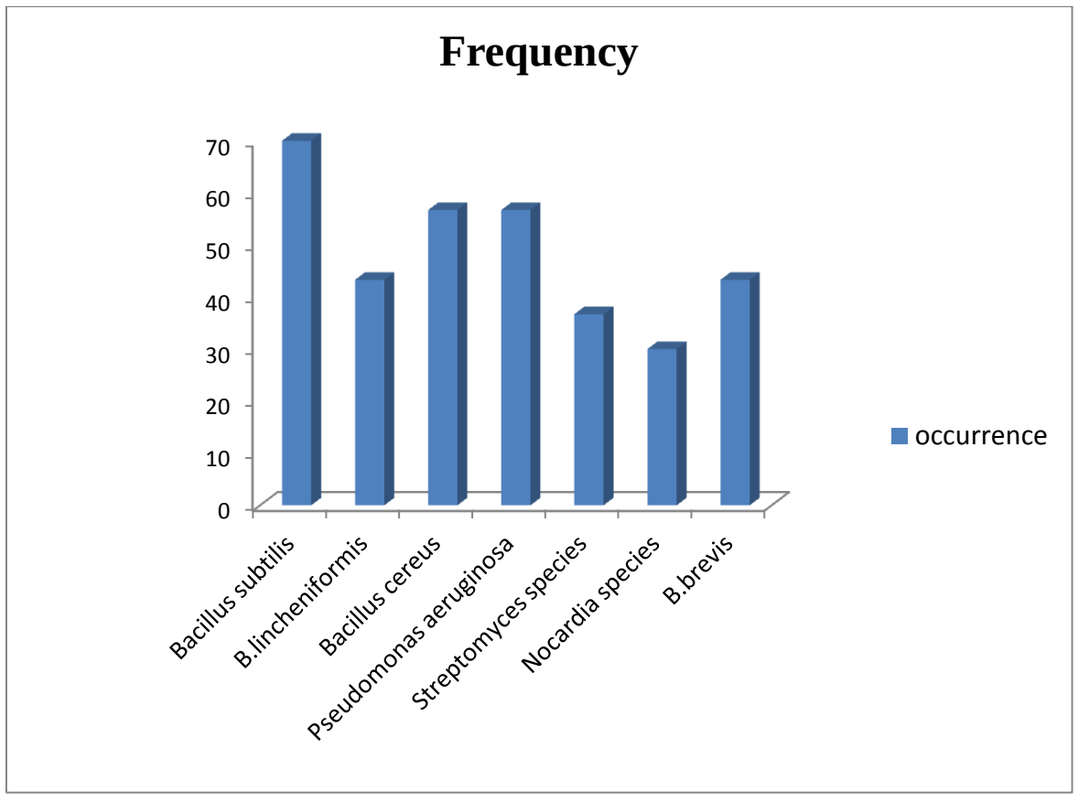
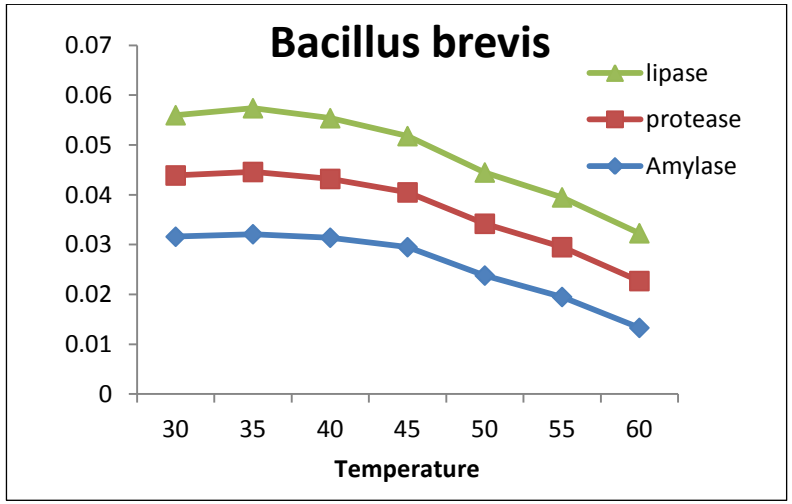
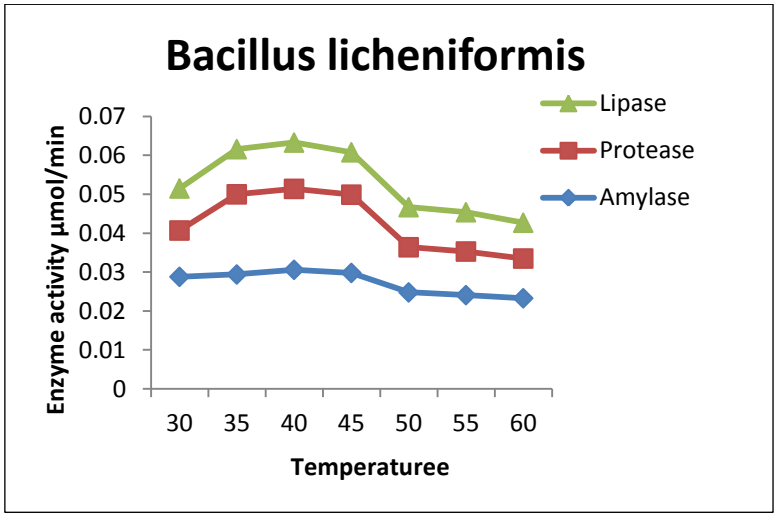
