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Research article

Isolation and characterization of potential multiple extracellular enzyme-producing bacteria from waste dumping area in Addis Ababa

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ABSTRACT

Extremozymes are innovative and robust biocatalysts produced by various microorganisms from harsh environments. As thermophilic organisms can only develop in a few places, studying them in geothermal environments has provided new insights into the origins and evolution of early life and access to significant bio-resources with potential biotechnology applications. The work aimed to isolate and identify likely multiple extracellular enzyme-producing thermophilic bacteria from an Addis Ababa landfill (Qoshe). The streaking approach was used to purify 102 isolates acquired by serial dilution and spread plate method. The isolates were morphologically and biochemically characterized. Thirty-five cellulases, 22 amylase, 17 protease, and nine lipase-producing bacteria were identified using primary screening methods. Further secondary screening using Strain safety evaluation; two bacterial strains (TQ11 and TQ46) were identified. Based on morphological and biochemical tests, they were found to be gram-positive and rod-shaped. Furthermore, molecular identification and phylogenic analysis of selected promising isolates confirmed the identity of the isolates, *Paenibacillus dendritiformis* (TQ11) and *Anoxybacillus flavithermus* (TQ46). The results indicated that, multiple extracellular enzyme-producing thermophilic bacteria isolated from a waste dumping area in Addis Ababa offer useful features for environmental sustainability in a wide range of industrial applications due to their biodegradability and specialized stability under extreme conditions, increased raw material utilization, and decreased waste.

1. Introduction

The microbial world is the planet's largest untapped source of biodiversity. It's an essential biological frontier getting a lot of attention (Preethi et al., 2022; Ranawat and Rawat, 2017). Extremozymes, innovative and robust biocatalysts, are produced by a

Abbreviations: NA, Nutrient Agar; BLAST, Basic Local Alignment Search Tool; NCBI, National Center for Biotechnology Information; rRNA, ribosomal Ribonucleic acid; TQ, Thermophilic Qoshe; EC, Electrical Conductivity; CFU, Colony Forming Units; CMC, Carboxy methyl Cellulose; HC, Hydrolysis Capacity.

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diverse range of microorganisms from harsh environments (Pathak et al., 2020). Many possible mesophilic microbial strains have been discovered from landfill soil and waste, including bacteria and fungi that can rapidly proliferate and biodegrade organic wastes despite high organic matter concentrations (Krishnan et al., 2021; Song et al., 2018). Extremophilic microorganisms are classified according to the type of extreme condition which they prefer to grow into seven families, thermophiles, psychrophiles, halophiles, acidophiles, alkaliphiles, metalophiles, and piezophiles (Taha et al., 2020). Extremophiles are involved in thermophilic microorganisms (those with an optimum growth temperature of 50 °C or higher) because they produced by thermostable enzymes (such as cellulases, amylases, pectinases, chitinases, proteases, lipases, xylanases, and DNA polymerases); all such enzymes have unique features that make them suitable for biotechnological processes at high temperatures (Mohammad et al., 2017). Extremophiles may reproduce and grow in severe conditions such as pH, temperature, salinity, etc. Extremophiles that can endure salinity extremes are known as halophiles (Ferreira et al., 2018; Ali et al., 2014). Temperature is one of the most critical elements influencing microorganism activity and evolution. Thermophiles are microorganisms that thrive in scorching environments, such as between 55 °C to 121 °C. The thermophiles' cellular components are extremely thermostable, and this, combined with their unique metabolic capacities, holds great potential for biotechnological applications (Sharma et al., 2013). Thermophilic organisms can only grow in a few places; therefore, studying them in geothermal situations has brought new insights into the origins and evolution of early life and access to significant bio-resources with potential applications in the biotechnology industry in Figure 1 (Yadav et al., 2018).

Kitchen waste is a mixture of organic substrates biodegraded by microbial enzymes. The thermophilic and mesophilic phases of the biodegradation process, which involve numerous microorganisms, are the most common. In several steps, microorganisms' biodegrade organic substrates into more stable and humidified products, heat generation as a metabolic waste product (Awasthi et al., 2018). Due to their high-temperature growth and unique macromolecular characteristics, thermophilic bacteria can have a greater metabolism and produce chemically and physically stable enzymes than equivalent mesophilic species (Zeikus, 1979).

Thermopiles are species that have evolved to thrive in hot environments. Because of their thermo-stability and thermo-activity, thermophilic enzymes have a variety of commercial applications. The extremozymes have an economic potential application in agriculture, food beverages, pharmaceutical, detergent, textile, leather, pulp and paper, and biomining industries. The expansion of new industrial processes based on extremozymes and the increasing demand of biotech industries for novel biocatalysts are of great interest for extremophile research (Dumorné et al., 2017). As a result, isolating thermophilic bacteria from natural sources and identifying them are essential steps in discovering new commercial enzymes (Panda et al., 2013). Thermophilic microorganisms are getting a lot of attention as a source of critical thermo-stable enzymes. However, the number of known strains is still tiny, and their most exciting biocatalysts are frequently intracellular or membrane-bound and generated in low quantities (Akassou and Groleau., 2019). As a result, this research aimed to isolate and characterize multiple enzymes to produce thermos-tolerance bacterial strains from the (Qoshe) Addis Ababa waste dump site.

