Journal of Tropical Biodiversity and Biotechnology
Volume 10, Issue 03 (2025): jtbb19548
DOI: 10.22146/jtbb.19548

Review Article

Diversity of Thermophilic Bacteria Isolated from Extreme Environments in Indonesia: A Perspective in Biotechnology Applications

Dyah Wulandari[1,5]*, Anto Budiharjo[2,5], Aurora Awalia Kirana Putri[4], R. Haryo Bimo Setiarto[3]
1)Department of Food Technology, Faculty of Agricultural Technology, Soegijapranata Catholic University, Jl. Rm. Hadisoebeno Sosro Wardoyo, Mijen, Semarang, Central Java, Indonesia, 50215
2)Biotechnology Study Program, Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang, Central Java, Indonesia, 50275
3)Research Center for Applied Microbiology, National Research and Innovation Agency (BRIN), Jl. Raya Jakarta-Bogor KM. 46, KST Soekarno, Cibinong, Bogor, Indonesia,16911
4)Molecular and Applied Microbiology Laboratory, Center of Research and Services, Diponegoro University, Jl. Prof. H. Soedarto, S.H., Tembalang, Semarang, Central Java, Indonesia, 50275
5)Research Collaboration Center for Thermophilic Enzyme Jl. Prof. H. Soedarto, S.H., Tembalang, Semarang, Central Java, Indonesia, 50275
* Corresponding author, email: dyahwulandari@unika.ac.id

Keywords:
Indonesian hot springs
Enzymes
Thermophilic bacteria
Thermostable enzymes
Geothermal biotechnology
Microbial diversity
Submitted:
24 January 2025
Accepted:
23 April 2025
Published:
12 September 2025
Editors:
Miftahul Ilmi
Liya Audinah

ABSTRACT

Indonesia, located along the Pacific Ring of Fire, hosts abundant geothermal sites and hot springs, creating ideal environments for thermophilic bacteria, which are microorganisms capable of thriving at elevated temperatures. These bacteria are recognized for producing thermostable enzymes, including amylases, proteases, cellulases, xylanases, and lipases, which are highly valuable for various industrial applications. This review compiles and analyses the diversity of thermophilic bacteria isolated from 13 geothermal locations across Indonesia, highlighting their enzymatic capabilities and potential applications in biotechnology. Notable genera include Bacillus, Geobacillus, Pseudomonas, Anoxybacillus, and Thermoanaerobacterium. These isolates demonstrate promising roles in bioenergy production, waste treatment, environmental bioremediation, food processing, agriculture, and pharmaceuticals. Additionally, several strains exhibit the capacity to produce bioactive compounds such as antimicrobial agents and natural pigments. The review also details standardized screening methods using selective solid media and outlines molecular identification techniques, including 16S rRNA gene sequencing and whole genome sequencing. Furthermore, it explores recombinant enzyme technologies applied to thermophiles, enabling enhanced expression, activity, and thermal stability of enzymes for industrial processes. Despite Indonesia's extensive geothermal resources, its microbial biodiversity remains largely untapped. This review not only serves as a scientific inventory of thermophilic strains but also emphasizes their relevance for biotechnological innovations. It aims to support future research, bioprospecting strategies, and industrial applications based on Indonesia's unique thermophilic microbial diversity, ultimately contributing to sustainable technological advancement and resource utilisation.

How to cite:
Wulandari, D. et al., 2025. Diversity of Thermophilic Bacteria Isolated from Extreme Environments in Indonesia: A Perspective in Biotechnology Applications. *Journal of Tropical Biodiversity and Biotechnology*, 10(3), jtbb19548. doi: 10.22146/jtbb.19548

INTRODUCTION

Indonesia, an archipelagic country with more than 17,000 islands along the Pacific Ring of Fire, is one of the most geologically active regions in the world. Around 40 % of Indonesia's geothermal energy is in its territory, which opens up great potential for utilizing and exploring geothermal energy sources (Masum & Akbar 2019). With more than 125 active volcanoes, over 250 hot springs, and extensive geothermal sites, Indonesia presents an exceptional opportunity for the exploration of thermophilic microorganisms. These extreme environmental conditions create ideal habitats for thermophilic bacteria, which can adapt to extreme temperatures, acidity, and mineral concentrations (Alam et al. 2013). Several regions in Indonesia, such as Java, Bali, and Sumatra, are known for their geothermal and volcanic activity. The Dieng Plateau in Central Java, for instance, is a well-known hotspot for thermal springs and acidic lakes, making it a prime location for thermophilic bacteria isolation. Similarly, the Kawah Ijen crater in East Java, with its sulfuric hot springs, provides another extreme environment where thermophiles are found in abundance (Ardhi et al. 2020). These extreme environments ranging from high-temperature sulfuric springs to volcanic soils, host diverse thermophilic bacteria capable of producing thermostable enzymes and bioactive compounds with significant industrial value. The Map and Pictures of Indonesian Hotsprings which had been investigated in this study is depicted in the Figure 1 and 2.

Thermophilic bacteria are a group of extremophilic microorganisms that thrive in high-temperature environments, typically ranging from 45 °C to over 80 °C (Benammar et al. 2020). Due to their unique adaptations, thermophilic bacteria produce enzymes known as thermozymes, such as proteases, cellulases, amylases, xylanases, chitinases, and lipases, which maintain catalytic activity under high temperatures (Zeldes et al. 2015; Ovando-Chacon et al. 2020). These enzymes exhibit exceptional thermal stability, resistance to chemical denaturation, and extended shelf life, making them valuable in industrial applications including biofuel production, waste treatment, food processing, biomining, and environmental bioremediation (Mawati et al. 2021). In the pharmaceutical sector, enzymes such as gelatinases derived from thermophiles are used for drug delivery systems and as antimicrobial agents (Mohammad et al. 2017).

The study of thermophilic microbes began in 1953 with the discovery of Taq polymerase from *Thermus aquaticus*, which became essential for the widely used PCR technique in molecular biology (Lischer et al. 2020). Until now, there have been many discoveries of new species of thermophilic microbes isolated from extreme areas, especially geothermal resources areas, generally around volcanoes. Identification and exploration of thermophilic microbes will open an understanding of the mechanism of adaptation of microbes to extreme environments, genetic traits, and potential metabolites produced (Schultz et al. 2022). The discovery of thermophilic microbes is certainly capable of being a new solution and encouragement for the fields of molecular, industrial, environmental, food, agricultural, and other fields related to microbial metabolites.

Recent studies have emphasized the importance of exploring thermophilic bacteria as sources of bioactive metabolites, enzymes, pigments, and vitamins with industrial potential (Zhu et al. 2020). Genetic engineering further enhances the potential of these organisms by enabling the optimization of enzyme yields and functionality (Thakur et al. 2022). In Indonesia, the widespread presence of geothermal features offers vast opportunities for the discovery and utilization of novel thermophilic microbes (Ifandi & Alwi 2015). Previous research on thermophilic bacteria has largely focused on well-known global geothermal sites, while studies specific to Indonesia have been sporadic and regionally limited. Despite its rich geothermal diversity, much of Indonesia's microbial potential remains underexplored.

The purpose of this review is to comprehensively compile and analyse the diversity of thermophilic bacteria isolated from extreme environments across Indonesia, particularly from volcanic and geothermal sites. This paper also evaluates their enzymatic potential, biotechnological applications, and outlines standardized screening methods. The unique contribution of this manuscript lies in its extensive geographic coverage across 13 Indonesian hot springs and its detailed correlation between bacterial species, enzyme types, and industrial relevance (Figure 3). This review serves not only as a scientific inventory but also as a strategic reference for future bioprospecting and enzyme-based innovation from Indonesian thermophiles.

THERMOPHILIC BACTERIA: CHARACTERISTICS AND IMPORTANCE

Thermophilic bacteria, thriving in high-temperature environments, play a crucial role in various biotechnological applications. Their ability to withstand extreme temperatures makes them valuable for industrial enzyme production, waste degradation, and biofuel generation (Ching et al. 2022). Ther-

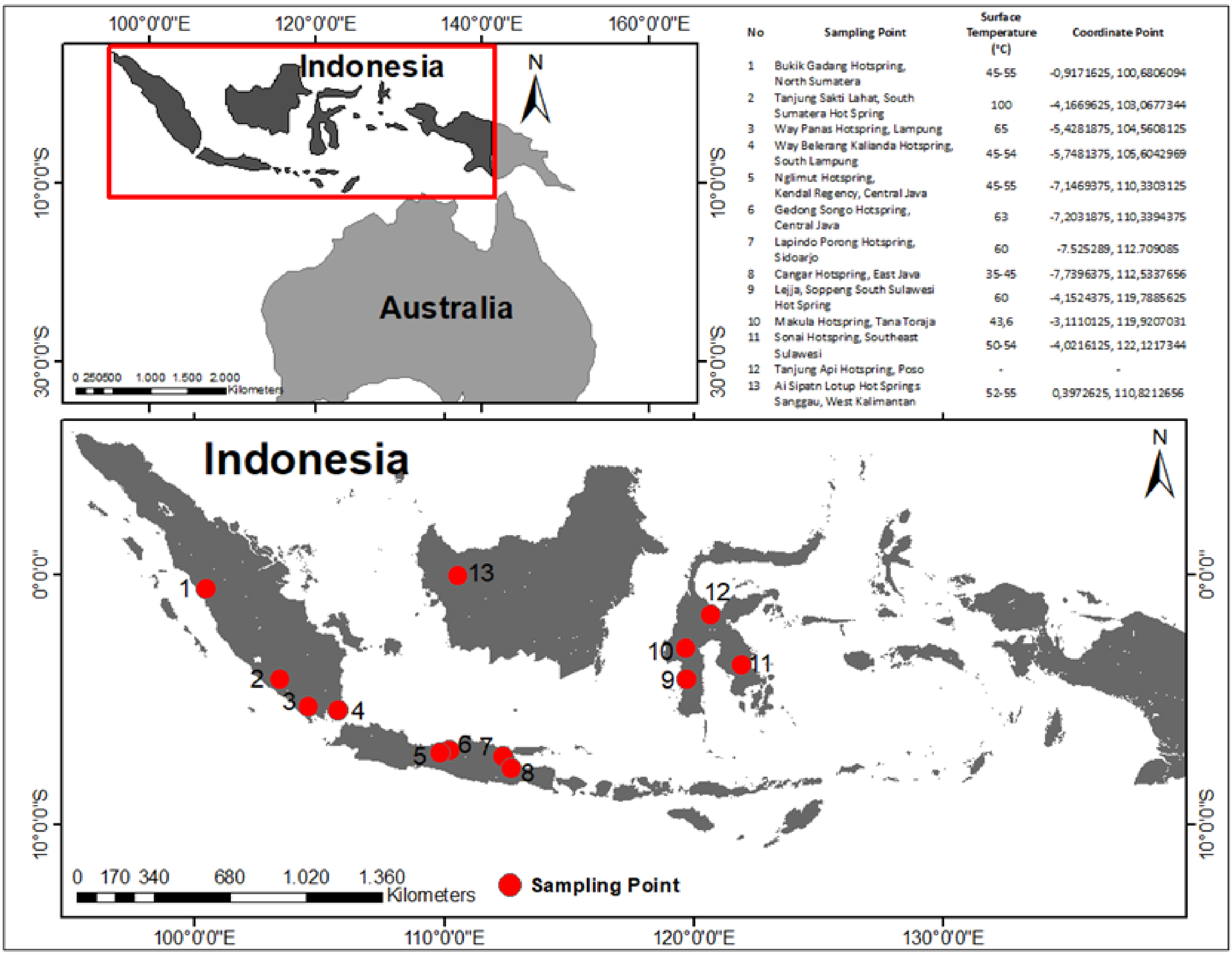

Figure 1. The Map of Indonesian Hotsprings which had been investigated in this study. Bukit Gadang Hotspring, North Sumatera (1). Tanjung Sakti Lahat, South Sumatera Hot Spring (2). Way Panas Hotspring, Lampung (3). Way Belerang Kalianda Hotspring, South Lampung (4). Nglimut Hotspring, Kendal Regency, Central Java (5). Gedong Songo Hotspring, Central Java (6). Lapindo Porong Hotspring, Sidoarjo (7). Cangar Hotspring, East Java (8). Lejja, Soppeng South Sulawesi Hot Spring (9). Makula Hotspring, Tana Toraja (10). Sonai Hotspring, Southeast Sulawesi (11). Tanjung Api Hotspring, Poso (12). Ai Sipatn Lotup Hot Springs Sanggau, West Kalimantan (13). The range of temperature were around 35-100 °C. Indonesia has more than 250 hotsprings, more than 265 geothermal sites and more than 125 active volcanoes. However, most of them still underexplored.

Figure 2. The pictures of Indonesian Hotsprings which had been investigated in this study (a-m).

Figure 2. Contd.