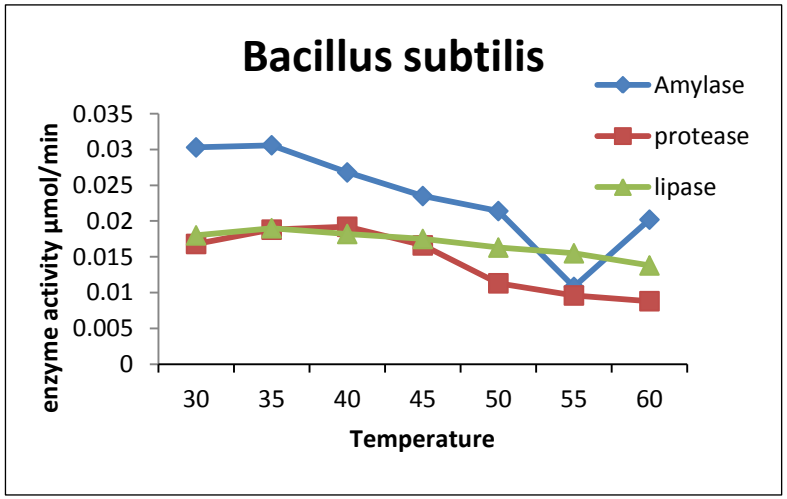
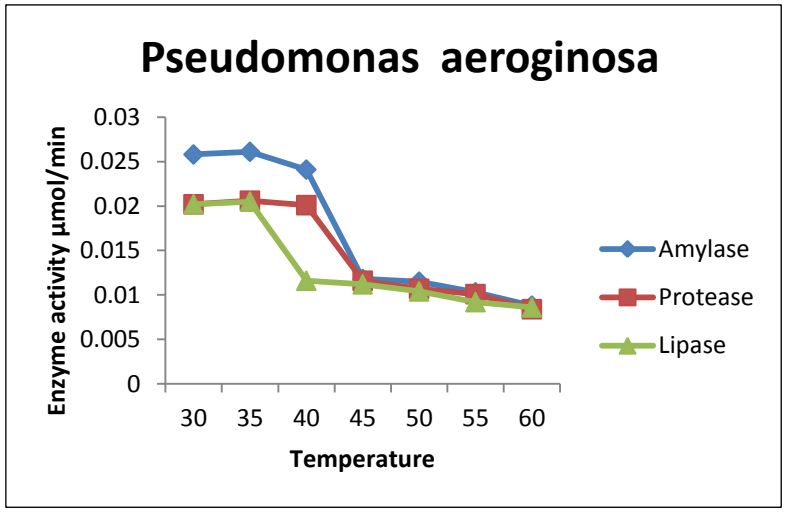