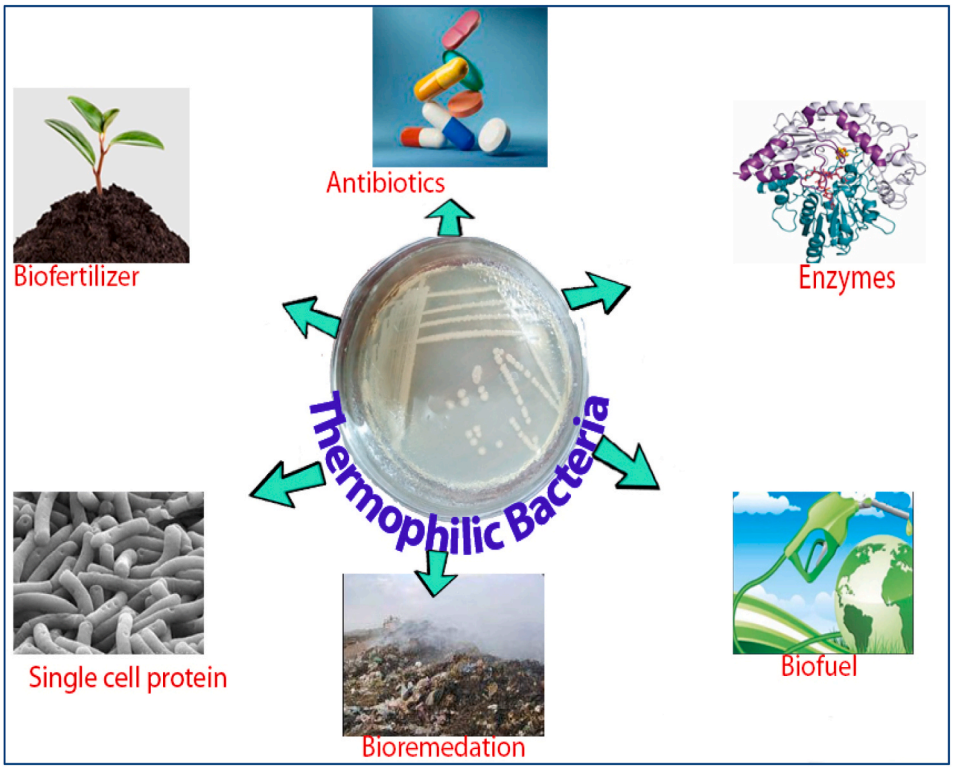

Figure 1. Applications of thermophilic bacteria.

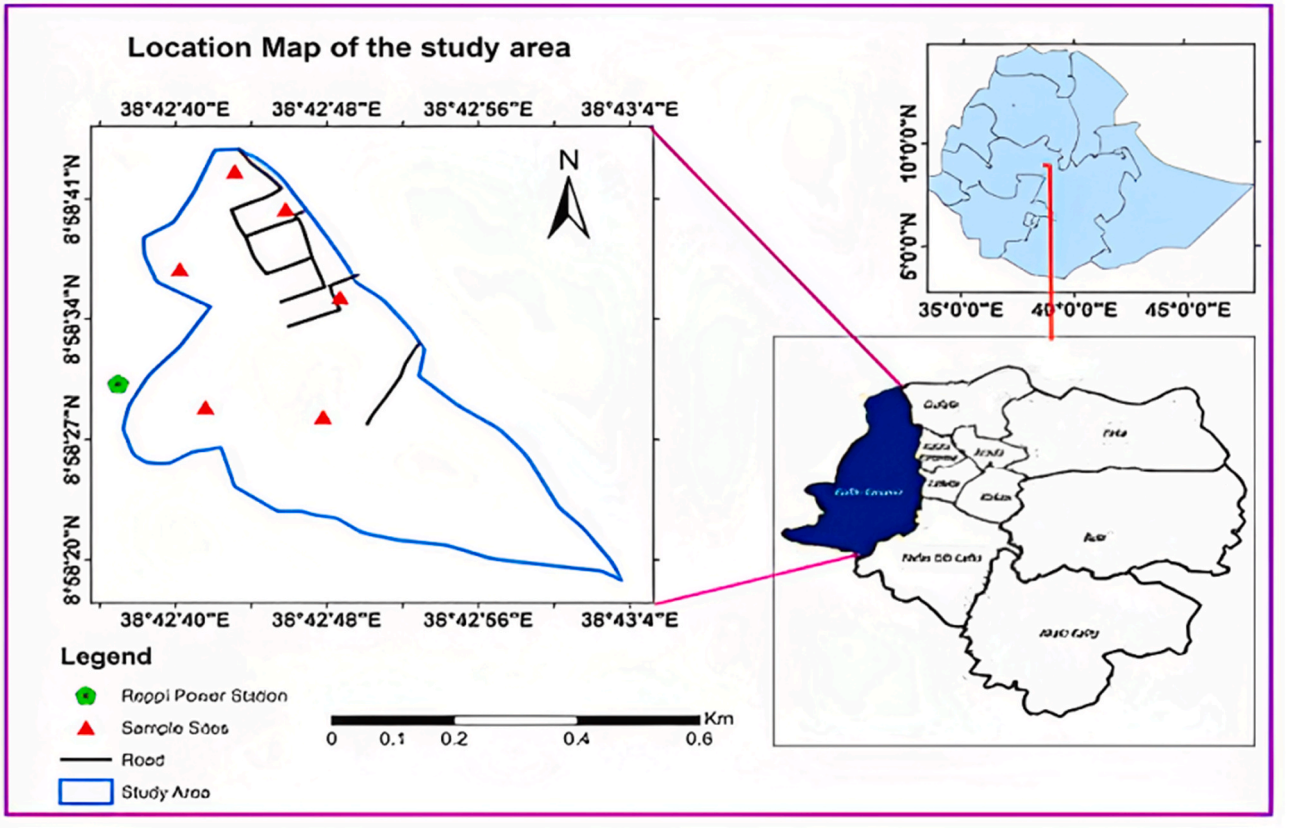

Figure 2. The map of the sampling area.

2. Material and methods

2.1. Collection of samples

Waste soil samples were collected from Qoshe in Addis Ababa around Ayertena, shown in Figure 2. The solid waste sample was brought from the waste dumping area: 500 g of sample was randomly taken in a glass bottle from different depths (10, 30, and 50 cm) of this site and mixed well. They were collected in sterile bottles and kept in a refrigerator at 4 °C. Then, the samples were taken to a lab to be tested for microorganisms.

2.2. Physicochemical characterization of the waste sample

Physicochemical parameters such as colour, odour, pH, Temperature, Electrical conductivity, and moisture content of sampling will be according to Masood et al. (2015).

2.3. Isolation of thermophilic bacteria

1 g of soil sample was then serially diluted up to 10^{-7}, with three replicates for each dilution. It's important to note that the tube was vortexes each time to make sure correct dilution and all work was done in an aseptic environment to avoid contamination. Subsequently, 100 µl of each dilution were inoculated into 20 ml of sterile, melted Nutrient Agar (N.A.) plates (solidified). The overall bacterial count was recorded as CFU g-1 after incubation of N.A. plates at 48 °C for 48 h; subsequent bacterial colonies were selected based on colony morphology and pigmentation as in Figure 3 (Prittesh et al., 2020; Masi and Naveen Kumar, 2016).

