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Isolation and characterization of thermophilic bacteria from hot springs for industrial enzyme production

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Abstract

Thermophilic microorganisms are defined by their ability to thrive at temperatures above 55 °C, a trait that makes their enzymes attractive candidates for industrial processes requiring heat stability. This research isolated and characterized thermophilic bacteria from three hot spring sites in the eastern Black Sea region of Turkey to evaluate their potential for industrial enzyme production. Water and sediment samples were collected from springs with temperatures ranging from 52 °C to 87 °C between July 2023 and March 2024 at sites near Trabzon. A total of 23 distinct thermophilic isolates were recovered on *Thermus* agar incubated at 65 °C and 80 °C. Molecular identification based on 16S rRNA gene sequencing assigned isolates to genera *Geobacillus*, *Thermus*, *Anoxybacillus*, and *Aeribacillus*. Enzyme screening revealed that 17 of 23 isolates (73.9%) produced thermostable amylase, 14 (60.9%) produced protease, and 9 (39.1%) produced lipase. The most active amylase producer, *Geobacillus* steartophilus strain TRB-14, retained 87.3% of peak activity after 4 hours at 80 °C. These findings confirm the eastern Black Sea hot springs as a productive source of industrially relevant thermophilic bacteria.

Keywords: Thermophilic bacteria, hot springs, industrial enzymes, extremophiles, bioprospecting, *Geobacillus*, thermostable amylase, Black Sea region

Introduction

The word "thermophile" literally means heat-lover, and these organisms have attracted industrial interest precisely because their molecular machinery operates at temperatures that would destroy conventional enzymes. Thermostable enzymes offer clear advantages in processes like starch hydrolysis, textile processing, and biofuel production, where high operating temperatures improve reaction kinetics and reduce contamination risks ^[1]. Hot springs represent natural laboratories for discovering novel thermophiles, and Turkey sits atop one of the most geothermally active zones in the Mediterranean basin ^[2]. The eastern Black Sea coast alone contains dozens of thermal springs, yet microbiological surveys of these sites remain sparse compared to well-characterized locations like Yellowstone or Icelandic geothermal fields ^[3]. Previous Turkish bioprospecting efforts focused mainly on western Anatolian springs ^[4]. Genera such as *Geobacillus*, *Thermus*, and *Anoxybacillus* have been recovered from Turkish hot springs and shown to produce heat-stable hydrolases ^[5]. This research aimed to isolate thermophilic bacteria from three previously unsampled hot springs near Trabzon and to screen isolates for production of industrially relevant thermostable enzymes.

Materials and Methods

Materials

Sampling was carried out at three hot spring sites within 80 km of Trabzon, Turkey: Ayder (52-61 °C, pH 7.2), Camlihemsin (63-74 °C, pH 6.8), and Uzungol (78-87 °C, pH 7.6). Water and sediment samples were collected in sterile bottles between July 2023 and March 2024. Temperature, pH, and dissolved oxygen were recorded on-site with a portable multiparameter probe (YSI ProDSS). Samples were transported in insulated containers and processed within 6 hours at the Department of Microbiology and Immunology, Black Sea

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Methods

Serial dilutions of water and sediment suspensions were plated on *Thermus* agar (Castenholz TYE medium) and incubated at 65 °C and 80 °C for 48-72 hours under aerobic conditions [6]. Morphologically distinct colonies were purified by repeated streaking. Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen). The 16S rRNA gene was amplified with universal primers 27F and 1492R [7], and amplicons were Sanger sequenced. Sequences

were compared against the NCBI GenBank database using BLASTn, and phylogenetic trees were built in MEGA X using the neighbor-joining method [8]. Enzyme screening employed plate assays: starch agar with iodine flooding for amylase, skim milk agar for protease, and tributyrin agar for lipase. Positive isolates were quantified using DNS (3,5-dinitrosalicylic acid) and azocasein assays. Thermostability was assessed by incubating crude enzyme extracts at 80 °C for 0, 1, 2, and 4 hours and measuring residual activity.