Fig. 1. Frequency of occurrence of bacterial isolates from dump sites


Bacillus brevis
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
30
35
40
45
50
55
60
Temperature
lipase
protease
Amylase


Bacillus licheniformis
Enzyme activity µmol/min
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
30
35
40
45
50
55
60
Temperaturee
Lipase
Protease
Amylase


Bacillus subtilis
enzyme activity µmol/min
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
30
35
40
45
50
55
60
Temperature
Amylase
protease
lipase


Pseudomonas aeroginosa
Enzyme activity µmol/min
0.03
0.025
0.02
0.015
0.01
0.005
0
30
35
40
45
50
55
60
Temperature
Amylase
Protease
Lipase

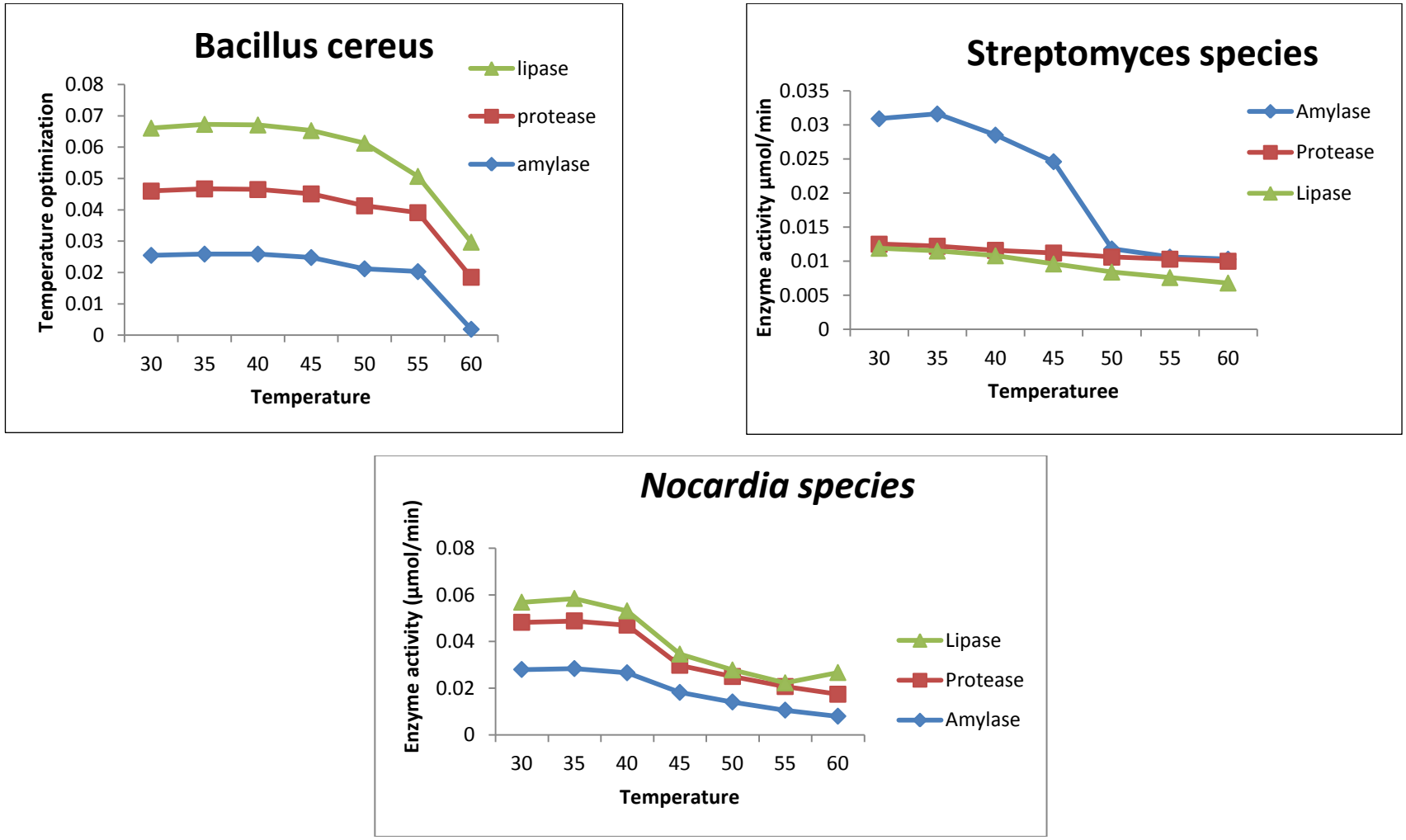
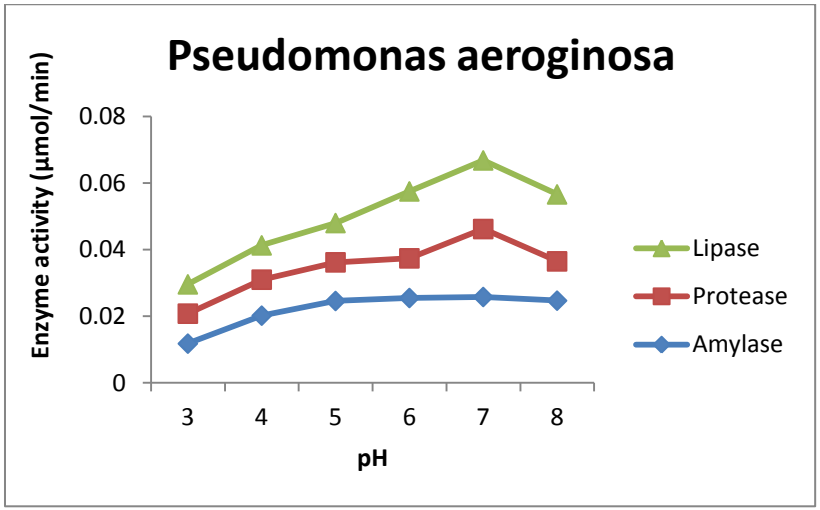
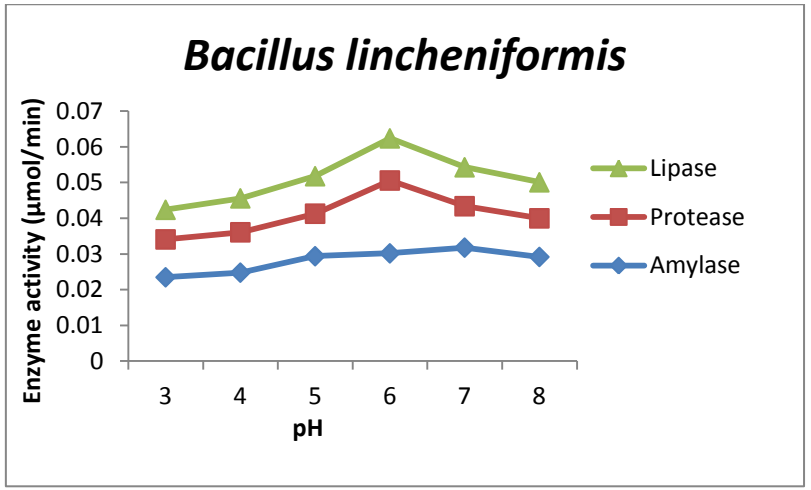
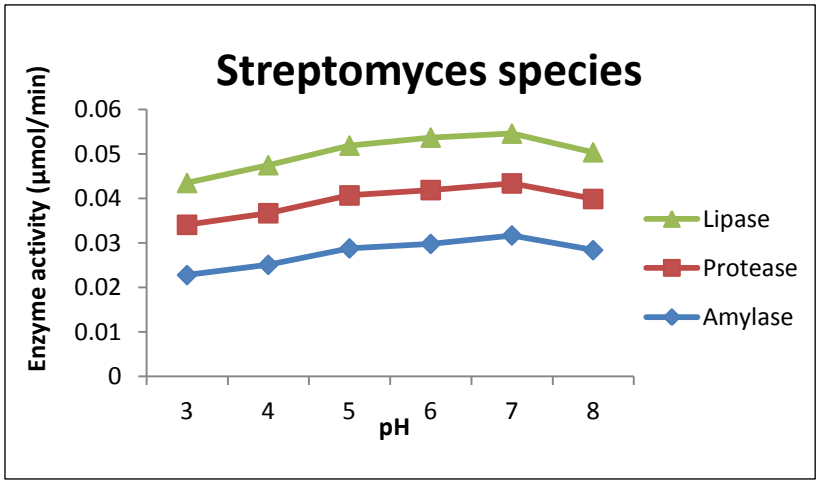
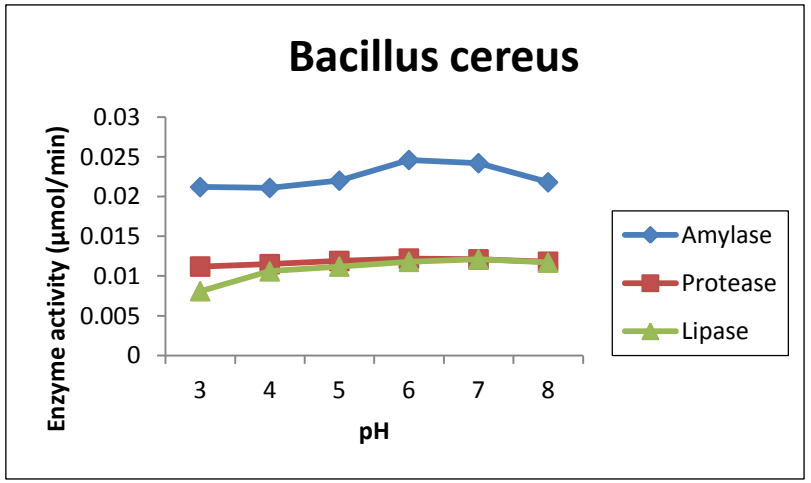