For the isolation of pure cultures, the isolated colonies were streaked on N.A. plates several times. Finally, the cleansed colonies were placed on N.A. slants and stored in the refrigerator for further study.

Colony-forming unit (cfu/ml) per milliliter was calculated with this formula (Iraboneye et al., 2021):

$$\text{Colony} - \text{forming unit (cfu / ml)} = \frac{\text{No of colonies} - \text{dilution factor (DF)}}{\text{Quantity plated}} \quad (1)$$

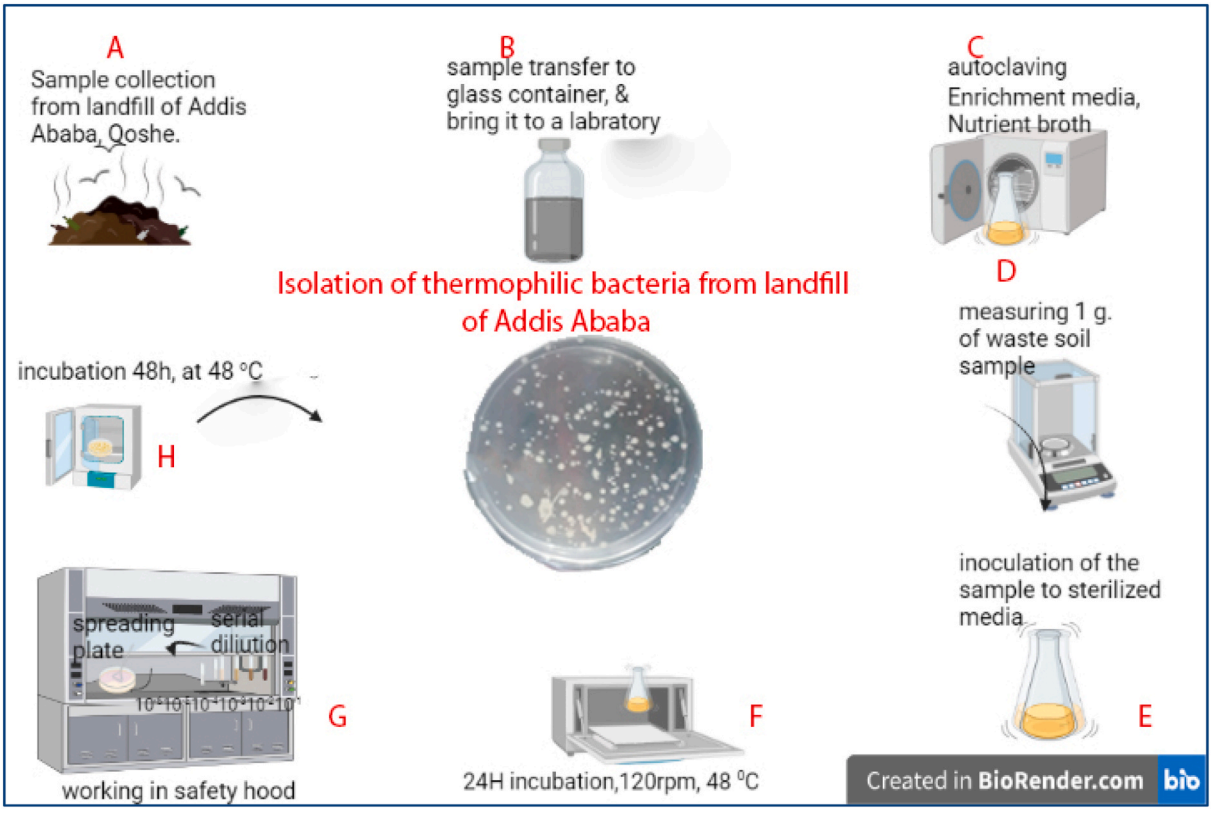

Figure 3. Isolation of thermophilic bacteria from the landfill of Addis Ababa.

2.4. Primary screening of multiple extracellular enzyme-producing bacteria

For the isolation of thermophilic bacteria from municipal waste, one gram of waste sample was measured on an analytical balance and inoculated into 100 ml of nutrient broth media supplemented with 1% each of casein, starch, tween 80, and cellulose to enrich bacteria that produce protease, amylase, lipase, and cellulase respectively (Yadav et al., 2018); Growth media: nutrient broth g/l: 5 g peptone, 5 g sodium chloride, 2 g yeast extract, 1 g beef extract, 1 L distilled water. It was then rapidly stirred to dissolve the waste sample and form a homogeneous solution, which was then incubated in a shaker incubator for 24 h at 120 rpm at 45 °C. Following that, the isolates were categorized based on their substrate specificity (starch, cellulose, casein, and tween 80). The inoculated plates were then incubated for 48 h at 45 °C, and a zone of clearing surrounding each bacterial isolate was evaluated. Following the identification of bacteria that make amylase and cellulase, these bacteria were tested for the ability to produce additional enzymes such as protease and lipase (Masi, 2020).

2.4.1. Screening for cellulase-producing bacteria

For screening of cellulase-producing bacteria, a cellulose hydrolysis test was done. First, bacterial isolates were grown in broth media with cellulose substrate. The resultant culture, about 50 μL was used for the hydrolysis zone in a CMC agar media plate well. Then, for the enhancement of bacterial activity, carboxymethyl cellulose (CMC) agar media containing (0.5 g KH_2PO_4, 0.25 g $MgSO_4$, 0.25 g cellulose, 15 g agar, and 2 g gelatin) was used (Islam and Roy, 2018). The Carboxymethyl cellulose (CMC) agar media plates were incubated for 24 h at 48 °C. Then, the plates were flooded with iodine solution using a dropper. The plates were then left undisturbed for a few minutes before being checked for the hydrolysis zone.

2.4.2. Screening for amylase-producing bacteria

A Starch hydrolysis test was used to screen for amylase-producing bacteria. Bacterial isolates were grown in broth media with the starch substrate, and the resultant culture, about 50 μL was used for the zone of hydrolysis in the well of the starch agar plate. Then the starch agar medium plates were incubated at 45 °C for 24 h (Ullah et al., 2021). With the help of a dropper, the Iodine solution was flooded into the plates after incubation. The plates were then left undisturbed for a few minutes before being checked for the hydrolysis zone.