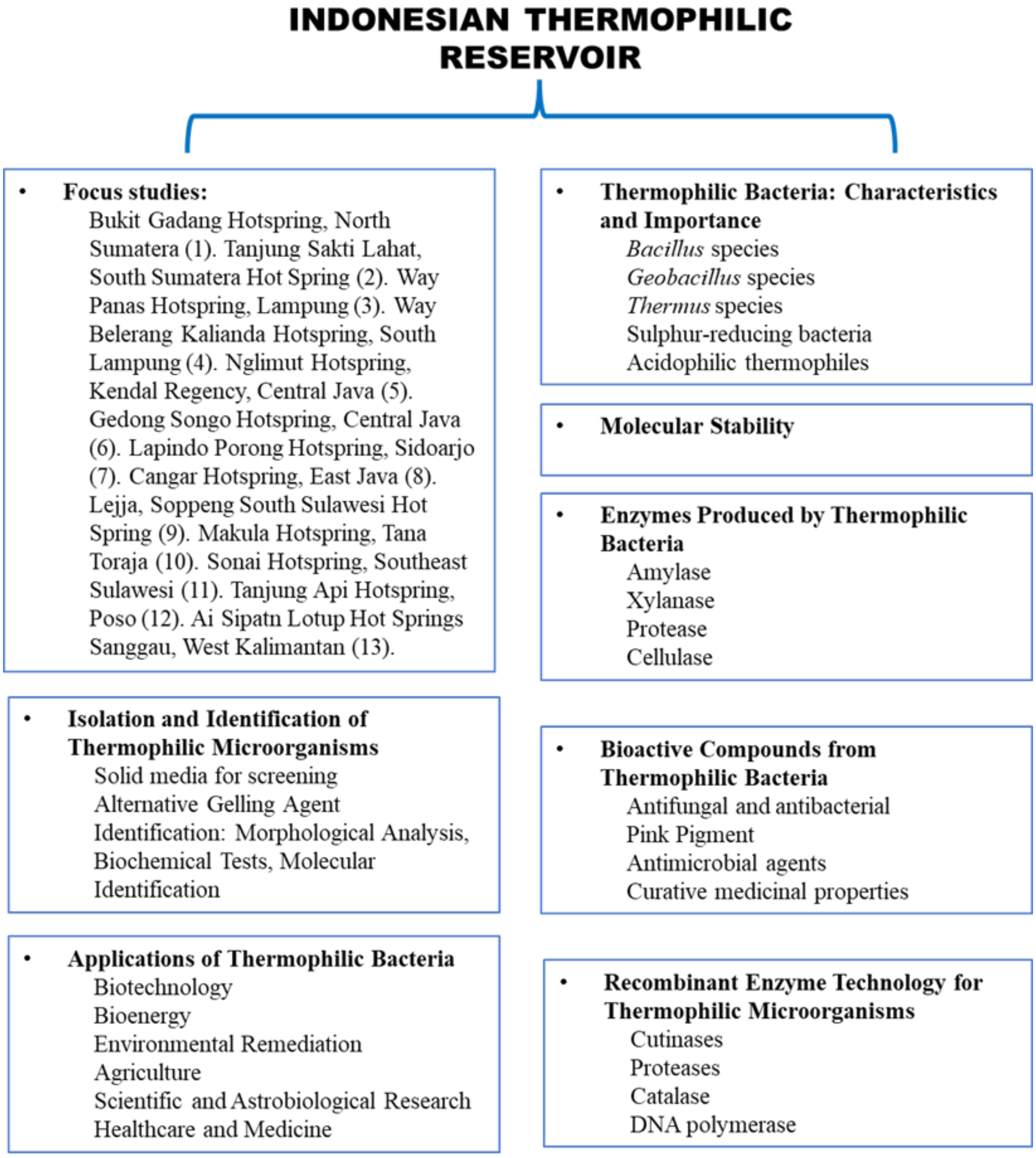

Figure 3. Schematic overview of the potential of Indonesian thermophilic reservoirs and their relevance for biotechnology. The left panel represents diverse extreme environments in Indonesia that harbor thermophilic bacteria, while the right panel outlines key application fields discussed in this review, including enzyme discovery, bioenergy, bioremediation, and other industrial uses.

mophilic bacteria survive and grow at temperatures unfavourable to most organisms by possessing specialized enzymes, proteins, and cellular structures that ensure stability and function at elevated temperatures. Many thermophiles also have unique metabolic pathways that enable them to utilize a wide range of substrates, including complex organic compounds. The significance of thermophilic bacteria extends beyond their ecological roles. They produce thermostable enzymes, such as amylases (Silaban et al. 2021; Soy et al. 2021; Widiana et al. 2022), cellulases (Fachrial et al. 2020; Budiharjo et al. 2024), proteases (Fachrial et al. 2020; Sabaria et al. 2024), lipases (Fang et al. 2021; Sürmeli et al. 2024), and DNA polymerases (Murtiyaningsih et al. 2022; Agustriana et al. 2023), which are valuable in industrial applications.

In addition, thermophilic bacteria have shown promise in the bioremediation of contaminated environments (Rakhmawati et al. 2021; Chen et al. 2021; Peng et al. 2024; Patil et al. 2024), the production of biofuels (Irdawati et al. 2023; Dai et al. 2023; Altinok et al. 2023), and the synthesis of various biochemicals (Özdemir et al. 2022; Marin-Sanhueza et al. 2022; Klein et al. 2023). The ongoing exploration of thermophilic bacteria in extreme environments is expected to yield new strains with novel properties.

Recent studies have revealed a rich diversity of thermophilic bacteria isolated from extreme environments across Indonesia. These bacteria belong to several different groups, including Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes. Some notable thermophilic strains found in Indonesia include:

Bacillus species: Several strains of *Bacillus* spp. have been isolated from hot springs and geothermal sites in Indonesia. These bacteria are known for producing thermostable enzymes, making them highly valuable for industrial processes. Some *Bacillus* strains from Indonesian geothermal areas have shown the ability to degrade complex organic compounds, such as lignocellulosic materials, which are important for biofuel production.

Geobacillus species: *Geobacillus* spp., thermophilic bacteria belonging to the family Bacillaceae, have been isolated from various geothermal habitats in Indonesia. These bacteria thrive at temperatures ranging from 50 °C to 75 °C and are notable for their ability to produce thermostable enzymes involved in carbohydrate and protein hydrolysis.

Thermus species: *Thermus* spp., which are members of the order Thermales, are another common group of thermophilic bacteria found in Indonesia. These bacteria are typically found in hot springs and are used in various biotechnological applications, particularly in the development of polymerase chain reaction (PCR) technologies.

Sulphur-reducing bacteria: In highly acidic and sulphur-rich environments, such as the Kawah Ijen crater, sulphur-reducing thermophilic bacteria play a significant role in sulphur metabolism. These bacteria can reduce sulphate to hydrogen sulphide, contributing to the sulphur cycle in these extreme environments.

Acidophilic thermophiles: Indonesia's hot springs, such as those found in the Dieng Plateau, often have highly acidic conditions that are favourable for acidophilic thermophiles. These bacteria can survive in environments with pH levels as low as 2.0-3.0, and some of these strains have demonstrated the ability to solubilize minerals, which can be beneficial for bioleaching processes in mining industries. Moreover, the distinctive features of Indonesian Geothermal environment and their species are described in the Table 1.

ISOLATION AND IDENTIFICATION OF THERMOPHILIC MICROORGANISMS

The process of isolating thermophilic bacteria from extreme environments typically involves collecting samples from high-temperature habitats such as hot springs, geothermal vents, or volcanic soils. Various growth media are used to isolate a wide range of thermophilic bacteria, including those that may require specific nutrients or conditions for optimal growth. Table 2 shows the requirements of the media for screening the thermophilic bacteria.

The alternative of Gelling Agent for the thermophilic microorganisms including Agar, consists of Linear polysaccharide of agarose and agaropectin. Agar forms a clear gel, that is stable over a wide temperature range and melts at 85 °C, making it suitable for growing mesophiles (McLachlan 1985; Becker et al. 1998). Gellan gum, forms a clear gel and consists of tetrasaccharide of two D-Glucose, L-rhamnose, and D glucoronic acid. Gellan gum can be used for growing thermophiles and it can be melted at 110 °C (Kang et al. 1982; Kuo et al. 2014). Xanthan gum, consists of pentasaccharide of two glucose, two mannose and glucuronic acid. Xanthan gum is stable over a wide range of temperature and pH levels. It can be used for growing various fungi and bacteria and melts at 270 °C (Babbar & Jain 2006; Prajapati et al. 2013). Guar gum, consists of galactomannan (galactose and mannose), forms a cloudy gel and can be used for growing various fungi and bacteria. It can be melted at 220 °C (Shimomura & Kamada 1986; Jain et al. 2005). Isubgol, consists of Xylose, arabinose, galacturonic acid, and traces of rhamnose and galactose. It can

Table 1. Comparative Study of Global Thermophilic Microbial Diversity vs. Indonesia.

Country / Region	Geothermal Environment	Dominant Thermophilic Species	Distinctive Features & Recent Findings	References
USA (Yellowstone)	Alkaline geysers, high-temp springs	*Thermus aquaticus*, *Sulfolobus acidocaldarius*, *Desulfurococcus* spp.	Home of Taq polymerase, revolutionized PCR; dominated by archaea; well-characterized enzyme systems.	Brock & Freeze 1969; Stetter 1996; Madigan et al. 1997
Iceland	Sulfuric hot springs, fumaroles	*Thermoproteus tenax*, *Pyrobaculum* spp., *Thermococcus litoralis*	Cold-climate geothermal systems with high microbial activity; emphasis on biohydrogen and biogas pathways.	Kristjánsson & Hreggvidsson 1995; Hreggvidsson et al. 2012
Japan (Kusatsu, Beppu)	Acidic and neutral thermal springs	*Thermus thermophilus*, *Geobacillus kaustophilus*, *Sulfolobus tokodaii*	Applications in polymerase enzymes and thermostable hydrolases; culture collections well curated.	Sako et al. 1996; Atomi et al. 2004
India (Manikaran, Bakreshwar)	Hot springs, volcanic zones Surajkund Hotsprings	*Geobacillus thermoleovorans*, *Bacillus licheniformis*, *Anoxybacillus flavithermus* Cultured & Uncultured (data metagenomic)	Diverse genera with enzyme production capacity, including cellulases and amylases for industrial starch processing. Diverse genera with enzyme production amylase, xylanase, and cellulase.	Pandey et al. 2015; Verma et al. 2020; Soy et al. 2023
Indonesia	Volcanic craters, acidic lakes, sulfur vents Angseri, Banjar, and Batur Hotspring Likupang Marine Hydrothermal, North Sulawesi	*Bacillus licheniformis*, *Geobacillus stearothermophilus*, *Pseudomonas stutzeri*, *Thermoanaerobacterium* spp., *Anoxybacillus* spp. undetected thermophilic taxa (data metagenomic) *Geobacillus thermoleovorans*; *Bacillus caldotenax*	Rich biodiversity; isolates from >250 hot springs; high potential in amylase, protease, xylanase, and catalase production. Novel strains reported in 2024 from Central Java, Sulawesi, Sumatra, Bali. Amylase producing bacteria	Saksono & Sukmarini 2010; Ifandi & Alwi 2015; Indriati & Megahati 2018; Ardhi et al. 2020; Ginting et al. 2021; Budiharjo et al. 2024; Wirajana et al. 2024
Malaysia	Poring Hot Spring Sabah, Malaysia	*Anoxybacillus flavithermus*	Potential for amylase-producing bacteria	Fazal et al. 2022
Turkey	Golan hot springs in Karakocan, Elazig.	*Bacillus*, *Geobacillus*, and *Thermomonas*	Heated groundwater, Diverse genera with enzyme production: cellulases and amylases.	Yildiz 2024
Russia	Kuril Island, Kunashir and Iturup Islands	*Sulfurihydrogenibium* and *Hydrogenobacter* sp., *Acidithiobacillus*, *Hydrogenobaculum* and Thiomonas Chloroflexota, Leptolyngbyaceae, and Oculatellaceae families	Shallow and terrestrial hot springs (pH 5.7–8.5 and temperature 40–79 °C)	Karaseva et al. 2024

Table 2. The Solid Media for screening the thermophilic microbes.

Source	Media	Temp (°C)	References
Halophilic Bacteria	Nutrient Agar (NA) (Difco, Sparks, MD, USA), Reasoner's 2A agar (R2A agar) (Difco, Sparks, MD, USA) Tryptic Soy Agar (TSA) (Difco, Sparks, MD, USA). + NaCl (w v^{-1}) concentration at 3-15 %	45-65	Lee et al. 2022
Amylase Production Bacteria	NA/R2A/TSA + 0.2 % (w v^{-1}) soluble starch (Difco, USA)	50-60	Cowan 1994; Marteinsson et al. 1996; Mahestri et al. 2021
Lipase Production Bacteria	NA/R2A/TSA + 1 % (v v^{-1}) Tween 80 (Sigma, St. Louis, MO, USA)	60	Rollof et al. 1987; Mohammad et al. 2017
Protease Production Bacteria	NA/R2A/TSA + 2 % (w v^{-1}) skim milk agar (Difco, Sparks, MD, USA)	60	Burke et al. 1991; Panda et al. 2013; Mahestri et al. 2021
Bacteria	Thermus medium (composition: 0.5 % NaCl, 0.5 % peptone, 0.4 % beef extract, 0.2 % yeast extract, and 2.0 % agar)	45-70	Welday et al. 2014
Cellulase Production Bacteria	NA + 1 % carboxymethyl cellulose	45-55	Mohammad et al. 2017; Budiharjo et al. 2024
Fungi	Nutrient Agar (NA), (Kenknight and Munaiers Agar, Potato Dextrose Agar (PDA), Tryptone Soya Agar (TSA), Pikovskaya Agar, and King's B Base.	30-80	Verma et al. 2018
Xylanase Production Bacteria	NA + 0.5-1 % Beechwood Xylan	50-60	Ahirwar et al. 2017; Irdawati et al. 2018
Bacteria	Nutrient Agar (NA), Tryptic Soy Agar (TSA), International Streptomyces Project medium No.2 (ISPII) containing glucose at 4 g L^{-1}; yeast extract at 4 g L^{-1}; and malt extract at 10 g L^{-1}	45	Rafiee et al. 2024
Thermophilic Fungi	Yeast Extract soluble starch agar (YpSs) medium (composition: starch, 15.0 g L^{-1}; magnesium sulphate, 1.0 g/l; dipotassium hydrogen phosphate, 1.0 g L^{-1} and yeast extract 4.0 g L^{-1}	45	Ahirwar et al. 2017
Mannanase production Fungi	Agar (YpSs) medium + 0.5 % mannan LBG	50	Ahirwar et al. 2017
Halophilic Archaea	High Salt Medium (composition: Peptone 10 g L^{-1}; $MgSO_4$-$7H_2O$ 2 g L^{-1}; KC1 2 g L^{-1} $CaCl_2$ 2 g L^{-1} $FeSO_4$-$7H_2O$ 0.005 g L^{-1} $MnCl_2$-$4H_2O$ 0.002 g L^{-1} NaCl 250 g L^{-1}	70	Hamana & Matsuzaki 1985
Halophilic Archaea	Medium for Halophilic archaea (DFMZ Medium 1184)	35-56	Verma et al. 2020
Haloarchaea	Haloarchaea Phosphate solubilisation Medium (HPS)	25-50	Shirling & Gottlieb 1966; Yadav et al. 2015
Halobacteria	Media for Dead Sea Halobacteria	35-50	Oren 1983
Archaea	Basal Media	20-60	Manikandan et al. 2009
Seawater Archaea	Marine salt (S.W.) containing Basal media	20-60	Manikandan et al. 2009
Archaea	Eimhjellen medium	40	Lizama et al. 2001
Archaea	Sehgal and Gibbons medium	30-50	Payne et al. 1960
Archaea	M.H. medium	30-40	Torreblanca et al. 1986
Archaea	HE medium (Hay extract media)	30-40	Torreblanca et al. 1986
Archaea	Mineral salts medium	40	Mevarech & Werczberger 1985; Cuadros-Orellana et al. 2006
Actinobacteria	International Streptomyces Project (ISP1, ISP2, ISP3) + 0.5-1 % Gellan Gum+$MgCl_2$ Bennett's medium + 0.5-1 % Gellan Gum+$MgCl_2$	45	Jones 1948; Shirling & Gottlieb 1966; Yadav et al. 2015
Deepsea microorganisms	EXP medium + (3.75 % w v^{-1} Gelrite (Phytagel, Sigma P8169, Sigma Chemical Corp., St. Louis, Mo.)	65-80	Sari et al. 2020

be melted at >100 °C (Sahay 1999; Jain 2011). Carrageenan consists of d-galactose and 3, 6-anhydro-galactose joined by a-1, 3 and B-1,4-glycosidic linkage. It can be used for growing the alkaliphiles and can be melted at 80 °C (Lines 1977; Das et al. 2015).