Fig. 2. Effect of temperature on bacterial enzyme activity


Pseudomonas aeroginosa
Enzyme activity (µmol/min)
0.08
0.06
0.04
0.02
0
3
4
5
6
7
8
pH
Lipase
Protease
Amylase


Bacillus lincheniformis
Enzyme activity (µmol/min)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
3
4
5
6
7
8
pH
Lipase
Protease
Amylase


Streptomyces species
Enzyme activity (µmol/min)
0.06
0.05
0.04
0.03
0.02
0.01
0
3
4
5
6
7
8
pH
Lipase
Protease
Amylase


Bacillus cereus
Enzyme activity (µmol/min)
0.03
0.025
0.02
0.015
0.01
0.005
0
3
4
5
6
7
8
pH
Amylase
Protease
Lipase

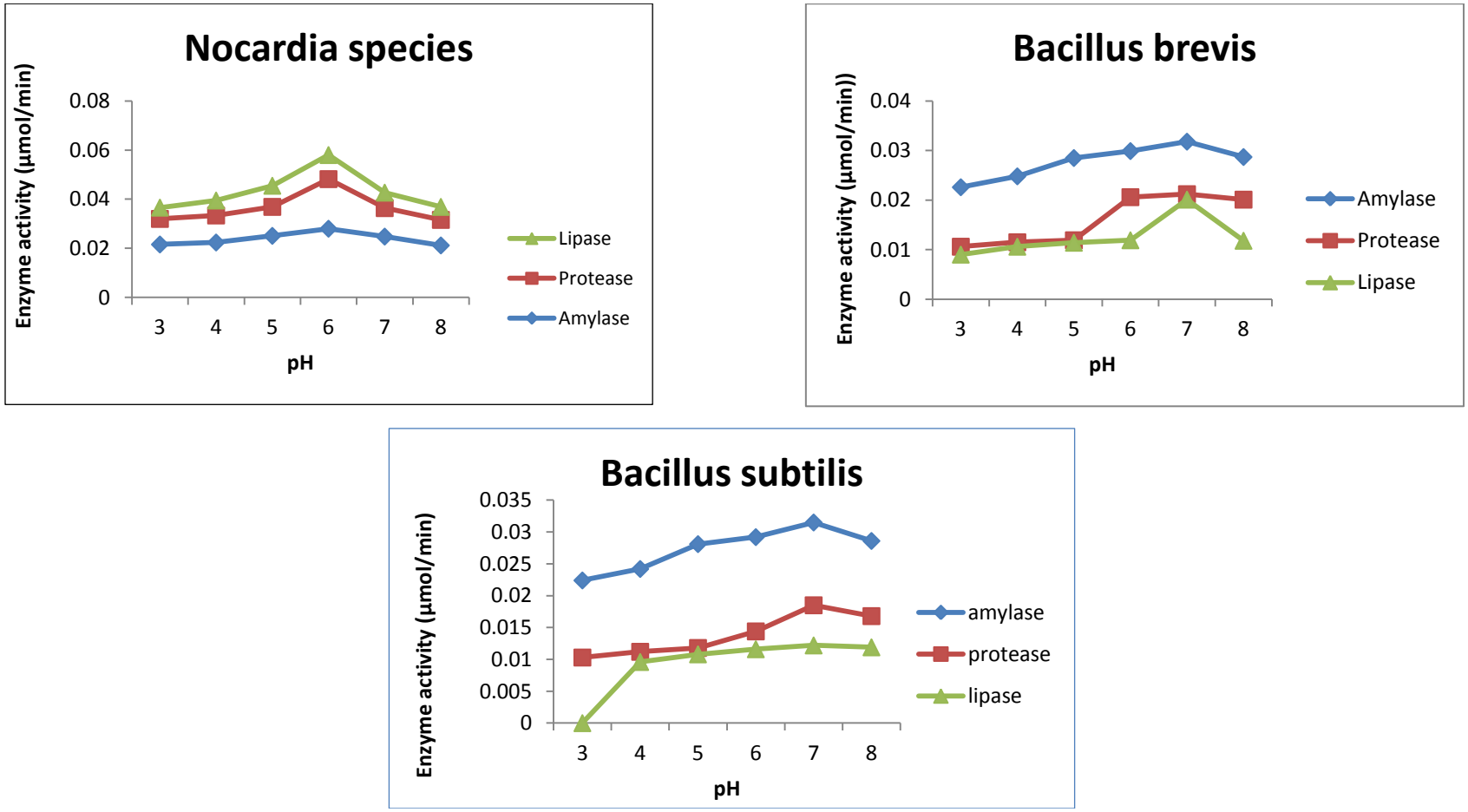


Fig. 3. Effect of pH on bacterial enzyme activities

4. DISCUSSION

The present research aimed at isolation of bacteria from waste dumpsites with enzymatic activities. Based on morphological and biochemical characterization, the dumpsites were found to harbour a variety of temperature tolerant and enzyme producing bacterial species which include *Bacillus subtilis, B. cereus, B. brevis, B. licheniformis, Pseudomonas aeruginosa*, *Nocardia* species and *Streptomyces* species. The isolates recovered from the waste dumpsites are in agreement with the findings of Obianefo et al. [13] who worked on dumpsites in Port Harcourt, South South Nigeria.

The occurrence of bacteria isolates have been reported to be associated with biodegradation of raw human faecal discharges and other wastes at the dumpsites. This observation compares favorably with the report of Hammond and Belies [14]. The micro flora of habitats is determined by the factors which include the availability of nutrients and suitable physical conditions of moisture, temperature etc. Microorganisms that produce amylases could be isolated from places such as soils around rice mills, cassava farms and flour markets (Fossi et al., 2005). Vaseekaran et al. [15] stated that amylase producing bacterial strains were isolated from the soils contaminated with decaying materials including kitchen waste, bakery waste, flour mill waste, soil receiving tea waste, hot white rice gruel and compost heap.