2.4.3. Screening for protease-producing bacteria

For screening of protease-producing bacteria, a skim milk hydrolysis test was done. Bacterial isolates were grown in broth media with casein substrate, and the resultant culture, about 50 μL was used for the zone of hydrolysis in the well of the skim milk agar plate. The plates were then incubated for 24 h at 45 °C. The zone of inhibition of bacterial proteolytic activity was measured and recorded (Masi et al., 2014; Abdollahi et al., 2021).

2.4.4. Screening for lipase-producing bacteria

A tween 80 hydrolysis test was done to screen Lipid producing bacteria. Bacterial isolates were grown in broth media. Then the resultant culture, about 50 μL was poured into tween 80 agar medium containing (g/L), peptone 10g; yeast extract 5.0; NaCl 5 g; agar 20 g and 10.0 ml tween 80, pH was adjusted to 7.5 and incubated at 45 °C for 48 h (Yusoff et al., 2020).

Determining the hydrolysis capacity (H.C.) value is more feasible for screening the degrading bacteria in large sample volumes than an enzymatic assay (Harnvoravongchai et al., 2020). The clear zones on the different mediums were used to conduct qualitative testing of extracellular enzyme-producing bacteria. As a result of the zone of hydrolysis result, bacteria with a large clear zone were selected for further research. The concentration and enzymatic activity produced were shown by the diameter of the hydrolysis zone (Khokhar et al., 2011; Gacesa, 1992).

2.5. Secondary screening for novel thermophilic bacteria

2.5.1. Strain safety evaluation

Hemolysis activity was examined by streaking the 18-h cultures on brain-heart infusion (BHI) broth supplemented with 5% (vol/vol) defibrinated sheep blood. After incubation for 24 h at 48 °C, hemolysis activity was classified as α-hemolysis (greenish halo surrounding colonies), β-hemolysis (clear halo surrounding colonies), and γ-hemolysis (absence of clearing zone surrounding colonies). α-Hemolysis or β-hemolysis indicated positive hemolytic activity, and γ-hemolysis was taken as a negative result (Zhang et al., 2016).

2.6. Identification of thermophilic bacteria

The bacteria were identified using standard procedures and the various tests necessary for identification.

2.6.1. Morphological and physio-biochemical characterization

Gram-stain, microbial motility, and biochemical properties were observed as morphological characteristics. Biochemical characteristics include the utilization of carbohydrates, gas production, sugar fermentation (sucrose, glucose, lactose, maltose), indole formation, citrate utilization, and H_2S production, which were all measured using semi-solid media according to Yadav et al. (2018) and methyl red-Voges-Proskauer (MR-VP), Urease production test (Verma et al., 2014).

2.6.2. Molecular identification and phylogeny

According to Mohammad et al. (2017), genomic DNA was extracted and purified. The universal bacterial primer 1492R (5′-TAC GGY TAC CTT GTT ACGACT T-3′) and the domain bacteria-specific primer 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) were used to sequence the isolate's 16S rRNA and amplify the target gene. Denaturation at 94 °C for 5 min was followed by 30 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 1.5 min, with a final extension at 72 °C for 10 min. Electrophoresis with a % agarose gel examined amplified PCR products from bacterial isolates. The QIA quick PCR purification kit was used to purify the PCR product (Qiagen). Genewiz Inc. in the United States sequenced the purified PCR products using a Genetic Analyzer (Applied Bio-systems 3130 XL, Switzerland). The obtained sequence was sent to a BLAST algorithm from the National Centre of Biotechnology in Bethesda, Maryland, United States, to identify similar sequences in Gene Bank. The NT system constructed a phylogenetic tree using distance matrix analysis. The BLAST database was used for database searches and comparisons. Mega X was used for phylogenetic analysis and tree construction (Mohammad et al., 2017).

2.7. Statistical analysis

Each experiment was performed in triplicates. The data obtained from the experiments were analyzed and expressed as mean and standard deviation.

3. Results

3.1. Physicochemical characterization of the waste sample

The sample was analyzed for physicochemical parameters such as colour, odour, pH, Temperature, Electrical conductivity (E.C.), and moisture content. The result was tabulated in Table 1. The physiochemical characteristics of the sample, at Qoshe, at the sampling site, and the soil temperature were 48 °C.

Morphologically, the colonies' colour, shape, and texture varied considerably between the isolates. However, the visual investigation of the sample showed that the colour of the waste soil samples from the municipal solid waste disposal site was dark and indicated there might be a high organic content that could be biodegraded. In addition, the odour was unpleasant and objectionable.

3.2. Isolation of thermophilic bacteria

Based on serial dilution (shape of cell, shape of colour, opacity, and surface), 102 thermophilic bacteria were isolated from municipal waste and then streaked on a nutrient agar plate for 48 h 48 °C. For further study, the pure selected isolates were preserved in nutrient broth and put in a refrigerator at 4 °C. Total thermophilic bacteria colony-forming units (CFU) were counted, then multiplied by the dilution factor and expressed in CFU/g of waste soil, obtaining 2.1 × 105 CFU/g of soil. The isolates were selected based on their colony morphological differences. For further investigation, isolated isolates with extracellular enzyme activity were selected.

3.3. Primary screening of multiple enzyme-producing thermophiles bacteria

Cellulose-degrading thermophilic bacteria were obtained after several sub-cultivations of isolated bacteria in the CMC agar plate holes. Grams' iodine solution was used to confirm the bacteria were cellulase positive: positive for the test showed a clear zone around the wells of media and measured by diameter, and those negative for the test showed blue-black: no zone of hydrolysis. Thirty-five thermophilic bacteria with cellulase activities (Figure 4a) were isolated from 102 thermophilic isolates. Following the screening of cellulase-producing bacteria, 22 amylase-producing bacteria (Figure 4b) were obtained out of the first 35 isolates. Seventeen Protease-producing bacteria (Figure 4c) were screened from 35 cellulase-producing bacteria. Nine lipases (Figure 4d) were positive from the screened 35 bacterial isolates. Finally, the ability to produce the four enzymes cellulase, amylase, protease, and lipase was confirmed in three isolates (TQ5, TQ11, and TQ46). Table 2 shows that most isolates produce more than one type of enzyme.