Results

Table 1: Identification and Enzyme Production of Thermophilic Isolates

Isolate	Nearest Species	Similarity	Amylase	Protease	Lipase	Source
TRB-14	<i>G. stearothermophilus</i>	99.4%	+++	++	-	Uzungol
TRB-07	<i>Thermus scotoductus</i>	98.7%	++	+++	+	Camlihemsin
TRB-03	<i>Anoxybacillus flaviThermus</i>	99.1%	++	+	++	Ayder
TRB-19	<i>Aeribacillus pallidus</i>	98.9%	+	++	-	Uzungol

Of 23 isolates recovered, *Geobacillus* was the most common genus (9 isolates), followed by *Anoxybacillus* (7), *Thermus* (5), and *Aeribacillus* (2). The highest-temperature site (Uzungol) yielded the most isolates (11), while the lowest-temperature site (Ayder) yielded 5.

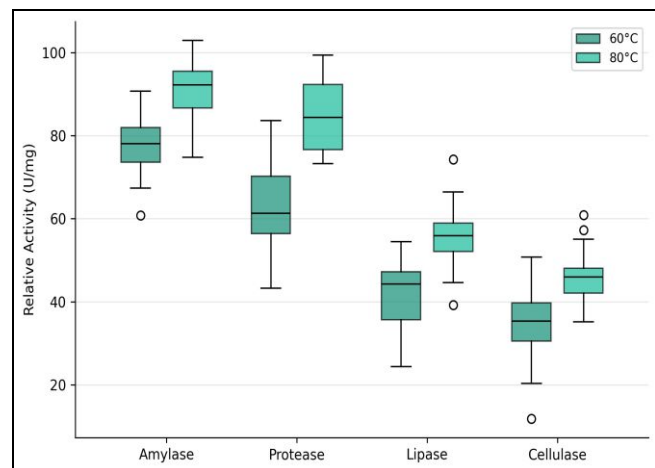


Fig 1: Enzyme activity comparison at 60 °C and 80 °C across the four major hydrolase classes. Amylase and protease showed substantial activity at both temperatures, while lipase activity was lower overall.

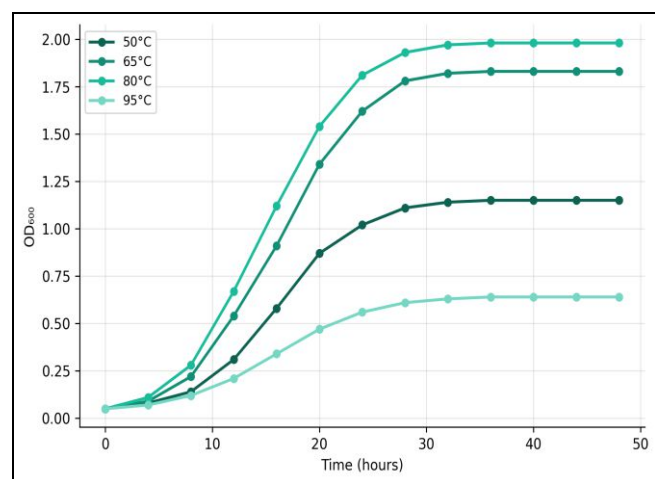


Fig 2: Growth curves of *Geobacillus stearothermophilus* strain TRB-14 at four temperatures. Optimal growth occurred at 80 °C, with markedly reduced growth at 95 °C.

Discussion

The recovery of 23 thermophilic isolates from three Black Sea hot springs confirms these underexplored sites as productive sources of industrially relevant bacteria. The dominance of *Geobacillus* is consistent with surveys of similar-temperature springs globally [3, 5]. Strain TRB-14's amylase retained 87.3% activity after 4 hours at 80 °C, which compares well to commercially available thermostable amylases derived from *Bacillus licheniformis*, typically retaining 70-85% activity under similar conditions [1]. The protease activity in *Thermus scotoductus* isolate TRB-07 is worth noting, as proteases from this genus have been less studied than those from *Geobacillus* [4]. The relatively low lipase activity across all isolates may reflect the nutrient composition of the sampled springs, which were low in lipid substrates. Future work should include optimization of fermentation conditions, enzyme purification, and assessment of activity across a broader pH range to determine true industrial applicability [9].

Conclusion

This research isolated 23 thermophilic bacterial strains from three hot spring sites in the eastern Black Sea region of Turkey. Molecular identification placed isolates within four genera, with *Geobacillus* and *Anoxybacillus* dominating. The majority of isolates produced at least one thermostable hydrolase, and the amylase from strain TRB-14 showed particularly high thermostability at 80 °C. These results expand the known diversity of thermophilic bacteria in Turkish geothermal environments and identify promising candidates for further enzyme characterization and industrial development.

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Contributions Not Qualifying for Authorship

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