Once the thermophilic microbes have isolated from various hot springs and geothermal sites, it can be further assessed and identified by molecular techniques. Molecular techniques, such as polymerase chain reaction (PCR) and 16S rRNA gene sequencing, have revolutionized the identification of thermophilic bacteria. These methods allow for precise identification of bacterial species based on their genetic makeup, providing insight into the phylogenetic diversity of the bacteria found in extreme environments. Additionally, the use of culture-independent techniques like metagenomics has further enhanced our understanding of microbial diversity in such habitats.

The isolation and identification of thermophilic bacteria are vital for harnessing their unique properties for scientific and industrial advancements. Their resilience and versatility offer immense opportunities for innovation across multiple domains. By overcoming current challenges and expanding research, thermophilic bacteria can play a pivotal role in shaping a sustainable and technologically advanced future.

APPLICATIONS OF THERMOPHILIC BACTERIA

Thermophilic bacteria have garnered significant attention for their diverse applications across industries and scientific research. These microorganisms produce thermostable enzymes and metabolites that function efficiently under extreme conditions, making them indispensable for various biotechnological, industrial, and environmental processes (Habibie et al. 2014). The diverse thermophilic bacteria found in Indonesia have potential applications in several fields:

a. Biotechnology: The enzymes produced by thermophiles, such as amylases, cellulases, proteases, lipases, chitinases, esterases, laccases, polymerases, and other enzymes are highly stable at elevated temperatures. Thermophilic enzymes, especially those produced by *Bacillus* (Zalma et al. 2021; Jeyabalan et al. 2025) and *Geobacillus* (Soy et al. 2021; Widiana et al. 2022; Özdemir et al. 2022; Agustriana et al. 2023; Sürmeli et al. 2024) species, are widely used in industrial processes. For example, thermostable amylases and cellulases are crucial for the production of biofuels from plant biomass. Additionally, thermostable proteases are used in laundry detergents, leather processing, and food industries.

b. Bioenergy: Thermophilic bacteria play a crucial role in the degradation of organic waste materials, which is an important step in the production of biogas, biodiesel, and biofuels (Irdawati et al. 2023; Dai et al. 2023; Altinok et al. 2023; Singh et al. 2025). The enzymatic breakdown of lignocellulose by thermophilic bacteria can help convert plant biomass into renewable energy sources (Silva et al. 2022; Panahi et al. 2022). In anaerobic digesters, thermophilic bacteria enhance the degradation of organic matter to produce methane and other biofuels.

c. Environmental Remediation: Thermophilic bacteria are being studied for their ability to degrade pollutants in high-temperature environments. These bacteria can break down complex organic compounds (Sharma & Leung 2021), including oils (Peng et al. 2024) and pesticides (Yang et al. 2020), in extreme conditions. Furthermore, sulfur-reducing thermophiles are being explored for use in the remediation of sulfur-contaminated environments (Frolov et al. 2018; Allioux et al. 2022), such as those found near industrial sites. Thermophilic bacteria accelerate the decomposition of organic waste into nutrient-rich compost (López et al. 2021; Zhang, J. et al. 2024). They thrive in the heat generated during composting, breaking down complex organic compounds into simpler forms (Li et al. 2023). Thermophiles enhance the breakdown of organic pollutants in wastewater at high temperatures, making treatment processes more efficient (Pugazhendi et al. 2017; Baker et al. 2021; Aragaw et al. 2022). Their heat-tolerant nature reduces the risk of contamination by pathogenic microorganisms.

d. Agriculture: The enzymes produced by thermophilic bacteria can also be used in agricultural processes, such as composting. They accelerate the breakdown of organic matter, leading to more efficient nutrient recycling (Zhu et al. 2021; Zhang, J. et al. 2024). Thermophilic bacteria produce compounds that act as biopesticides, offering eco-friendly alternatives to chemical pesticides. They help fix nitrogen (Nishihara et al. 2018; Kato et al. 2018) and decompose organic matter (Cao et al. 2019; López et al. 2021), enriching soil fertility.
e. Scientific and Astrobiological Research: Thermophilic bacteria are valuable models for studying life's adaptability to extreme conditions, offering insights into evolutionary biology (Alexandraki et al. 2019) and the potential for extraterrestrial life (von Hegner 2020). The study of thermophiles reveals how organisms adapt to extreme environments through specialized proteins, enzymes, and membrane structures. Thermophilic bacteria serve as analogs for potential extraterrestrial life forms that might exist on planets or moons with harsh environments, such as Mars or Europa (Carré et al. 2022). Thermophiles are used to engineer novel genetic circuits and metabolic pathways, creating organisms with enhanced functionalities for industrial applications (Finch & Kim 2018; Kong et al. 2022).
f. Healthcare and Medicine: Thermophilic bacteria and their products have made ground-breaking contributions to healthcare and diagnostics. They produce thermostable enzymes like Taq polymerase, which is crucial for the polymerase chain reaction (PCR) (Lischer et al. 2020), a widely used technique in genetic testing, medical diagnostics, and forensic science. Thermophilic bacteria are also a rich source of novel bioactive compounds with potential therapeutic applications. Thermophiles produce unique antibiotics that can combat resistant pathogens (Alrumman et al. 2019; Octarya et al. 2022). Some thermophilic bacteria produce secondary metabolites with anticancer properties (Obeidat & Al-Shomali 2023; Satarzadeh et al. 2024). Thermophilic bacteria-derived enzymes are used in vaccine production processes, particularly for stabilizing and enhancing the delivery of vaccines (Liu, H. et al. 2023).

MOLECULAR STABILITY AND IDENTIFICATION OF THERMOPHILIC BACTERIA

To evaluate their potential in industrial applications, thermophilic microbes require precise and efficient identification methods. Three commonly used approaches for identifying thermophilic bacteria include culture-based and morphological identification, 16S rRNA gene sequencing, and whole genome sequencing. Each of these methods provides unique insights and levels of resolution, and they are often used in a complementary manner.

Initial identification of thermophiles typically begins with bacterial culture and morphological observations, which offer a straightforward and cost-effective screening method (Lischer 2021). This process involves cultivating isolates on nutrient-rich media that imitate their natural high-temperature environmental conditions. Optimizing bacterial growth in a medium can be done by engineering the carbon source, agitation speed, substrate concentration, and substrate stability (Indriati & Megahati 2018). However, designing appropriate solid media for thermophiles poses a challenge. Standard agar solidifies well at temperatures below 65–70 °C, but begin to lose integrity at higher temperatures. To address the challenge, a thermally stable medium incorporating polypropylene was developed in 1997, enabling bacterial cultivation at 80–84 °C for up to 24 hours. Pre-incubating the media at 35–40 °C further enhances its durability (D'Souza et al. 1997). Furthermore, solid media are essential not only for bacteria isolation, but also for assessing enzyme production (Sharma et al. 2019).

While culture-based methods provide phenotypic information, molecular techniques offer greater resolution. The creation of 3-domain classification of living things in 1977, pioneered by Carl Woese and colleagues, and the development of 16S rRNA gene sequencing has revolutionized microbial taxonomy by allowing phylogenetic relationships to be inferred from conserved genetic sequences (Koonin 2010). The 16S rRNA gene is particularly useful due to its conserved nature interspersed with hypervariable regions that distinguish different species. The unique sequences, relatively constant properties, and the occurrence of small mutations, making it a superior identification strategy. It also circumvents the limitation of only fewer than 1 % of environmental microbes which are culturable under laboratory conditions. Bioinformatic analysis of 16S rRNA sequences allows accurate bacterial classification down to the species level (Suddin et al. 2019).

Recent advancements have extended this approach to community-level analysis through next-generation sequencing (NGS). For example, MiSeq-based 16S amplicon sequencing, as demonstrated in studies of several hot springs in Sri Lanka, accommodated the characterization of the entire microbial communities as well as predicted their metabolic capabilities. These include nitrogen fixation, ammonia oxidation, methanogenesis, dehalogenation, and the degradation of various pollutants such as aromatic compounds, chlorophenols, atrazines, sulphur, and naphtalenes (Sadeepa et al. 2022). This high-throughput method saves significant amount of time and cost while revealing potential functional traits of thermophilic communities. Advances in sequencing using advanced generations have further opened up knowledge and information about microbial interactions and their abiotic environment, which is advantageous in industrial applications.

For deeper insights into microbial function and adaptation, whole genome sequencing (WGS) provides the most comprehensive data about the base sequence of bacterial thermophiles. Although costlier, WGS allows complete genomic analysis and is often used to confirm species identity and explore adaptive mechanisms (Lischer et al. 2020). For instance, *Parageobacillus caldoxylosilyticus* ER48, originally misidentified as *Geobacillus caldoxylosilyticus*, was reclassified based on genome data obtained using the PacBio RSII platform in 2001, which revealed a 3.9 Mbp genome with a GC content of 44.31 % (Ching et al. 2022). Earlier genomic studies of *Geobacillus caldoxylosilyticus* in 2004 also successfully identified genes involved in thermotolerance, such as prokaryotic protamine P1, polyamine synthase, polyamine ABC transporter, and RNA methylase, which were contained in 839 unique genes. The study shed light on the molecular strategies used by thermophiles to form stable nucleic acids at elevated temperatures (Takami et al. 2004). Whole genome sequencing not only enables the identification and verification of thermophilic bacteria, but also enhances the overall efficiency of exploration by saving time and reducing energy consumption.

ENZYMES PRODUCED BY THERMOPHILIC BACTERIA

Thermophilic microorganisms are attracting great attention in industry because they are difficult to denature, have a longer shelf life, minimize contamination problems, and increase chemical resistance (Drejer et al. 2018). Some microorganisms produce thermostable enzymes such as amylase, cellulase, chitinase, pectinase, xylanase, protease, lipase, and DNA polymerase (Mohammad et al. 2017). Currently, enzymes obtained from microbes are preferred over plants and animals because the ability to produce in large quantities and the ease of engineering to obtain enzymes are desirable characteristics (Drejer et al. 2018).

Amylase

Amylase enzyme isolated from thermophilic bacteria possess thermostable properties, making them suitable for industrial applications requiring high temperatures, for example, in gelatinization, liquefaction, and saccharification processes at high (Mehta & Satyanarayana 2016). Thermostable amylase reduces the risk of contamination and external cooling costs and increases the rate of diffusion (Fossi et al. 2014). Thermophilic alpha-amylase is widely applied in sugar production, brewing, and starch processing (Ullah et al. 2021). Several *Bacillus* species, such as *Geobacillus stearothermophilus* (Al-Qodah 2006; Fincan & Enez 2014), *Bacillus subtilis* (Asgher et al. 2007; Al-Johani et al. 2017), *Bacillus licheniformis* (Shukla & Singh 2015), *Anoxybacillus* (Jabeen et al. 2019; Sharif et al. 2023), and *Bacillus amyloliquefaciens* (Devaraj et al. 2019), are known to produce starch hydrolyzing enzymes.

Hot springs have different physical, chemical, and nutritional properties. This allows for microbial biodiversity, including thermostable bacteria (Chan et al. 2017), which have the potential to produce thermostable enzymes important for industry. Exploration of thermophilic amylase enzyme in Way Panas hot spring, South Lampung, identified isolates of A.WP.50.4 had an inhibition zone of 11.83 mm, which was incubated at 50 °C. After morphological, biochemical, and molecular testing, the bacterial isolate A.WP.50.4 was a species of *Bacillus cereus* (Mahestri et al. 2021). In the same place, research conducted by Mawati et al. (2021) found that out of five isolates, isolate A.WB.50.1 had an inhibition zone diameter of 15.44 mm, which was incubated at 50 °C. Based on BLAST analysis obtained from RNA sequences, the isolate A.WB.50.1 was *Pseudomonas stutzeri* bacteria. Exploration of the hot springs of Hangar, East Java, is known to have amylolytic activity. The isolates were incubated at 50 °C, and they showed a clear zone after being added with iodine. The isolate was suspected to be *Bacillus subtilis* subsp. Inaquosorum. The subspecies inaquosorum also has previously been isolated from the United States, South Korea, and India. The discovery of several stable amylase enzymes at 50 °C can potentially develop as additional enzymes in the manufacture of detergents (Knight et al. 2018).

Simair et al. (2017) proves that strains of *Bacillus* sp. SM 01-05 had the highest enzyme activity in molasses medium for 60 hours at 50 °C and pH 8.0 and showed performance in cleaning-stained fabrics. It has great potential in the detergent industry and saccharification of starchy materials. The search for thermophilic amylase enzymes in Bukit Gadang hot springs, North Sumatra, found that LBKURCC190 isolate produced the largest clear zone (2.78 ± 0.38), which was incubated at 50 °C. The results of 16S rRNA sequencing analysis showed that LBKURCC190 isolates had the highest similarity (>98 %) with *Bacillus licheniformis* found in GenBank (Ardhi et al. 2020). Similar results were carried out by the study of Msarah et al. (2020) on the amylolytic activity of *B. licheniformis* HULUB1 isolated from Dusun Tua Hot Springs, Malaysia. The isolate produced the highest amylase enzyme at pH 6.0, a temperature of 45 °C, after 18 hours of growth. These isolates can be used as bioremediation for food waste treatment because they can hydrolyze organic compounds and decompose food waste.