Table 1
Physicochemical Characterization of the waste sample.

Physico-chemical Parameters	Observation
Color	Dark
Odor	The unpleasant, rotting smell
pH	7.6
Temperature (°C Average)	48
EC.(μs/cm)	2100
Moisture (%)	54

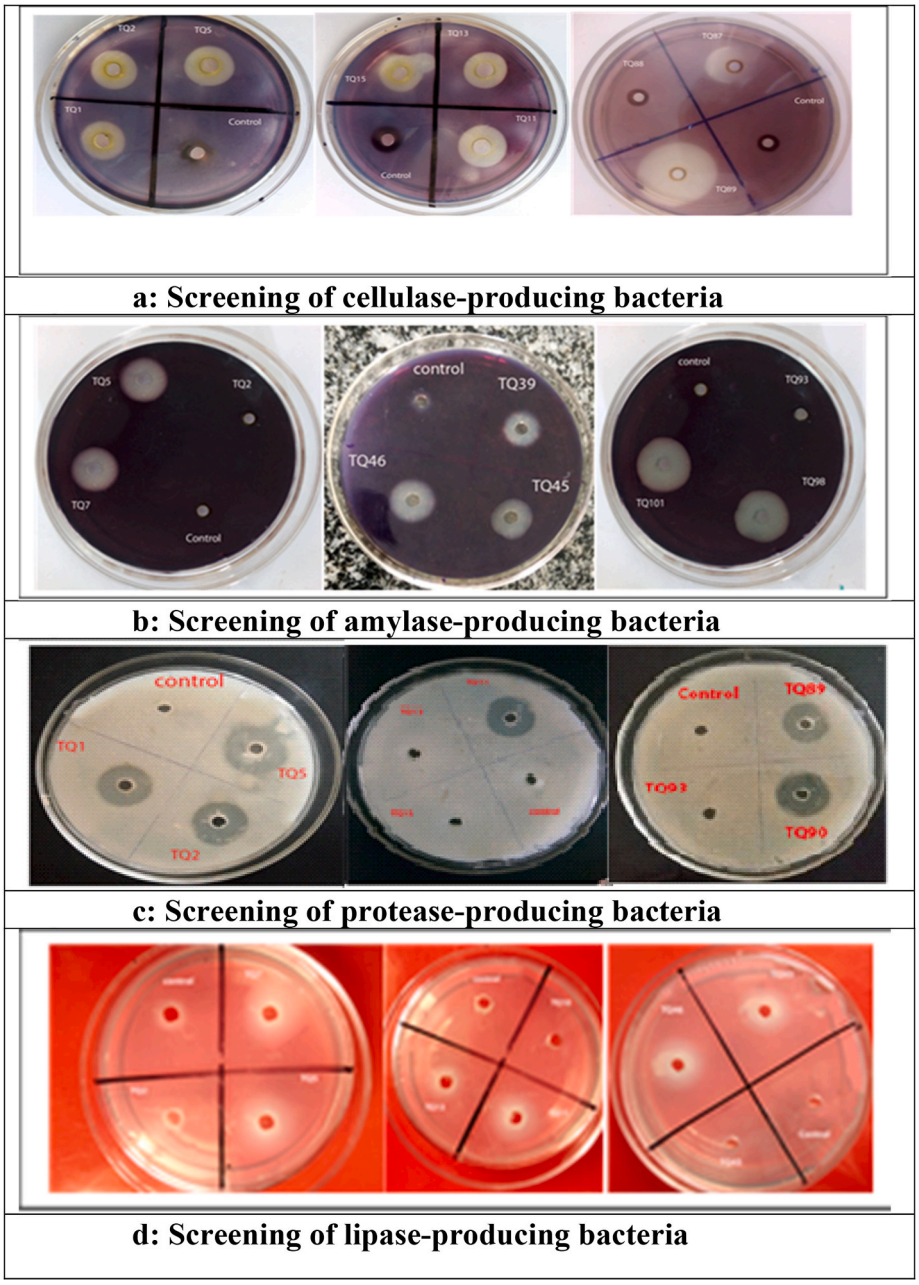

Figure 4. (a) Screening of cellulase-producing bacteria. (b) Screening of amylase-producing bacteria.(c) Screening of protease-producing bacteria. (d) Screening of lipase-producing bacteria.

3.4. Secondary screening of thermophiles bacteria

3.4.1. Strain safety evaluation

The hemolysis pattern of the three bacteria showed that TQ11 and TQ46 are gamma hemolysis since they showed no lysis of red blood cells in the culture media, and TQ5 had alpha hemolysis since the colony was surrounded by a greenish discoloration in Figure 5. Thus, the first two strains (TQ11 and TQ46) are generally considered safe strains and used for further study; the TQ5 strain was rejected since it might cause disease to humans and the environment.

Table 2
Primary screening of multiple enzyme-producing thermophiles bacteria.

Sample ID	Cellulase test	Amylase test	Protease test	Lipase test
TQ1	+	−	+	−
TQ2	+	−	+	−
TQ5	+	+	+	+
TQ7	+	+	−	+
TQ9	+	+	−	−
TQ10	+	−	+	−
TQ11	+	+	+	+
TQ13	+	−	−	+
TQ15	+	+	−	−
TQ18	+	−	−	+
TQ19	+	+	+	−
TQ23	+	−	−	−
TQ25	+	+	+	−
TQ27	+	−	−	+
TQ28	+	+	+	−
TQ29	+	+	−	+
TQ33	+	−	−	−
TQ36	+	−	+	−
TQ38	+	+	−	−
TQ39	+	+	−	−
TQ45	+	+	−	−
TQ46	+	+	+	+
TQ54	+	+	−	−
TQ59	+	+	+	−
TQ64	+	−	−	−
TQ69	+	+	+	−
TQ71	+	+	+	−
TQ78	+	+	−	−
TQ87	+	−	−	−
TQ89	+	−	+	−
TQ 90	+	+	+	−
TQ93	+	−	−	+
TQ98	+	+	+	−
TQ101	+	+	−	−
TQ102	+	+	+	−

'+' positive for the test, '−' negative for the test.