The ability of bacteria to grow at high temperatures is indicative of their potentials to be used at industrial level application. The application of microbial based enzymes at high temperature is desirable in the food industries where thermal treatments are applicable for the control of industrial microorganisms in the product [9]. Considering these facts, the thermophilic bacteria were found to possess amylase, protease and lipase activity. Rath et al., [16] have also reported various extracellular enzymes from some thermophilic bacteria isolated from the same hot spring. However, several workers have reported protease activity from thermophilic *Bacillus* species (Guangrong et al., [17] and Vijayalakshmi et al. [18]).

Proteases are essential constituents of all forms of life on earth including prokaryotes, fungi, plants and animals and are highly exploited enzymes in food, leather, detergent, pharmaceutical, diagnostics, waste management and silver recovery [19]. *Bacillus*' protease is of special importance because of its wide applications in various industries like pharmaceutical, leather, food and waste processing industries [20].

The variations in the optimum temperature for the production of different enzymes as recorded in this work is attributed to genetics of the different isolates. Reports have shown that temperature affects both the production and activities of enzymes. Notwithstanding, organisms can be very active over a wide temperature ranges as thermophiles and extreme thermophiles produce enzymes of industrial purposes. The findingshere show that these organisms produce enzymes at elevated temperatures (50^oC, 55^oC and 60^oC, Table 2) and as such can be useful for commercial use. Also the ability to produce enzyme at such temperature could be utilized in the control of contaminations by microorganisms.

Lipase production by the organisms imply potential use in both food industry and laundry for the removal of strong oil stains on fabrics.Furthermore, the results show that there were variations in the optimum pH for the production of the different enzyme types (amylase, protease and lipase). *Bacillus* cereusand *subtilis* had optimum pH for amylase production at pH 6.0 while the optimum pH for *Bacillus brevis* and *Bacillus lincheniformis* was 7.0 and both had exactly the same rate of amylase production (0.0318µmol/min) at this pH.

Pseudomonas aeruginosa produced amylase optimally at pH 7.0 with production rate of 0.0258 µmol/min. The optimum pH for amylase by *Nocardia* species and *Streptomyces* species isolates was 6.0 for both while their rate of enzyme production were 0.0280 µmol/min and 0.0298 µmol/min respectively.

Protease production rate varied among the bacteria isolate in terms of optimum pH and rate of production. The optimum pH for protease production was 6.0 for four bacteria isolates: *Bacillus cereus, Bacillus lincheniformis*, *Nocardia* species and *Streptomyces* species. However there rates of production varied (Fig. 3). *Bacillus brevis* and *Pseudomonas aeruginosa* recorded optimum protease at pH of 7.0 with the production rates of 0.0212 µmol/min and 0.0204 µmol/min respectively. This agrees with the findings of Mrunmaya (2013).

The rate of Lipase production by the bacterial isolates was comparatively lower than those of

amylase and protease productions. The variations in the enzyme production cut across the different organisms for a particular type of enzyme as well as variation between the same organisms for different enzyme types.

Some enzymes were active over a range of pH values. Vihinen et al. [21] reported that some enzymes are active and stable over a wide range of pH 4.0 to 11.0. However, extreme pH values have been reported to inhibit enzyme activity [22,23].

5. CONCLUSION

The results obtained in this project work showed that dump site soil harboured a wide range of bacteria which were confirmed to be thermophiles. Most of the bacterial isolates demonstrated ability to produce enzymes including amylase, protease and lipase at elevated temperatures. Results also showed variations in the optimum conditions for enzyme production. The optimum pH and temperature of enzymes production reveals that the isolates have potentials as source of different enzymes that could be of various industrial benefits.

6. RECOMMENDATIONS

Waste dumpsites are good sources of industrially beneficial bacterial that could be assessed for various industrial applications. The search for such organisms from waste dumpsites could furnish scientists and industrialists with better performing bacteria in the future.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Dumpsite

Positive Amylase Test

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