Xylanase

Thermostable xylanases are widely used as biocatalysts in the industry because of their ability to withstand extreme conditions without denaturation at high temperatures, alkaline or acid treatment, or solvents. Thermophilic xylanase is in great demand because it reduces costs by extending the life of the biocatalyst. Thermophilic xylanase is needed in the food and feed industry, paper and pulp technology, textile production, and biofuels (Knapik et al. 2019). The *Geobacillus* sp. strain WSUCF1 has attracted attention because it produces highly heat-resistant xylanase with excellent thermostability, having half-lives of 18 days at 60 °C and 12 days at 70 °C (Bhalla et al. 2014). The exploration of thermophilic microbes producing xylanase enzymes conducted by Saksono and Sumarini (2010) in Tanjung Api, South Sumatra, also found *Geobacillus stearothermophilus* T-6 which has been well characterised from the genetic level to the protein structure. The xylanase enzyme produced by these bacteria has thermostability under temperatures up to 70 °C and alkaline stability at pH 7.0.

Exploration of thermophilic microbes taken from the Makula hot spring, Tana Toraja, isolate suspected to be *Bacillus stearothermophilus* SL3S. The crude extract of xylanase enzyme can hydrolyse xylan from corn cobs at optimum pH 7.0 and temperature 45 ºC, activated by Ca^{2+}, Mg^{2+}, Ni^{2+} and inhibited by CO^{2+} ions (Putri et al. 2017). Sonai hot springs, Sulawesi, showed the highest xylanase activity in media containing rice husks, temperature 50 ° C, pH 9.0, and agitation speed of 150 rpm. The analysis of the similarity of the 16S rRNA gene sequences of isolate IIA-3 had a 92 % similarity with *Pseudomonas aeruginosa* strain RSB3 (Susilowati et al. 2012). Meanwhile, in the Lapindo hot mud in Porong, Sidoarjo, showed that the maximum activity of the xylanase enzyme from isolate C211 was 3.95 U mL^{-1} under incubation at 50 °C. After molecular identification of 16S rRNA was carried out, isolate C211 had a genetic closeness with *Bacillus licheniformis* with a degree of homologous similarity of 99 % for each (Habibie et al. 2014). Similar results were also obtained by Raj et al. (2018) who isolated thermophilic bacteria from paper mill waste contaminated with soil and identified the bacteria as species *B. licheniformis.* Xylanase activity increased to 5.26 mg mL^{-1} at 60 °C and pH 9.0. This is because the enzyme activity is stimulated by Ca^{2+}, Fe^{2+}, and Mg^{2+} and inhibited by Cd^{2+}, Hg^{2+}, and Cu^{2+}. GC-MS analysis of filtrate from xylanase-treated pulp showed variations in the presence of derivative organic compounds, which can be applied in paper production by making it a cleaner and environmentally friendly process.

Protease

Protease is one of the largest and most refined enzymes in the human proteome. Protease enzymes are enzymes that are essential for human life because proteases are important in the synthesis of all proteins and regulate the composition, shape, and size. Protease enzymes function to catalyse the hydrolysis of peptide bonds in proteins. Protease enzymes are important enzymes and have high economic value because of their wide application. Examples of industries that use protease enzymes include the detergent (Mahakhan et al. 2022; Neog et al. 2024), leather processing (Moonnee et al. 2021; Khan et al. 2023), textile (Zhang et al. 2025; Ariaeenejad & Motahar 2025), food (Christensen et al. 2022; Zhang, X. et al. 2024), dairy (Yang et al. 2021; Kaur et al. 2023), pharmaceutical (Pan et al. 2019; Tang et al. 2022), beer (Lin et al. 2022, 2023), and waste industries (Ariaeenejad et al. 2022; Majithiya & Gohel 2025). Sources of protease enzymes that have been known come from a variety of organisms, including animals (Magalhães et al. 2007), plants (Yadav et al. 2006; Akhtaruzzaman et al. 2012), fungi (Sharma et al. 2015; Maitig et al. 2018), and bacteria (Das & Prasad 2010; Uddin et al. 2014), both intracellular and extracellular. Plants are the largest source of protease enzymes (43.85 %), followed by bacteria (18.09 %), fungi (15.08 %), animals (11.15 %), algae (7.42 %) and viruses (4.41 %) (Zafrida et al. 2022). Thermophilic bacteria isolated from the environment at high temperatures are one of the producers of protease enzymes, so they become thermo-protease enzymes. Protease enzymes produced from thermophilic bacteria are known to be more widely used in industry because of their thermostable nature, which allows them to produce more efficient products (Vaidya et al. 2018). Its thermostable nature can also reduce the possibility of microbial contaminants because it is used at high temperatures. In addition, using thermostable protease enzymes reduces the cost of cooling in large-scale fermentation (Johnvesly & Naik 2001; Nascimento & Martins 2004; Liu, D. et al. 2023).

The exploration of thermophilic bacteria that produce protease enzymes has been carried out by several researchers (Table 3), including thermophilic bacteria from the Lejja hot spring in Soppeng, South Sulawesi, by Hafsan et al. (2021). Three isolates of thermophilic bacteria were taken, namely *B. coagulans*, *B. stearoformis*, *B. licheniformis* and their growth was observed based on the optical density (OD) of the production media at certain time intervals. A constant OD value indicates that bacterial cell growth has reached a stationary phase, a phase where cell growth remains and a good time to isolate proteases produced by bacteria. After being observed for some time, it was found that the three thermophilic bacteria achieved optimum activity at the same pH but at different temperature ranges with relatively high activity and potential to be applied for various industrial purposes. Gedong Songo Ungaran hot spring, Central Java, has a pH of 6.0 with a temperature of 68 °C. Isolated thermophilic bacteria *Thermoanaerobacterium* sp. by 78-86 % are anaerobic bacteria, gram-negative rods, and have extracellular enzymes - amylase and proteases (Nuritasari et al. 2017). The hot springs of Sungai Penuh, Jambi, have temperatures between 50-78 °C with a pH of around 8.5. Total of 70 colonies of thermophilic bacteria were isolated, and after proteolytic testing, 39 isolates were obtained which had a proteolytic index between 0.13 - 7.89 mm, which was indicated by the formation of a clear zone around the colonies growing on the media. The MII2.1 isolate had the highest index of 7.89, and it was isolated from a location with a temperature of 77 °C and a pH of 8.71. In the protease enzyme activity test, MI2.3 isolate was found with the highest value of 13,592 U mL^{-1}. The character of the MI2.3 bacterial isolate was gram -positive, rod-shaped cells, and non-motile. The optimum growth temperature is 50 °C, and the optimum pH is 9.0 (Wahyuna et al. 2012). Furthermore, from Tanjung Sakti Lahat hot spring, South Sumatra, four isolates of thermophilic bacteria have been isolated, which have protease activity with the highest proteolytic index on isolate TA4, which is 0.77. Microscopically round cells, Gram-positive and lacking endospores, non-motile and biochemical tests, and all isolates showed different physiological properties. Based on these characteristics, all isolates belonged to the genus *Saccharococcus*. *Saccharococcus* can live at a temperature of 78 °C and in natural habitats (Muharni et al. 2013).

Cellulase

Cellulase is an enzyme that can degrade cellulose into glucose, cellobiose, and cello-oligosaccharides. Cellulase is an inductive enzyme. The production of cellulase enzymes by microbes requires the presence of an inducer in the fermentation medium. The inducer stimulates the production of cellulase enzymes in microbial cells. The amount of enzymes present in the cell is not fixed, depending on the inducer. The amount will increase several times if the medium contains an inducing substrate. The inducer compound required is generally in the form of the enzyme substrate (Wezyah 2013). Cellulases can be applied to pulp refining in the paper industry, fabric brightening in textiles, food quality enhancement, organic matter decomposition, feed improvement, bioconversion of cellulose to valuable chemicals, and reducing environmental pollution. Cellulase enzymes are also used in the fermentation process from biomass into biofuels such as bioethanol. They are also used as a substi-

Table 3. Biotechnology Applications of Thermophilic Bacteria: Enzymes.

Produce Enzyme	Collection site	Thermophilic Bacteria	References	Biotechnological Applications
Amylase	Way Panas Hotspring, Lampung	*Bacillus cereus* WP.50.4.	Mahestri et al. 2021	Thermostable α-amylase is widely used in the starch industry involving gelatinization, liquefaction, and saccharification processes in sugar production, brewing, and starch processing.
	Way Belerang Kalianda Hotspring, South Lampung	*Pseudomonas stutzeri* A.WB.50.1	Mawati et al. 2021	
	Bukit Gadang Hotspring, North Sumatera	*Bacillus licheniformis* LBKURCC190	Ardhi et al. 2020	
	Gedong Songo Hotspring, Central Java	*Anoxybacillus* sp. dan *Thermoanaerobacterium* sp.	Nuritasari et al. 2017	
	Cangar Hotspring, East Java	*Bacillus subtilis* subsp. inaquosorum CGR-1	Geraldi et al. 2022	
	Tanjung Api Hotspring, Poso	*Geobacillus stearothermophilus* T-6	Saksono & Sukmarini 2010	
	Lapindo Porong Hotspring, Sidoarjo	*B. licheniformis* C211	Habibie et al. 2014	
	Sonai Hotspring, Southeast Sulawesi	*Pseudomonas aeruginosa* IIA -3	Susilowati et al. 2012	
	Makula Hotspring, Tana Toraja	*Bacillus stearothermophilus* SL3S	Putri et al. 2017	
	Nglimut Hotspring, Kendal Regency, Central Java	*Bacillus amyloliquefaciens* *Bacillus licheniformis*	Budiharjo et al. 2024	
Xylanase	Tanjung Api Hotspring, Poso	*Geobacillus stearothermophilus* T-6	Saksono & Sukmarini 2010	Thermostable xylanase is applied to enzymatic saccharification of biomass in biofuel production, pulp bioleaching, increasing bread volume and quality, fabric biopolishing, and improving digestibility and quality of animal feed.
	Lapindo Hotspring Porong, Sidoarjo	*B. licheniformis* C211	Habibie et al. 2014	
	Sonai Hotspring, Sulawesi Tenggara	*Pseudomonas aeruginosa* IIA -3	Susilowati et al. 2012	
	Makula Hotspring, Tana Toraja	*Bacillus stearothermophilus* SL3S	Putri et al. 2017	
Protease	Lejja, Soppeng South Sulawesi Hot Spring	*Bacillus licheniformis, Bacillus stearoformis, Bacillus coagulans*	Hafsan et al. 2021	Protease enzymes are enzymes that are able to hydrolyze proteins into their constituent amino acids. Gelatin as a protein compound in the presence of a protease enzyme (gelatinase) will decompose into its amino acids. Gelatin is a complex protein compound that solidifies in the cooling process. In the presence of proteases, the peptide bonds in gelatin are broken, causing gelatin to degrade. The effect of gelatin degradation is when the gelatin does not solidify during the cooling process.
	Gedong Songo Hotspring, Ungaran, Central Java	*Thermoanaerobacterium* sp.	Nuritasari et al. 2017	
	Tanjung Sakti Lahat, South Sumatera Hot Spring	*Saccharococcus* sp.	Muharni et al. 2013	

Table 3. Contd.

Produce Enzyme	Collection site	Thermophilic Bacteria	References	Biotechnological Applications
Cellulase	Agricultural Compost from Desa Bayat, Klaten	Cellulolytic bacteria isolate KB and KK	Alam et al. 2013	Cellulase is applied to refine pulp in the paper industry, keep the color of fabrics bright in the textile industry, improve quality in the food industry, decompose organic materials, improve animal feed nutrition, play an important role in the bioconversion of cellulose into various commodity chemical compounds and can reduce negative impact of waste pollution in the environment.
	Soil from Cowshed, Institut Pertanian Bogor	Isolate KS 0.1, KS 0.7, KS 9.1	Sembiring 2019	
	Agricultural and Plantation Waste, Andalas University	NG2 Bacteria	Ramadhan et al. 2020	
	Nglimut Hotspring, Kendal Regency, Central Java	*Bacillus amyloliquefaciens* *Bacillus licheniformis*	Budiharjo et al. 2024	

tute for chemicals in the process of making alcohol from materials containing cellulose. The presence of cellulase enzymes in a reaction can maximize the conversion of cellulose into simple sugars and higher ethanol yields (Nababan et al. 2019). Cellulase enzymes can be isolated from thermophilic cellulolytic bacteria. The advantage is the acquisition of cellulase enzymes with heat-resistant characteristics so that they can be used in industrial fields that use high temperatures. Industrial applications require cellulase enzymes that can be produced in large quantities and with high activity but at an economical cost. Cellulase produced by thermophilic bacteria shows stability at high temperatures, so it is very useful.

Thermostable enzymes can tolerate higher temperatures, so they are advantageous in industrial processes (Prasad et al. 2014). The advantages of bacteria as cellulase producers are high growth rates, expression of multi-enzyme complexes, stability at extreme temperatures and pH, less feedback inhibition, and the ability to withstand environmental stresses (Sharma et al. 2013). In a study by Alam et al. (2013), thermophilic bacteria producing cellulase were isolated from agricultural compost in Bayat Village, Klaten. KB colonies were bone white, large, fused spheres with irregular edges, while KK colonies were clear, small, and spread out with irregular edges.