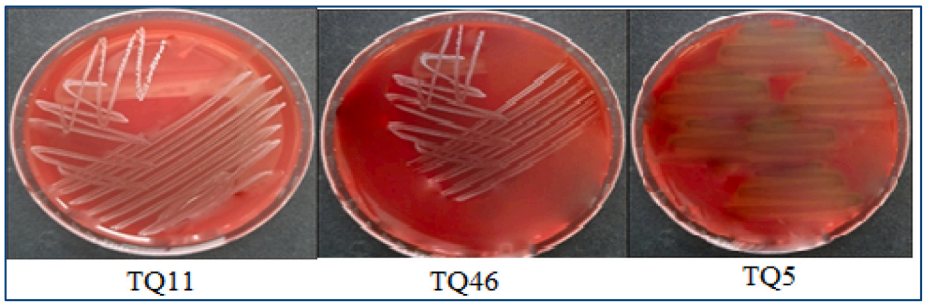

Figure 5. The hemolysis patterns of TQ11 & TQ46 are gamma; TQ5 is alpha hemolysis on blood-based agar media.

3.5. Identification of the thermophiles bacteria

3.5.1. Morphological and biochemical characterization

Finally, based on secondary screening, we selected two thermophilic bacteria isolates, TQ11 and TQ46, cultured on nutrient agar medium to characterize their morphological futures and presented in Figure 6 and Table 3. According to the morphology analysis, both isolates were Gram-positive, rod shape cells, round colonies, smooth, motile, and endospore-forming. TQ46 isolate was a yellowish colony and opaque, whereas TQ11 was translucent and white-grey.

Gram staining was performed on two isolates and reference bacterium. They were all considered Gram-positive cells since they were violet cells under the light microscope. All of the isolates had a rod form to them. Figure 7 depicts the appearances of various gram-positive cells. Many biochemical tests revealed that both isolates were catalase, oxidase, and methyl red positive and did not produce hydrogen sulfide. Isolate TQ46 was citrate positive and negative for urease and indole tests.

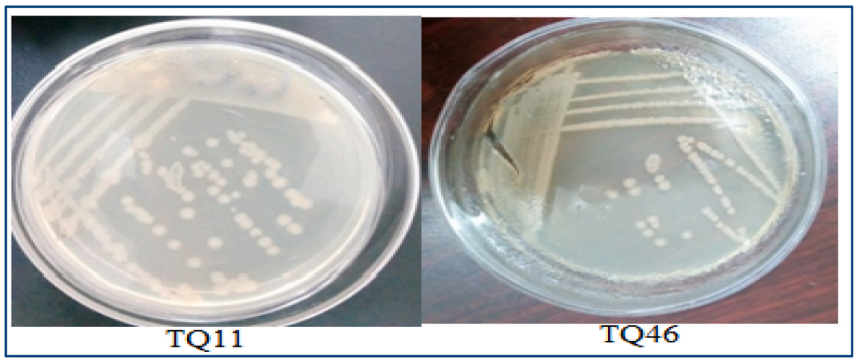

Figure 6. Bacterial colony morphology of TQ11 & TQ46 cultured on NA media.

Table 3
Morphology of isolate TQ11 and TQ 46.

Characteristics	TQ11	TQ46
Color	White	Yellowish
Shape of cell	Rod	Rod
Shape of colony	Round	Round
Surface	Smooth	Smooth
Opacity	Translucent	Opaque
Gramm staining	Positive	Positive
Motility	Motile	Motile
Endospore staining	Positive	Positive

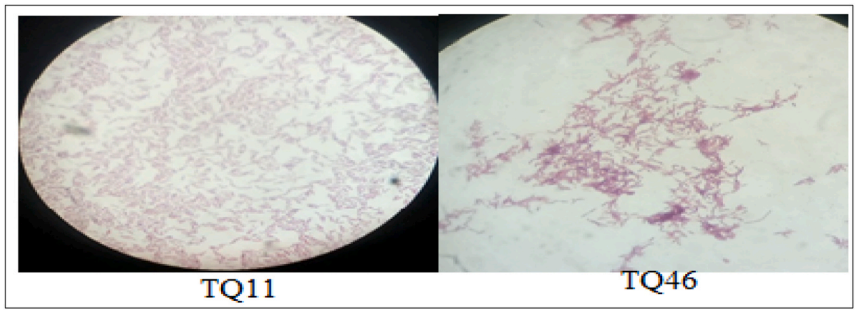

Figure 7. Gram staining reaction of TQ11 &TQ46.

Table 4
Biochemical test of the isolate TQ11 and TQ46.

Biochemical test	TQ11	TQ46
Catalase test	+	+
Oxidase test	+	+
Hydrogen sulfide test	−	−
Citrate test	−	+
Urease test	+	−
Methyl red	+	+
Voges-Proskauer test	−	−
Indole test	+	−
Carbohydrate fermentation		
Glucose	+	+
Lactose	+	−
Maltose	−	+
Sucrose	+	+
fructose	−	+
Starch	+	+
Dextrose	−	+

In contrast, isolate TQ11 was citrate negative and positive for urease and indole production. The Carbohydrate fermentation ability of the isolates was evaluated and tabulated in Table 4. Both isolates ferment glucose, sucrose, and starch; isolate TQ46 further utilized maltose, fructose, and dextrose but did not utilize lactose, whereas TQ11 isolate utilized lactose but not maltose, fructose, and dextrose.

3.5.2. Identification of isolates

The 16S rRNA gene sequence was used to isolate two isolates for further molecular characterization. The 16sRNA, PCR product DNA fragments with a size of roughly 1.5 kb, were amplified (Figure 8). In the BLAST analysis of the 16S rRNA gene revealed high homology with the taxa *Peanibacillus dendritiformis* (TQ11) and *Anoxybacillus flavithermus* (TQ46). Phylogenetic trees were used to establish the closest relationship between each sequence. The amplified DNAs were sequenced, and isolates were identified by comparing the sequences to those in the Gene Bank database, and strains *Peanibacillus dendritiformis* (TQ11) *Anoxybacillus flavithermus* (TQ46) were identified using the BLAST search software, and sequences were submitted to NCBI. OK560696.1 (*Anoxybacillus flavithermus*) and OK560694.1 (*Anoxybacillus flavithermus*) have been assigned to the submitted sequences (*Peanibacillus dendritiformis*). Figure 9 depicts their phylogenetic tree based on 16S rRNA gene sequences. By 16S rRNA characterization studies and partial sequence comparison by NCBI BLAST; the isolates were finally identified up to species level, whereas *Peanibacillus dendritiformis* (TQ11) and *Anoxybacillus flavithermus* (TQ46) were identified up to species level. MEGA11 method was used to analyze evolutionary distances using the maximum composite likelihood approach.