BIOACTIVE COMPOUNDS OF THERMOPHILIC BACTERIA

Thermophilic bacteria produce bioactive compounds with numerous benefits (Table 4). Hot springs serve as reservoirs for thermophilic microorganisms. Bacteria are an inexhaustible source of chemical compounds, which produce a wide variety of active secondary metabolites. Secondary metabolites these bacteria produce can be antimicrobials, antitumour agents, immunosuppressants, herbicides, pesticides, anti-parasitic agents, and enzymes. In addition, thermophilic bacteria can be used to produce bioethanol and other industrial chemicals (Gurumurthy et al. 2020). Environmental pressures, predators competition, and reproduction cause the production of bioactive compounds. Recently, seven species of thermophilic cyanobacteria isolated from Geno hot springs (Bandar Abbas province, Republic of Iran) were found, namely: *Oscillatoria subbrevis*, *Oscillatoria tenius*, *Oscillatoria limentica*, *Oscillatoria angusta*,

Table 4. Botechnology Applications of Thermophilic Bacteria: Bioactive Compounds.

Collection site	Thermophilic Bacteria	References	Biotechnological Applications
Ai Sipatn Lotup Hot Springs Sanggau, West Kalimantan	*Thermoactinomyces* sp. (H21), *Thermoactinomyces* sp. (H24), *Thermobifida* sp. (S311), *Streptomyces* sp. (S211), *Actinomadura* sp. (S21(2), and *Nocardiopsis* sp. (H22*1)	Manalu et al. 2019	Antifungal and antibacterial
Ai Sipatn Lotup Hot Springs, West Kalimantan	*Microbispora* sp. (S311A) and *Streptomyces* sp. (H2232)	Manalu et al. 2019	Antifungal
Gedong Songo, Jawa Tengah Hot Spring	*Rhodococcus* sp. Chr-9.	Kusdiyantini et al. 2017	Pink Pigment
Hot springs in Ayas, Turkey	*Synechococcus* sp. and *Phormidium* sp.	Sadettin & Dönmez 2006	Dye bioaccumulation
Geno Hot Spring, Bandar Abbas, Iran	*Oscillatoria subbrevis*, *Oscillatori tenuis*, *O. limentica*, *O. angusta*, *O. articulate*, *Synechocystis aquatilis*, *S. cerdorum*	Heidari et al. 2012	Antimicrobial agents against five Gram-positive bacteria, three Gram-negative bacteria and two fungi
Jakrem hot water spring in West Khasi Hill District, Meghalaya, India	*Mastigocladus* sp. and *Microcoleus* sp.	Siangbood & Ramanujam 2011	Curative medicinal properties

Articulated *Oscillatoria*, *Synechocystis aquatilis*, and *Synechoccous cerdrorum* (Heidari et al. 2012). Isolation performed on cyanobacteria obtained methanol extract, which showed high antibacterial activity against *Bacillus subtilis* and *Bacillus pumilus*. This extract was then filtered to determine its antibacterial activity against various microorganisms. It was found to inhibit the growth of gram-positive and gram-negative bacteria and fungal species such as *Candida albicans* and *Cladosporium resinae*. In contrast, the extract was inactive against other cyanobacterial species. Siangbood and Ramanujam (2011) also isolated several thermophilic Cyanophyceae species, namely *Mastigocladus* and *Microcoleus*, from Jakrem hot springs in Bukit Khasi Barat (Meghalaya district, India), which showed curative properties. In addition, different organic solvents and aqueous extracts (including extracellular polysaccharides) from the thermophilic freshwater algae *Cosmarium* sp. isolated from the Aïn-Echeffa hot spring in northern Tunisia, were tested for antibacterial activity, including antioxidant and cytotoxic activity, against gram-positive and gram-negative bacteria. The algal biomass showed a significant antibacterial effect, with the minimum inhibitory concentration (MIC) ranging from 28 to 85 g mL^{-1}. In contrast, extracellular polysaccharides had a MIC of 50-150 g mL^{-1}, and aqueous extracts of extracellular polysaccharides showed moderate antioxidant activity (24.97 %) (Challouf et al. 2012).

Actinomycetes also have the potential to be antifungal agents. The compounds produced include kasugamycin by members of the *Streptomyces kasugaensis* species (Umezawa et al. 1965) and polyoxins B and D by members of the *Streptomyces cacaoi* (Isono et al. 1967). The resulting metabolites inhibit the fungal cell wall synthesis process. The potential of Actinomycetes is also described in studies conducted by several scientists. Deepa et al. (2014) reported as many as 16 isolates isolated in the Indian region as potential antimicrobial agents, four of which have potential as antifungal agents, namely *Streptomyces griseoflavus*, *Streptomyces cyaneus*, *Streptomyces exfoliatus* and *Streptomyces albus*. Based on the results of the isolation carried out by Manalu et al. (2019) at the Ai' Sipatn Lotup hot spring, 11 members of the Actinomycetes bacteria were found. The isolate was suspected of having antifungal potential. Based on the antifungal activity test results, two isolates were able to inhibit the growth of the test fungus: isolates S311A (*Microbispora* sp.) and H2232 (*Streptomyces* sp.). The inhibition was indicated by the formation of a clear zone around the paper disc. Actinomycetes are also known as antibiotic-producing agents. About 70 % of antibiotics found are produced by Actinomycetes, mainly from members of the genus *Streptomyces*.

The use of natural pigments as dyes has been increasing due to their safety properties. Natural pigments are secondary metabolites produced by plants, animals, and microorganisms. Many natural pigments have commercial potential as antioxidants. Exploration of pigment-producing microorganisms continues to identify potential isolates for industrial applications. One of the pigment-producing microorganisms is bacteria. Extreme environments, such as hot springs, are one place worth exploring for pigmented bacteria (Tkáčová et al. 2015). Some bacteria have great potential to produce various types of pigments; for example, *Vogesella* sp. produces a blue pigment (Cardona-Cardona 2010). In addition, Cyanobacteria produce phycobilin pigments, and *Serratiamarcescens* produce prodigiosin pigments (Vora et al. 2014). In addition to bacteria, some microalgae (*Haematococcus pluvialis*) and yeast (*Phaffiarhodozyma*) produce the pigment astaxanthin (Gramza-Michałowska & Stachowiak 2010).

RECOMBINANT ENZYME TECHNOLOGY FOR THERMOPHILIC MICROORGANISMS

Recombinant enzyme technology has greatly enhanced the study and application of thermophilic microorganisms in various industrial and biotechnological fields. The recombinant technology enables faster and simpler production of thermostable enzymes by allowing expression on wide range of hosts with faster growth and higher enzymes yield compared to native thermophiles. This approach facilitates protein engineering (e.g., site-directed mutagenesis) to enhance catalytic efficiency or substrate specify, enables purification and functional studies under controlled conditions, and also permits mass production with consistent quality and performance. This section highlights five specific enzyme categories— cutinase, protease, catalase, and DNA polymerase—chosen for their industrial significance, thermal stability, and prevalence in recombinant research. Although numerous other recombinant thermozymes, such as lipases, xylanases, and cellulases, have been investigated, the enzymes selected here represent distinct functional groups (hydrolases, oxidoreductases, and polymerases) and provide well-documented cases of heterologous expression, purification, and practical use (Table 5).

Cutinases

Cutinases are enzymes that are mainly obtained from thermophilic actinomycetes, such as *Thermobifida fusca* in 2005. They are categorised into two groups based on their thermostabilities: higher thermostabilities: (>70 °C) and lower thermostabilities: (<70 °C) (Dresler et al. 2006; Oda et al. 2021). Cutinases are members of the serine hydrolase family and share the catalytic triad Ser-His-Asp. They have several unique properties, including metal ion -binding on the enzyme's surface, elevation of melting temperatures, and activation of the enzyme (Kawai et al. 2020; Sui et al. 2023). Cutinases are able to degrade cutin, which is part of the cuticular layer in leaves, or the suberin part of tree bark. They can also hydrolyse polyesters, such as polyethylene terephthalate (PET), aliphatic polyester (PCL), and aliphatic-aromatic co-polyester (PBSA) (Weber et al. 2021). In order to obtain thermostable polyester-degrading enzymes for applications in biodegradable plastic recycling, textile processing, and detergent formulations, recombinant technology is applied to cutinases. This approach will simplify the expression and purification in non-pathogenic hosts, supporting directed evo-

Table 5. Recombinant Technology of Thermophilic Bacteria: Enzyme Technology.

Type	Enzyme	Origin	Recombinant Technology	Results	References
Cutinase	Cutinase	*Thermobifida cellulosylitica*	Cloning and expression using pET25b(+) vector in *E. coli* (DH5α) and BL21 strain (DE3) (IPTG Induction)	Protein (29kDa) with highest activity at an optimum pH of 9 and thermal stability up to 60 °C	Usman et al. 2023
	Cutinase (mutantEst1 DM)	*Thermobifida alba*	Cloning and expression using pQE80L-est1 in *E. coli* cells Rosetta-gami B (DE3) (IPTG Induction)	High yield of recombinant Est1DM, more than 120 mg per liter of *E. coli* culture	Kitadokoro et al. 2018
Protease	Recombinant protease 1147	*Cohnella* sp. A01	cloning and expression using pET26b(+) plasmid in *E. coli* strains DH5α and BL21 (DE3) (IPTG induction)	Protein (~18 kDa) with excellent tolerance to high temperature and a broad range of pH, highest activity at 60°C and a pH of 7	Tarrahimofrad et al. 2020
	Serine protease Tcsp	*Thermomonospora curvata* Henssen ATCC 19,995	Cloning and expression of Tcsp using pET25b(+) vector in *E. coli* BL21 (DE3) (IPTG induction)	Protein (~40 kDa) with higher stability than commercial protease from *B. licheniformis* (Blsp) at high temperatures, optimum activity at 70 °C around pH 10	Sittipol et al. 2019
	Subtilisin (serine protease)	*Chaetomium thermophilum*	Procurement of C. thermophilum gene pro cDNA fragment via RT-PCR; Cloning and expression in *E. coli* BL21 (DE3) and Pischia pastoris	Recombinant protease with optimum catalytic activity at 60 °C and pH 8	Li & Li 2009
	alkaline serine protease	*Geobacillus stearothermophilus* B-1172	cloning and expression using pET22b(+) vector in *E. coli* strains BL21 (DE3)	Thermostable protein (39 kDa) with specific activity of 97.5 U mg^{-1}, and a recovery of 23.6 %, optimum activity at 90 °C at pH 9 (73.8 U mg^{-1})	Iqbal et al. 2015
	protease	*Bacillus stearothermophilus*	Cloning and expression in *Bacillus subtilis* DB104, under the control of the sacB gene promoter.	Protein (35 kDa) with optimal temperature and pH at 65 °C and 7.5 and specific activity of 16530 U mg^{-1}, 80 % activity after 1 h reaction at 65 °C	Zhang M. et al. 2008
	subtilisin (serine protease) TTHA0724	*Thermus thermophilus* HB8	Cloning and expression in *E. coli* Transetta (DE3)	Thermostable protein with optimum activity between 65 and 85 °C at pH 7.5, maintaining 50 % activity after 48 h at 75 °C and >78 % activity across the pH range 5.0–9.5, and demonstrated broad substrate specifity	Xie et al. 2019

Table 5. Contd.

Type	Enzyme	Origin	Recombinant Technology	Results	References
Catalase	Recombinant Cat-IIGt	*Geobacillus thermopakistaniensis*	Cloning and expression using pTZ57 R/T plasmid in *E. coli* BL21-CodonPlus (DE3)-RIL cells (IPTG induction)	The enzyme activity increased gradually with increasing temperature up to 70 °C with a half-life of 30 min at 100 °C, highest catalase activity at pH 10.0 with specific activity of 40,529 µmol min^{-1} mg^{-1}.	Shaeer et al. 2022
	Catalase-peroxidase Tx_CP	*Thermobacillus xylanilyticus* strain XE	Cloning and expression using pET-28b (+) plasmid in *E. coli* DH5α and Tuner DE3	Tx-CP showed the highest stability at pH 7.0 with 100 % of its activity conserved after 24 h , optimum temperature for peroxidase activity 55 °C	Fall et al. 2023
DNA Polymerase	DNA polymerase Tth	*Thermus thermophilus*	Cloning and expression using pD861-His and pD861-MBP plasmids in *E. coli* BL21 (DE3) (L-rhamnose induction)	The total protein concentration of His-Tth DNA polymerase is 3.9095 mg mL^{-1} while for MBP-Tth DNA polymerase it is 33.541 mg mL^{-1};	Maksum et al. 2022
	DNA polymerase	*Thermus aquaticus*	Cloning and expression of recombinant protein using T7-induced promoters, plasmid pD451-SR in *E. coli* DH5α (autoinduction)	A high protein yield of approximately 83.5 mg L^{-1} culture of active Taq-pol	Laksmi et al. 2025
	DNAP-1	*Bacillus licheniformis* strain NWMF1	Cloning and expression using PET28a+ vector in *E. coli* BL21 (DE3)pLysS (IPTG Induction)	Active His-tag purified recombinant DNAP-1 for PCR-amplification of the alkaline protease gene (1140 bp) from *B. licheniformis* with DNA fragment of expected size	Mudiyanselage et al. 2021

lution or mutagenesis to improve the enzyme's activity.

Proteases

Proteases are enzymes that break down proteins into smaller units, such as peptides or amino acids. They are used in many industrial applications, including detergents, laundry, leather, and pharmaceuticals. Thermophilic bacteria are advantageous for industrial use because they have high growth rates, a reduced risk of microbial contamination, and other benefits (Kambourova 2018; Mushtaq et al. 2024). The recombinant technology approach is applied to produce heat-stable proteases for use in detergents, food processing, pharmaceuticals, and waste treatment under extreme conditions. This will provide high-yield protease production that is not achievable from native strains, and also facilitate the modification of enzyme properties for specific industrial processes.

For instance, *Streptomyces thermovulgaris* produces a metalloprotease that exhibits stability across a broad range of pH levels and temperatures. The protease was purified using precipitation and affinity chromatography

(Mushtaq et al. 2024). Another example is *Geobacillus thermoglucosidasius* SKF4, which produces a thermostable alkaline serine protease known as SpSKF4. The cloned gene was successfully expressed in *E. coli* (Allison et al. 2023). Additionally, *Aeribacillus pallidus* P18 has had its protease gene cloned into the pET SUMO expression vector, with the target gene's coding sequence amplified via PCR (Saadati et al. 2024). From *Cohnella* sp. A01, a thermostable protease gene was isolated and expressed recombinantly, with subsequent characterization of its biophysical and biochemical properties (Tarrahimofrad et al. 2020). Furthermore, a protease gene was cloned from *Fervidobacterium pennivorans* (Tarrahimofrad et al. 2020), as well as from *Thermoanaerobacter yonseiensis* (Li et al. 2007), exhibiting the extensive implementation of recombinant technology.