4. Discussion

The physical, chemical, biological, and mechanical properties and behavior of waste and liner materials in landfills are affected by temperature. Temperatures are affected by seasonal fluctuations, waste placement, waste age, waste depth and location, and available moisture. Many findings are in line with this claim that landfill areas are a repository for complex microbial diversity responsible for the biodegradation of solid waste and a potential source of industrially essential microbes (Jha, 2020; Thakur et al., 2020).

The sample's moisture content was 54% using the dry oven method of moisture determination. High bacterial density is seen with the high moisture level, and the optimal activity of aerobic bacteria occurs between 50 and 75% moisture content (Sarkar and Chourasia, 2017). Thus, the sample moisture content is in this range and could be a habitat for a diverse range of microbes. In various situations, including seawater, food, wood, biofilms, landfills, and soils, the high moisture content may inhibit microbial activity (Stark and Firestone, 1995). Low water availability can also restrict microbial activity by lowering intracellular water potential, lowering enzyme hydration, and inhibiting enzyme function (Guo et al., 2016).

The soil's acidity or basicity (alkalinity) is measured by its pH. Therefore, soil pH is an important characteristic used to make quantitative and qualitative analyses of soil characteristics. The waste soil sample had a pH of 7.6, slightly alkaline. The cause for this could be due to the soil's high metal content, the metal ions (such as Fe, Mn, Ca, Mg, Zn, etc.) usually hydrolyze water and produce proton (H^+), which causes acidity (Kebede et al., 2016).

Electrical conductivity (E.C.) measures a solution's ability to transport electric charge or total dissolved salts. It is a factor that determines a plant's ability to absorb water. Soil E.C. depends on salts and mineral ion content levels (Artiola et al., 2019). Uncontrolled waste outflow causes a considerable increase in E.C., harming plants and hindering them from obtaining water from the soil.

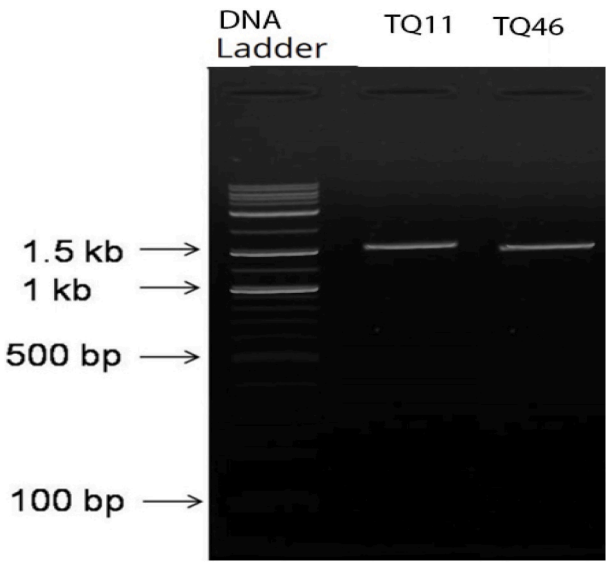

Figure 8. PCR amplification product of bacterial Genomic DNA from selected bacterial isolates (TQ11 and TQ 46). Electrophoresis was performed on a 1.0% Agarose gel stained with gel red.

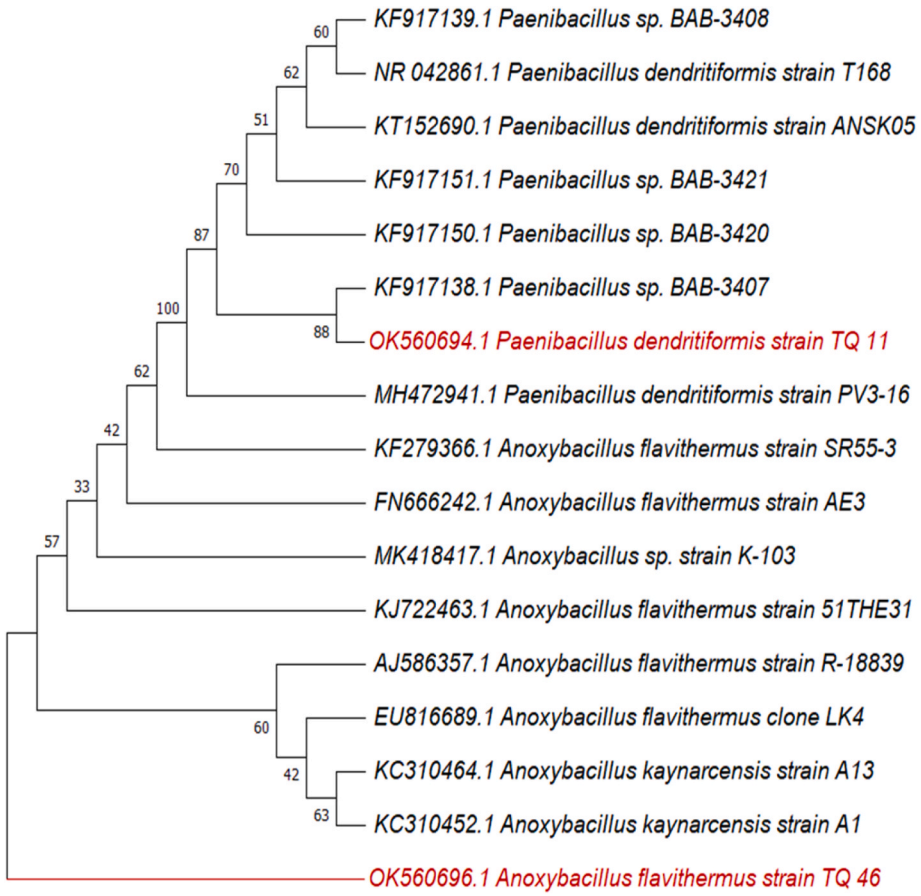

Figure 9. The phylogenetic tree was constructed based on 16S rRNA gene sequences of Anoxybacillus flavithermus (TQ46) and Paenibacillus dendritiformis (TQ11) with other Bacterial species obtained from the NCBI gene bank database.