Catalase

Catalase is an enzyme that breaks down hydrogen peroxide into water and oxygen molecules. It has many applications (Jia et al. 2016), including: Food industry: Removes hydrogen peroxide from milk before cheese production and prevents food from oxidising in food wrappers and Textile industry: Removes hydrogen peroxide from fabrics. Catalase cloning enzymes from thermophilic bacteria, including *Geobacillus* sp. CHB1: This thermophilic bacteria's Kat gene was cloned, expressed, purified, and characterised. The recombinant enzyme was stable and active over a wide range of temperatures, from 10 °C to 90 °C (Jia et al. 2016). *Thermus* sp. YS 8-13: A heat-stable catalase was purified from this thermophilic bacterium (Kagawa et al. 1999). *Metallosphaera hakonensis*: An alkali-tolerant catalase was purified from this thermophilic bacterium (Ebara & Shigemori 2008).

DNA polymerase.

Thermostable DNA polymerases are enzymes that come from thermophilic bacteria or archaea and are used in the polymerase chain reaction (PCR). They can withstand high temperatures that would denature most proteins. The cloning of thermostable DNA polymerase genes can be done by amplifying the gene with specific primers, isolating and purifying the amplified fragment, and ligating it into a cloning vector. The recombinant plasmid can then be transformed into competent cells using a heat shock method (Witasari et al. 2010; Briones 2023). Taq polymerase is extracted from the thermophilic bacterium *Thermus aquaticus*. It was originally isolated in 1976 by Chinese scientists Alice Chien et al. Taq polymerase is often used in PCR to automate repetitive steps and amplify specific DNA sequences. Pfu: This is another thermostable enzyme isolated from thermophilic bacteria. Bst DNAP enzyme is from Bacillus stearothermophilus (now categorized as *Geobacillus stearothermophilus*). It is often used in isothermal amplification techniques like loop-mediated isothermal amplification (LAMP) and whole genome amplification (WGA) (Wang et al. 2022; Briones 2023).

CONCLUSIONS

The diversity of thermophilic bacteria found in Indonesia’s extreme environments is a testament to the adaptability of life in harsh conditions. These bacteria play not only vital ecological roles in the sulphur and carbon cycles but also offer numerous practical applications in industries ranging from biotechnology to environmental remediation. As research into thermophilic bacteria continues to expand, it is likely that new species with unique properties will be discovered, offering even greater potential for industrial applications. The ongoing exploration of Indonesia’s geothermal and volcanic habitats promises to be a rich source of novel thermophilic bacteria with promising biotechnological uses. Thermophilic microbes have been widely studied for their potential in biotechnology. The discovery of thermophilic microbes is certainly a new solution for meeting the enzyme needs in the molecular, industrial, environmental, food, and agricultural sectors. The unique thermophilic nature of microbial enzymes allows the catalytic process to proceed rapidly and be biodegradable, adding value for future applications.

AUTHOR CONTRIBUTION

D.W. and A.B. designed the study; D.W., A.A.K.P. and R.H.B.S. wrote the manuscript.

ACKNOWLEDGMENTS

DW would like to thank (i) BIMA Fundamental research: Pemetaan Mikrobiota dan Senyawa Bioaktif dalam Makanan Fermentasi Dengke Naniura melalui Pendekatan Multi-Omics, Grant No: 127/C3/DT.05.00/PL/2025. (ii) Diponegoro University for the WCU Program, Indonesia Endowment Fund for Education Grant No: 61/UN7.A/HK/XII/2024. AB would like to thank (iii) Diponegoro University for the RPIBT Grant No: 225-44/UN7.6.1/PP/2022 and WCU IJR FSM Scheme Grant No:650/UN7.F8/PP/III/2025. (iv) RAR grant no: 222-055/UN7.D2/PP/IV/2025.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests that could have appeared to influence the work reported in this paper.

REFERENCES

Agustriana, E. et al., 2023. Optimized expression of large fragment DNA polymerase I from *Geobacillus stearothermophilus* in *Escherichia coli* expression system. *Preparative Biochemistry & Biotechnology*, 53(4), pp.384-393. doi: 10.1080/10826068.2022.2095573

Ahirwar, S. et al., 2017. Isolation and screening of thermophilic and thermotolerant fungi for production of hemicellulases from heated environments. *Mycology*, 8(3), pp.125-134. doi: 10.1080/21501203.2017.1337657

Akhtaruzzaman, M. et al., 2012. Isolation and characterization protease enzyme from leguminous seeds. *Agricultural Science Research Journals*, 2(8), pp.434-440.

Al-Johani, N.B., Al-Seeni, M.N. & Ahmed, Y.M., 2017. Optimization of alkaline α-amylase production by thermophilic Bacillus subtilis. *African Journal of Traditional, Complementary and Alternative Medicines*, 14(1), pp.288-301. doi: 10.21010/ajtcam.v15i4.6

Al-Qodah, Z., 2006. Production and characterization of thermostable α-amylase by thermophilic *Geobacillus stearothermophilus*. *Biotechnology Journal: Healthcare Nutrition Technology*, 1(7-8), pp.850-857. doi: 10.1002/biot.200600033

Alam, M.S., Sarjono, P.R. & Aminin, A.L., 2013. Isolasi Bakteri Selulolitik Termofilik Kompos Pertanian Desa Bayat, Klaten, Jawa Tengah. *Chem Info*, 1(1), pp.90-195.

Alexandraki, V. et al., 2019. Comparative genomics of Streptococcus thermophilus support important traits concerning the evolution, biology and technological properties of the species. *Frontiers in microbiology*, 10, 2916. doi: 10.3389/fmicb.2019.02916

Allioux, M. et al., 2022. Genome analysis of a new sulphur disproportionating species Thermosulfurimonas strain F29 and comparative genomics of sulfur-disproportionating bacteria from marine hydrothermal vents. *Microbial Genomics*, 8(9), 000865. doi: 10.1099/mgen.0.000865

Allison, S.D., AdeelaYasid, N. & Shariff, F.M., 2023. Molecular Cloning, Characterization, and Application of Organic Solvent-Stable and Detergent-Compatible Thermostable Alkaline Protease from Geobacillus thermoglucosidasius SKF4. *Journal of Microbiology and Biotechnology*, 34 (2), 436. doi: 10.4014/jmb.2306.06050.

Alrumman, S.A. et al., 2019. Antimicrobial activity and GC-MS analysis of bioactive constituents of Thermophilic bacteria isolated from Saudi hot springs. *Arabian Journal for Science and Engineering*, 44, pp.75-85. doi: 10.1007/s13369-018-3597-0

Altinok, F. et al., 2023. Application of Anoxybacillus gonensins UF7 lipase as a catalyst for biodiesel production from waste frying oils. *Fuel*, 334, 126672. doi: 10.1016/j.fuel.2022.126672

Aragaw, T. A., Bogale, F. M. & Gessesse, A., 2022. Adaptive response of thermophiles to redox stress and their role in the process of dye degradation from textile industry wastewater. *Frontiers in Physiology*, 13, 908370. doi: 10.3389/fphys.2022.908370

Ardhi, A. et al., 2020. Molecular identification of amylase-producing thermophilic bacteria isolated from Bukit Gadang Hot Spring, West Sumatra, Indonesia. *Biodiversitas Journal of Biological Diversity*, 21(3), pp.994-1000. doi: 10.13057/biodiv/d210319

Ariaeenejad, S. & Motahar, S.F.S., 2025. A collagen-hydrolyzing halotolerant protease for enhanced dye decolorization and toxicity reduction in high-salinity textile tannery wastewater. *Journal of Hazardous Materials Advances*, 18, 100669. doi: 10.1016/j.hazadv.2025.100669

Ariaeenejad, S. et al., 2022. Simultaneous hydrolysis of various protein-rich industrial wastes by a naturally evolved protease from tannery wastewater microbiota. *Science of The Total Environment*, 815, 152796. doi: 10.1016/j.scitotenv.2021.152796

Asgher, M. et al., 2007. A thermostable α-amylase from a moderately thermophilic Bacillus subtilis strain for starch processing. *Journal of food engineering*, 79(3), pp.950-955. doi: 10.1016/j.jfoodeng.2005.12.053

Atomi, H. et al., 2004. Description of T*hermococcus kodakaraensis* sp. nov., a well studied hyperthermophilic archaeon previously reported as *Pyrococcus* sp. KOD1. *Archaea*, 1(4), pp.263-267. doi: 10.1155/2004/204953

Babbar, S.B. & Jain, R., 2006. Xanthan gum: an economical partial substitute for agar in microbial culture media. *Current microbiology*, 52, pp.287–292. doi: 10.1007/s00284-005-0225-5.

Baker, B.A. et al., 2021. Efficiency of thermophilic bacteria in wastewater treatment. *Arabian Journal for Science and Engineering*, 46, pp.123-128. doi: 10.1007/s13369-020-04830-x

Becker, A. et al., 1998. *Xanthan gum* biosynthesis and application: a biochemical/genetic perspective. *Applied Microbiology and Biotechnology*, 50(2), pp.145-152. doi: 10.1007/s002530051269.

Benammar, L. et al., 2020. Diversity and enzymatic potential of thermophilic bacteria associated with terrestrial hot springs in Algeria. *Brazilian Journal of Microbiology*, 51(4), pp.1987-2007. doi: 10.1007/s42770-020-00376-0.

Bhalla, A. et al., 2014. Novel thermostable endo-xylanase cloned and expressed from bacterium *Geobacillus* sp. WSUCF1. *Bioresource Technology*, 165, pp.314-318. doi: 10.1016/j.biortech.2014.03.112.

Briones, C., 2023. Polymerase Chain Reaction. In *Encyclopedia of Astrobiology*. Berlin, Heidelberg: Springer Berlin Heidelberg, pp.2441-2442.

Brock, T.D. & Freeze, H., 1969. Thermus aquaticus gen. n. and sp. n., a non-sporulating extreme thermophile. *Journal of bacteriology*, 98(1), pp.289-297. doi: 10.1128/jb.98.1.289-297.1969

Budiharjo, A. et al., 2024. Bioprospecting and Molecular Identification of Amylase and Cellulase Producing Thermophilic Bacteria from Sediment of Nglimut Hot Springs, Kendal Regency. *Journal of Tropical Biodiversity and Biotechnology*, 9(3), 86756. doi: 10.22146/jtbb.86756.

Burke, V. et al., 1991. Longitudinal studies of virulence factors of Pseudomonas aeruginosa in cystic fibrosis. *Pathology*, 23(2), pp.145-148. doi: 10.3109/00313029109060814.

Cao, Y. et al., 2019. Contributions of thermotolerant bacteria to organic matter degradation under a hyperthermophilic pretreatment process during chicken manure composting. *BioResources*, 14(3), pp.6747-6766.

Cardona-Cardona, V.Z., 2010. *Molecular analysis, physiological study and biotechnological capabilities of Blue Pigmented Bacteria from Puerto Rico.* University of Puerto Rico.

Carré, L. et al., 2022. Relevance of earth-bound extremophiles in the search for extraterrestrial life. *Astrobiology*, 22(3), pp.322-367.

Challouf, R. et al., 2012. Antibacterial, antioxidant and cytotoxic activities of extracts from the thermophilic green alga, *Cosmarium* sp. *African Journal of Biotechnology*, 11(82), pp.14844-14849. doi: 10.5897/AJB12.1118.

Chan, C.S. et al., 2017. Effects of physiochemical factors on prokaryotic biodiversity in Malaysian circumneutral hot springs. *Frontiers in Microbiology*, 8, 1252. doi: 10.3389/fmicb.2017.01252.

Chen, G. et al., 2021. Detoxification of azo dye Direct Black G by thermophilic Anoxybacillus sp. PDR2 and its application potential in bioremediation. *Ecotoxicology and Environmental Safety*, 214, 112084. doi: 10.1016/j.ecoenv.2021.112084

Ching, X.J. et al., 2022. Complete genome sequence data of tropical thermophilic bacterium Parageobacillus caldoxylosilyticus ER4B. *Data in Brief*, 40, 107764. doi: 10.1016/j.dib.2021.107764

Christensen, L.F. et al., 2022. Extracellular microbial proteases with specificity for plant proteins in food fermentation. *International Journal of Food Microbiology*, 381, 109889. doi: 10.1016/j.ijfoodmicro.2022.109889

Cowan, D.A., 1994. Industrial enzymes. In B*iotechnology: The Science and the Business, 2nd ed.* New York, NY, USA: Harwood Academic Publishers.

Cuadros-Orellana, S., Pohlschröder, M. & Durrant, L.R., 2006. Isolation and characterization of halophilic archaea able to grow in aromatic compounds. *International Biodeterioration & Biodegradation*, 57, pp.151–154. doi: 10.1016/j.ibiod.2005.04.005.

Dai, K. et al., 2023. Metabolic engineering of Thermoanaerobacterium aotearoense strain SCUT27 for biofuels production from sucrose and molasses. *Biotechnology for Biofuels and Bioproducts*, 16(1), 155. doi: 10.1186/s13068-023-02402-3

Das, G. & Prasad, M. P., 2010. Isolation, purification & mass production of protease enzyme from Bacillus subtilis. *International Research Journals of microbiology*, 1(2), pp.26-31.

Das, N. et al., 2015. Progress in the development of gelling agents for improved culturability of microorganisms. *Frontiers in Microbiology*, 6, 698. doi: 10.3389/fmicb.2015.00698

D'Souza, D.R. et al., 1997. Isolation of thermophilic bacteria using bacteriological grade agar at temperatures above 80°C. *Biotechniques*, 22(6), pp.1078. doi: 10.2144/97226bm14.

Deepa, S., Kanimozhi, K. & Panneerselvam, A., 2014. 16S rDNA phylogenetic analysis of actinomycetes isolated from marine environment associated with antimicrobial activities. *Journal for Drugs and Medicines*, 5(2), pp.43 -50.