The E.C. value of the waste soil sample was high, 2100 μs/cm. It indicatesmany dissolved inorganic substances in an ionized form in and around solid waste dumpsites (Ayub and Siddiqui, 2015).

Furthermore, a high E.C. value indicates the presence of pollutants like sulfate and potassium (Nazir et al., 2015). When the average conductivity value in the waste sample was examined, it was found that the soil had a significant amount of iodine. In addition, at least one hydrolase activity was found in the isolated strain's thermostable enzymes cellulase, lipase, amylase, lipase, and protease.

Isolation of primary screening of thermophilic bacteria, the similar work from the Kahrizak landfill of Tehran, a bio-surfactant producing thermophilic bacteria, was isolated (Sharafi et al., 2014). At a constant temperature of 48 °C, the resulting isolate was successfully purified and maintained in a stable growth state. Therefore, the observed isolates are thermophilic bacteria (Simandjuntak and Mokosuli, 2018). Furthermore, the soil waste sample from which thermophilic bacteria was isolated was high and consistedof organic materials; thus, bacteria isolated from such places may have better potential to produce critical industrial compounds under adverse conditions (Harnvoravongchai et al., 2020).

Wasteland soils support numerous types of microorganisms, including cellulolytic, amylolytic, proteolytic, and lipolytic organisms belonging to *Bacillus, Cellulomonas, Cytophaga, Pseudomonas, Sporocytophaga, Streptomyces*, etc. (Yadav et al., 2018). Landfills are an essential source of cellulase-producing bacteria because they accept a variety of home trash with high cellulose content (Uyar et al., 2011). The amylase production was confirmed by flooding the plate with gram iodine solution. Iodine will form blue colouration by reaction with starch (Lakshmi et al., 2020). In another study, 14 amylase-positive thermophilic bacteria were screened out of 33 thermophilic bacteria (Cai et al., 2020). Protease activity is characterized by forming transparent zones around the colony of microorganisms (Masi et al., 2021). From the 19 isolates, eleven isolates were found to be protease-positive thermophilic bacteria from 33 isolates (Cai et al., 2020).

Similarly, three strains that produced alkaline proteases having activities at pH 10.5 and above 70 °C were selected from decaying soil samples (Anpalagan et al., 2020). Many earlier studies have been done to screen and isolate novel bacterial sources for lipase production. Yasar et al. (2020) reported that the five most efficient lipase producer strains were chosen.

Strains that produce no zone are γ-hemolysis, green zones are considered α-hemolysis, clear zones are ß-hemolysis, and only strains with γ-hemolysis are considered safe (Mangia et al., 2019). TQ5 had alpha hemolysis; this type of hemolysis represents a partial decomposition of the haemoglobin of the red blood cells (Ogunshe and Falode, 2021). According to the morphology analysis, both

isolates were Gram-positive and rod-shaped cells. Also, the same result was obtained by Anpalagan et al. (2020) using the *Paenibacillus* area Gram-positive rod. *Bacillus* is a large and diversified genus of rod-shaped, Gram-positive to Gram-variable, endospore-forming, aerobic, and facultatively anaerobic bacteria (Yavuz et al., 2004).

The intrinsic thermostability of thermostable enzymes refers to long shelf life, increased tolerance to organic solvents, decreased risk of microbiological contamination, reduced activity losses during processing even at elevated temperatures, and a potential advantage in pre-treatments of ionizable material (Yadav et al., 2018). *Anoxybacillus flavithermus* and *Paenibacillus dendritiformis* were found in the cultivated microbial community in Qoshe's landfill. The isolated microbes belong to the domain bacteria class bacilli and order *bacillus*. Shahinyan et al. (2017) also investigated *Anoxybacillus flavithermus* strain AK1 was isolated from Armenian geothermal springs. The TQ11 isolate's 16S rRNA gene sequence shared 88%identity with the *Paenibacillus* strain in GenBank. According to Yohandini (2015) rRNA sequence similarity values of less than 95% indicated discovering a new species.

According to several publications, the sequence similarity of 96%–98% indicated the presence of a new or different species. In some reports, the sequence similarity of 96%–98% indicated the presence of a unique or distinct species. *Anoxybacillus spp*. enzymes have been shown to degrade various substrates, including starches, cellulose, lipids, and proteins. In *Anoxybacillus* spp., many carbohydrate-encoding genes have been identified (Grady et al., 2016). In addition, Glucanases, chitinases, cellulases, and proteases produced by soil-dwelling *Paenibacillus* species have been linked to the degradation of eukaryote cell walls (Verma et al., 2014; Ginting et al., 2021).

5. Conclusion

This study established the importance of thermophile bacteria from an Addis Ababa landfill that were probably generating extracellular enzymes (Qoshe). 102 isolates obtained by serial dilution and the spread plate method were purified using the streaking method. The isolates underwent biochemical and morphological characterization. Using primary screening techniques, 35 cellulases, 22 amylases, 17 proteases, and 9 bacteria-generating lipases were discovered. TQ11 and TQ46 were discovered after additional secondary screening employing strain safety evaluation. The inquiry also uncovered a set of potential candidates whose enzyme characteristics and production optimization are still being studied but who could provide a source of thermozymes with significant biotechnological applications. The agricultural, food and beverage, pharmaceutical, detergent, textile, leather, pulp and paper, and biomining industries all have potential commercial uses for the thermophile extremozymes. Extremophile research is very interested in the growth of new industrial processes based on extremozymes and the rising needs of the biotech industries for novel biocatalysts.

Declarations

Author contribution statement

Chandran Masi: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Abel Tebiso: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Selva Kumar K V: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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No data was used for the research described in the article.

Declaration of interest's statement

The authors declare that they have no competing interests.

Additional information

No additional information is available for this paper.

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