Devaraj, K. et al., 2019. Production of thermostable multiple enzymes from Bacillus amyloliquefaciens KUB29. *Natural product research*, 33(11), pp.1674-1677. doi: 10.1080/14786419.2018.1425857

Drejer, E.B. et al., 2018. Genetic tools and techniques for recombinant expression in thermophilic Bacillaceae. *Microorganisms*, 6(2), 42. doi: 10.3390/microorganisms6020042.

Dresler, K., et al., 2006. Production of a recombinant polyester-cleaving hydrolase from Thermobifida fusca in *Escherichia coli. Bioprocess and Biosystems Engineering*, 29, pp.169-183. doi: 10.1007/s00449-006-0069-9.

Ebara, S. & Shigemori, Y., 2008. Alkali-tolerant high-activity catalase from a thermophilic bacterium and its overexpression in *Escherichia coli. Protein Expression and Purification*, 57(2), pp.255-260. doi: 10.1016/j.pep.2007.09.015

Fachrial, E.D.Y. et al., 2020. Molecular identification of cellulase and protease producing Bacillus tequilensis UTMSA14 isolated from the geothermal hot spring in Lau Sidebuk Debuk, North Sumatra, Indonesia. *Biodiversitas Journal of Biological Diversity*, 21(10), pp.4719-4725. doi: 10.13057/biodiv/d211035

Fall, I. et al., 2023. A thermostable bacterial catalase-peroxidase oxidizes phenolic compounds derived from lignins. *Applied Microbiology and Biotechnology*, 107(1), pp.201-217. doi: 10.1007/s00253-022-12263-9

Fang, Y. et al., 2021. Preparation and characterization of a novel thermostable lipase from Thermomicrobium roseum. *Catalysis Science & Technology*, 11(22), pp.7386-7397. doi: 10.1039/D1CY01486B

Fazal, B.Z. et al., 2022. Screening, isolation, and characterization of amylase-producing bacteria from Poring Hot Spring Sabah, Malaysia. *Biodiversitas Journal of Biological Diversity*, 23(6), pp.2807-2815. doi:10.13057/biodiv/d230604

Fincan, S.A. & Enez, B., 2014. Production, purification, and characterization of thermostable α-amylase from thermophilic Geobacillus stearothermophilus. *Starch-Stärke*, 66(1-2), pp. 182-189. doi: 10.1002/star.201200279

Finch, A.J. & Kim, J.R., 2018. Thermophilic proteins as versatile scaffolds for protein engineering. *Microorganisms*, 6(4), 97. doi: 10.3390/microorganisms6040097

Fossi, B.T. et al., 2014. Microbial interactions for enhancement of α-amylase production by *Bacillus amyloliquefaciens* 04BBA15 and *Lactobacillus fermentum* 04BBA19. *Biotechnology Reports*, 4, pp.99-106. doi: 10.1016/j.btre.2014.09.004.

Frolov, E.N. et al., 2018. Desulfothermobacter acidiphilus gen. nov., sp. nov., a thermoacidophilic sulfate-reducing bacterium isolated from a terrestrial hot spring. *International Journal of Systematic and Evolutionary Microbiology*, 68(3), pp.871-875. doi: 10.1099/ijsem.0.002599

Geraldi, A. et al., 2022. Isolation and characterization of thermophilic *Bacillus subtilis* subsp. inaquosorum CGR-1 from Cangar hot springs. *Journl of Bio-Molecule Research and Engineering*, 1(1), pp.32-39. doi: 10.20473/jbiome.v1i1.35860.

Ginting, E.L. et al., 2021. Isolation and identification of thermophilic amylolytic bacteria from Likupang Marine Hydrothermal, North Sulawesi, Indonesia. *Biodiversitas Journal of Biological Diversity*, 22(6), pp.3326-3332. doi.: 10.13057/biodiv/d220638.

Gramza-Michałowska, A. & Stachowiak, B., 2010. The antioxidant potential of carotenoid extract from Phaffia rhodozyma. *Acta Scientiarum Polonorum Technologia Alimentaria*, 9(2), pp.171-188.

Gurumurthy, D.M. et al., 2020. Cyanoxanthomycin, a Bacterial Antimicrobial Compound Extracted from Thermophilic *Geobacillus* sp. Iso5. *Jordan Journal of Biological Sciences*, 13, pp.725-729.

Habibie, F.M., Wardani, A.K. & Nurcholis, M., 2014. Isolation and molecular identification of thermophilic microorganism producing xylanase from Hot Mud Disaster Lapindo. *Jurnal Pangan dan Agroindustri*, 2(4), pp.231 -238.

Hafsan, H., Ramadani, K. & Abbas, A., 2021. Protease Activity Of Thermophilic Bacteria From Lejja Hot Springs In Soppeng South Sulawesi. *JST (Jurnal Sains dan Teknologi)*, 10(2), pp.211-219.

Hamana, K. & Matsuzaki, S., 1985. Further study on polyamines in primitive unicellular eukaryotic algae. *The Journal of Biochemistry*, 97(5), pp.1311-1315. doi: 10.1093/oxfordjournals.jbchem.a135182.

Heidari, F. et al., 2012. Antimicrobial activity of cyanobacteria isolated from hot spring of Geno. *Middle-East Journal of Scientific Research*, 12(3), pp.336-339. doi: 10.5829/idosi.mejsr.2012.12.3.64169.

Hreggvidsson, G.O. et al., 2012. Microbial speciation in the geothermal ecosystem. In *Adaption of microbial life to environmental extremes: novel research results and application.* Vienna: Springer Vienna, pp.37-67. doi: 10.1007/978-3-211-99691-1_3.

Ifandi, S. & Alwi, M., 2015. Isolation of thermophilic bacteria from Bora hot springs in Central Sulawesi. *Biosaintifika: Journal of Biology & Biology Education*, 10(2), pp.291-297. doi: 10.15294/biosaintifika.v10i2.14905.

Indriati, G. & Megahati, R.R.P., 2018. Isolation of thermophilic bacteria and optimizing the medium growth conditions. *International Journal of Current Microbiology and Applied Science*, 7(1), pp.1457-1464. doi: 10.20546/ijcmas.2018.701.177

Iqbal, I. et al., 2015. Purification and characterization of cloned alkaline protease gene of Geobacillus stearothermophilus. *Journal of Basic Microbiology*, 55(2), pp.160-171. doi: 10.1002/jobm.201400190

Irdawati, I. et al., 2018. Screening of Thermophilic Bacteria Produce Xylanase from Sapan Sungai Aro Hot Spring South Solok. *IOP Conference Series: Materials Science and Engineering*, 335(1), 012021. doi: 10.1088/1757-899X/335/1/012021.

Irdawati, I. et al., 2023. Effect of the Thermophilic Bacterial Biculture Consortium from Mudiak Sapan Hot Springs on Biofuel Production. *Jurnal Penelitian Pendidikan IPA*, 9(10), pp.9032-9037. doi: 10.29303/jppipa.v9i10.3597

Isono, K. et al., 1967. Studies on polyoxins, antifungal antibiotics: Part v. isolation and characterization of polyoxins c, d, e, f, g, h and i. *Agricultural and Biological Chemistry*, 31(2), pp.190-199. doi: 10.1080/00021369.1967.10858788

Jabeen, F. et al., 2019. Isolation of thermophilic Anoxybacillus beppuensis JF84 and production of thermostable amylase utilizing agro–dairy wastes. *Environmental Progress & Sustainable Energy*, 38(2), pp.417-423. doi: 10.1002/ep.12991

Jain, R., Anjaiah, V. & Babbar, S.B., 2005. Guar gum: a cheap substitute for agar in microbial culture media. *Letters in Applied Microbiology*, 41, pp.345–349. doi: 10.1111/j.1472-765X.2005.01760.x.

Jain, R., 2011. Evaluation of blends of alternative gelling agents with agar and development of xanthagar, a gelling mix, suitable for plant tissue culture media. *Asian Journal of Biotechnology*, 3, pp.153-164. doi: 10.3923/ajbkr.2011.153.164.

Jeyabalan, J., Veluchamy, A. & Narayanasamy, S., 2025. Production optimization, characterization, and application of a novel thermo-and pH-stable laccase from Bacillus drentensis 2E for bioremediation of industrial dyes. *International Journal of Biological Macromolecules*, 308(Pt 3), 142557. doi: 10.1016/j.ijbiomac.2025.142557

Jia, X. et al., 2016. Cloning, expression, and characterization of a novel thermophilic monofunctional catalase from *Geobacillus* sp. CHB1. *BioMed Research International*, 2016(1), 7535604. doi: 10.1155/2016/7535604.

Johnvesly, B. & Naik, G.R., 2001. Studies on production of thermostable alkaline protease from thermophilic and alkaliphilic Bacillus sp. JB-99 in a chemically defined medium. *Process biochemistry*, 37(2), pp.139-144. doi: 10.1016/S0032-9592(01)00191-1

Jones, K.L., 1948. Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. *Journal of Bacteriol*, 57, pp.141–145. doi: 10.1128/jb.57.2.141-145.1949.

Kagawa, M. et al., 1999. Purification and cloning of a thermostable manganese catalase from a thermophilic bacterium. *Archives of Biochemistry and Biophysics*, 362(2), pp.346-355. doi: 10.1006/abbi.1998.1041.

Kambourova, M., 2018. Thermostable enzymes and polysaccharides produced by thermophilic bacteria isolated from Bulgarian hot springs. *Engineering in Life Sciences*, 18(11), pp.758-767. doi: 10.1002/elsc.201800022.

Kang, K.S. et al., 1982. Agar-like polysaccharide produced by a *Pseudomonas* species: production and basic properties. *Applied and Environental Microbiology*, 43, pp.1086–1091. doi: 10.1128/aem.43.5.1086-1091.1982.

Karaseva, A.I. et al., 2024. Microbial diversity of hot springs of the Kuril Islands. *BMC microbiology*, 24(1), 547. doi: 10.1186/s12866-024-03704-8.

Kato, S. et al., 2018. Long-term cultivation and metagenomics reveal ecophysiology of previously uncultivated thermophiles involved in biogeochemical nitrogen cycle. *Microbes and environments*, 33(1), pp.107-110. doi: 10.1264/jsme2.ME17165

Kaur, S., Vasiljevic, T. & Huppertz, T., 2023. Milk Protein Hydrolysis by Actinidin—Kinetic and Thermodynamic Characterisation and Comparison to Bromelain and Papain. *Foods*, 12(23), 4248. doi: 10.3390/foods12234248

Kawai, F., Kawabata, T. & Oda, M., 2020. Current state and perspectives related to the polyethylene terephthalate hydrolases available for biorecycling. *ACS Sustainable Chemistry & Engineering*, 8(24), pp.8894-8908. doi: 10.1021/acssuschemeng.0c01638

Khan, Z. et al., 2023. Protease from Bacillus subtilis ZMS-2: Evaluation of production dynamics through Response Surface Methodology and application in leather tannery. *Journal of King Saud University-Science*, 35 (4), pp.102643. doi: 10.1016/j.jksus.2023.102643

Kitadokoro, K. et al., 2018. Expression, Purification and Crystallization of Thermostable Mutant of Cutinase Est1 from Thermobifida alba. *Advances in Bioscience and Biotechnology*, 9(05), 215. doi: 10.4236/abb.2018.95015

Klein, V.J. et al., 2023. Metabolic engineering of thermophilic Bacillus methanolicus for riboflavin overproduction from methanol. *Microbial Biotechnology*, 16(5), pp.1011-1026. doi: 10.1111/1751-7915.14239

Knapik, K., Becerra, M. & González-Siso, M.I., 2019. Microbial diversity analysis and screening for novel xylanase enzymes from the sediment of the Lobios Hot Spring in Spain. *Scientific Reports*, 9(1), pp.11195. doi: 10.1038/s41598-019-47637-z.

Knight, C.A. et al., 2018. The first report of antifungal lipopeptide production by a *Bacillus subtilis* subsp. inaquosorum strain. *Microbiological Research*, 216, pp.40-46. doi: 10.1016/j.micres.2018.08.001.

Kong, L. et al., 2022. CRISPR/dCas9-based metabolic pathway engineering for the systematic optimization of exopolysaccharide biosynthesis in Streptococcus thermophilus. *Journal of Dairy Science*, 105(8), pp.6499-6512. doi: 10.3168/jds.2021-21409

Koonin, E.V., 2010. The two empires and three domains of life in the post-genomic age. *Nature Education*, 3(9), 27.

Kristjánsson, J.K. & Hreggvidsson, G.O., 1995. Ecology and habitats of extremophiles. *World Journal of Microbiology & Biotechnology*, 11(1), pp.17–25. doi: 10.1007/BF00339134

Kuo, S.M. et al., 2014. Evaluation of the ability of xanthan gum/gellan gum/hyaluronan hydrogel membranes to prevent the adhesion of postrepaired tendons. *Carbohydrate Polymers*, 114, pp.230-237. doi: 10.1016/j.carbpol.2014.07.049.

Kusdiyantini, E. et al., 2017. Molecular and biochemical characterization of pink-pigmented thermophile bacteria (GDG IX) from Gedongsongo hot -spring in Bandungan–Semarang. *Advanced Science Letters*, 23(7), pp.6421-6423. doi: 10.1166/asl.2017.9641

Laksmi, F.A. et al., 2025. A robust strategy for overexpression of DNA polymerase from Thermus aquaticus using an IPTG-independent autoinduction system in a benchtop bioreactor. *Scientific Reports*, 15(1), 5891. doi: 10.1038/s41598-025-89902-4

Lee, Y.J. et al., 2022. Isolation and characterization of thermophilic bacteria from hot springs in Republic of Korea. *Microorganisms*, 10(12), pp.2375. doi: 10.3390/microorganisms10122375.

Li, A.N. & Li, D.C., 2009. Cloning, expression and characterization of the serine protease gene from Chaetomium thermophilum. *Journal of applied microbiology*, 106(2), pp.369-380. doi: 10.1111/j.1365-2672.2008.04042.x

Li, A.N. et al., 2007. Purification and characterization of two thermostable proteases from the thermophilic fungus Chaetomium thermophilum. *Journal of Microbiology and Biotechnology*, 17(4), pp.624-631.

Li, T. et al., 2023. Effect of inoculating thermophilic bacterial consortia on compost efficiency and quality. *Waste Management*, 170, pp.341-353. doi: 10.1016/j.wasman.2023.09.023

Lin, C.L. et al., 2022. Towards lager beer aroma improvement via selective amino acid release by proteases during mashing. *Journal of the Institute of Brewing*, 128(1), pp.15-21. doi: 10.1002/jib.682

Lin, C.L. et al., 2023. Increasing Higher Alcohols and Acetates in Low-Alcohol Beer by Proteases. *Molecules*, 28(11), pp.4419. doi: 10.3390/molecules28114419

Lines, A.D., 1977. Value of the K+ salt of carageenan as an agar substitute in routine bacteriological media. *Applied and Environmental Microbiology*, 34, pp.637-639. doi: 10.1128/aem.34.6.637-639.1977.

Lischer, K. et al., 2020. The emergence and rise of indigenous thermophilic bacteria exploration from hot springs in Indonesia. *Biodiversitas Journal of Biological Diversity*, 21(11), pp.5474-5481. doi: 10.13057/biodiv/d211156.

Lischer, K., 2021. Identification of Thermophilic Bacteria from Tirta Lebak Buana Hot Spring in Serang, Banten, Indonesia. *Makara Journal of Technology*, 25(3), pp.142-1466. doi: 10.7454/mst.v25i3.3993.

Liu, D., Guo, Y. & Ma, H., 2023. Production of value-added peptides from agro-industrial residues by solid-state fermentation with a new thermophilic protease-producing strain. *Food Bioscience*, 53, pp.102534. doi: 10.1016/j.fbio.2023.102534

Liu, H., Kheirvari, M. & Tumban, E., 2023. Potential applications of thermophilic bacteriophages in one health. *International Journal of Molecular Sciences*, 24(9), pp.8222. doi: 10.3390/ijms24098222

Lizama, C. et al., 2001. Taxonomic study of extreme halophilic archaea isolated from the "Salar de Atacama", Chile. *Systematic and Applied Microbiology*, 24(3), pp.464-474. doi: 10.1078/0723-2020-00053.

López, M. J. et al., 2021. Characterization of thermophilic lignocellulolytic microorganisms in composting. *Frontiers in microbiology*, 12, 697480. doi: 10.3389/fmicb.2021.697480

Madigan, M.T., Martinko, J.M. & Parker, J., 1997. *Brock Biology of Microorganisms, 8th edn*, Englewood Cliffs: Prentice-Hall Inc.

Magalhães, A. et al., 2007. Purification and properties of a coagulant thrombin-like enzyme from the venom of Bothrops leucurus. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 146(4), pp.565-575. doi: 10.1016/j.cbpa.2005.12.033

Mahakhan, P. et al., 2022. Alkaline protease production from Bacillus gibsonii 6BS15-4 using dairy effluent and its characterization as a laundry detergent additive. *Journal of Microbiology and Biotechnology*, 33(2), pp.195-202. doi: 10.4014/jmb.2210.10007

Mahestri, L., Harpeni, E. & Setyawan, A., 2021. Isolation and Screening of Amylolytic and Proteolytic Thermophilic Bacteria from Way Panas Hot Spring, Kalianda, South Lampung. *Jurnal Perikanan dan Kelautan*, 26(3), pp.161-168.

Maitig, A.M.A., Alhoot, M.A. & Tiwari, K., 2018. Isolation and screening of extracellular protease enzyme from fungal isolates of soil. *Journal of Pure & Applied Microbiology*, 12(4), pp.2059-2067. doi: 10.22207/JPAM.12.4.42

Majithiya, V.R. & Gohel, S.D., 2025. Agro-industrial Waste Utilization, Medium Optimization, and Immobilization of Economically Feasible Halo-Alkaline Protease Produced by Nocardiopsis dassonvillei Strain VCS-4. *Applied Biochemistry and Biotechnology*, 197(1), pp.545-569. doi: 10.1007/s12010-024-05057-4

Maksum, I. P. et al., 2022. Overexpression of soluble recombinant Thermus thermophilus (Tth) DNA polymerase in *Escherichia coli* BL21 (DE3) using an MBP fusion tag as a solubility enhancer. *Journal of Applied Pharmaceutical Science*, 12(9), pp.017-024. doi: 10.7324/JAPS.2022.120903

Manalu, J., Rahmawati & Hidayat, N., 2019. Antifungal activity of Actinomycetes isolates from hot springs Ai Sipatn Lotup Sanggau against isolate *Hortaea werneckii* (T1). *Protobiont*, 8(1), pp.69-77.

Manikandan, M., Pašić, L. & Kannan, V., 2009. Optimization of growth media for obtaining high-cell density cultures of halophilic archaea (family Halobacteriaceae) by response surface methodology. *Bioresource Technology*, 100(12), pp.3107-3112. doi: 10.1016/j.biortech.2009.01.033.

Marin-Sanhueza, C. et al., 2022. Stress dependent biofilm formation and bioactive melanin pigment production by a thermophilic bacillus species from Chilean hot spring. *Polymers*, 14(4), 680. doi: 10.3390/polym14040680

Marteinsson, V.T. et al., 1996. Numerical taxonomic study of thermophilic *Bacillus* isolated from three geographically separated deep-sea hydrothermal vents. *FEMS Microbiology Ecology*, 21(4), pp.255-266. doi: 10.1111/j.1574-6941.1996.tb00122.x.

Masum, M. & Akbar, M.A., 2019. The Pacific ring of fire is working as a home country of geothermal resources in the world. *IOP Conference Series: Earth and Environmental Science*, 249, 012020. doi: 10.1088/1755-1315/249/1/012020.

Mawati, S.D., Harpeni, E. & Fidyandini, H.P., 2021. Screening of Amylolitic Potential Thermophilic Bacteria From Way Belerang Hot Spring Kalianda Lampung Selatan. *Journal of Aquatropica Asia*, 6(1), pp.1-7.

McLachlan, J., 1985. Macroalgae (seaweeds): industrial resources and their utilization. *Plant Soil*, 89, pp.137-157. doi: 10.1007/BF02182240.

Mehta, D. & Satyanarayana, T., 2016. Bacterial and archaeal α-amylases: diversity and amelioration of the desirable characteristics for industrial applications. *Frontiers in Microbiology*, 7, 1129. doi: 10.3389/fmicb.2016.01129.

Mevarech, M. & Werczberger, R., 1985. Genetic transfer in Halobacterium volcanii. *Journal of Bacteriology*, 162, pp.461–462. doi: 10.1128/jb.162.1.461-462.1985.

Mohammad, B.T. et al., 2017. Isolation and characterization of thermophilic bacteria from Jordanian hot springs: *Bacillus licheniformis* and Thermomonas hydrothermalis isolates as potential producers of thermostable enzymes. *International Journal of Microbiology*, (1), pp.6943952. doi: 10.1155/2017/6943952

Moonnee, Y.A. et al., 2021. Keratinolytic protease from Pseudomonas aeruginosa for leather skin processing. *Journal of Genetic Engineering and Biotechnology*, 19, 53. doi: 10.1186/s43141-021-00149-8

Msarah, M.J. et al., 2020. Optimisation and production of alpha amylase from thermophilic *Bacillus* spp. and its application in food waste biodegradation. *Heliyon*, 6(6), e04183. doi: 10.1016/j.heliyon.2020.e04183.

Mudiyanselage, J.M.B.W.W. et al., 2021. Cloning of DNA Polymerase-I Gene from Thermophilic *Bacillus licheniformis* Strain NWMF1 into an *E. coli* Expression System. *Journal of Microbiology, Biotechnology and Food Sciences*, 2021, pp.1317-1319. doi: 10.15414/jmbfs.2019.8.6.1317-1319

Muharni, M., Juswardi, J. & Prihandayani, I., 2013. Isolasi dan identifikasi bakteri termofilik penghasil protease dari sumber air panas Tanjung Sakti Lahat Sumatera Selatan. *Prosiding SEMIRATA FMIPA Universitas Lampung*, 1(1), pp.139-143.

Murtiyaningsih, H., Arum, L. S. & Anggriawan, R., 2022. Selection of Polymerase-Producing Thermophilic Bacteria from Ijen Crater, East Java. *Indonesian Journal of Biotechnology and Biodiversity*, 6(1), pp.26-32.

Mushtaq, A. et al., 2024. Cloning, Expression, and Characterization of a Metalloprotease from Thermophilic Bacterium Streptomyces thermovulgaris. *Biology*, 13(8), 619. doi: 10.3390/biology13080619.

Nababan, M., Gunam, I.B. & Wijaya, I.M., 2019. Produksi enzim selulase kasar dari bakteri selulolitik. *Jurnal Rekayasa dan Manajemen Agroindustri*, 7(2), pp.190-199.

Nascimento, W.C.A.D. & Martins, M.L.L., 2004. Production and properties of an extracellular protease from thermophilic Bacillus sp. *Brazilian journal of microbiology*, 35, pp.91-96. doi: 10.1590/S1517-83822004000100015

Neog, P. R., Saini, S. & Konwar, B. K., 2024. Purification, and characterization of detergent-compatible serine protease from Bacillus safensis strain PRN1: A sustainable alternative to hazardous chemicals in detergent industry. *Protein Expression and Purification*, 219, 106479. doi: 10.1016/j.pep.2024.106479

Nishihara, A. et al., 2018. Nitrogenase activity in thermophilic chemolithoautotrophic bacteria in the phylum Aquificae isolated under nitrogen-fixing conditions from Nakabusa hot springs. *Microbes and environments*, 33(4), pp.394-401. doi: 10.1264/jsme2.ME18041

Nuritasari, D., Sarjono, P.R. & Aminin, A.L., 2017. Isolasi bakteri termofilik sumber air panas gedongsongo dengan media pengaya MB (Minimal Broth) dan TS (Taoge Sukrosa) serta identifikasi fenotip dan genotip. *Jurnal Kimia Sains dan Aplikasi*, 20(2), pp.84-91. doi: 10.14710/jksa.20.2.84-91.

Obeidat, M. & Al-Shomali, B., 2023. Moderately Thermophilic Bacteria from Jordanian Hot Springs as Possible Sources of Thermostable Enzymes and Leukemia Cytotoxic Agents. *Jordan Journal of Biological Sciences*, 16 (3), pp.413-424. doi: 10.54319/jjbs/160304

Octarya, Z., Nugroho, T.T. & Nurulita, Y., 2022. Molecular Identification, GC-MS Analysis of Bioactive Compounds and Antimicrobial Activity of Thermophilic Bacteria Derived from West Sumatra Hot-Spring Indonesia. *HAYATI Journal of Biosciences*, 29(4), pp.549-561. doi: 10.4308/hjb.29.4.549-561

Oda, M. et al., 2021. Cutinases from thermophilic bacteria (actinomycetes): From identification to functional and structural characterization. *Methods in Enzymology*, 648, pp.159-185. doi: 10.1016/bs.mie.2020.12.031.

Oren, A., 1983. *Halobacterium sodomense* sp. nov., a Dead Sea halobacterium with an extremely high magnesium requirement. *International Journal of Systematic and Evolutionary Microbiology*, 33(2), pp.381-386. doi: 10.1099/00207713-33-2-381.

Ovando-Chacon, S.L. et al., 2020. Characterization of thermophilic microorganisms in the geothermal water flow of el chichón volcano crater lake. *Water*, 12(8), 2172. doi: 10.3390/w12082172.

Özdemir, F.İ. et al., 2022. Biochemical characterization and detection of antitumor activity of l-asparaginase from thermophilic Geobacillus kaustophilus DSM 7263T. *Protein Expression and Purification*, 199, 106146. doi: 10.1016/j.pep.2022.106146

Pan, X. et al., 2019. Study on molecular mechanisms of nattokinase in pharmacological action based on label-free liquid chromatography–tandem mass spectrometry. *Food Science & Nutrition*, 7(10), pp.3185-3193. doi: 10.1002/fsn3.1157

Panahi, H.K.S. et al., 2022. Engineered bacteria for valorizing lignocellulosic biomass into bioethanol. *Bioresource technology*, 344, 126212. doi: 10.1016/j.biortech.2021.126212

Panda, M.K., Sahu, M.K. & Tayung, K., 2013. Isolation and characterization of a thermophilic Bacillus sp. with protease activity isolated from hot spring of Tarabalo, Odisha, India. *Iranian Journal of Microbiology*, 5(2), pp.159-165.

Pandey, A. et al., 2015. Thermophilic bacteria that tolerate a wide temperature and pH range colonize the Soldhar (95 C) and Ringigad (80 C) hot springs of Uttarakhand, India. *Annals of microbiology*, 65, pp.809-816. doi: 10.1007/s13213-014-0921-0

Patil, A. et al., 2024. Biochemical, Molecular Characteristics, and Bioremediation Properties of Mn2+-Resistant Thermophilic Bacillus Strains. *Waste and Biomass Valorization*, 16, pp.175–190. doi: 10.1007/s12649-024-02713-y

Payne, J.I., Sehgal, S.N. & Gibbons, N.E., 1960. Immersion refractometry of some halophilic bacteria. *Canadian Journal of Microbiology*, 6(1), pp.9-15. doi: 10.1139/m60-002

Peng, L. et al., 2024. Metagenomic analysis of a thermophilic bacterial consortium and its use in the bioremediation of a petroleum-contaminated soil. *Chemosphere*, 360, 142379. doi: 10.1016/j.chemosphere.2024.142379

Prajapati, V.D. et al., 2013. An insight into the emerging exopolysaccharide gellan gum as a novel polymer. *Carbohydrate polymers*, 93, pp.670-678. doi: 10.1016/j.carbpol.2013.01.030.

Prasad, P., Tanuja, B.S. & Bedi, S., 2014. Characterization of a novel thermophilic cellulase producing strain Streptomyces matensis strain St-5. *International Journal of Current Microbiology and Applied Sciences*, 3(3), pp.74-88.

Pugazhendi, A. et al., 2017. Biodegradation of low and high molecular weight hydrocarbons in petroleum refinery wastewater by a thermophilic bacterial consortium. *Environmental technology*, 38(19), pp.2381-2391. doi: 10.1080/09593330.2016.1262460

Putri, L.D., Natsir, H. & Dali, S., 2017. Thermophilic Xylanase Production Rom Isolates of Macula'Hot Springs Bacteria Using Corn Cobs Waste Media. *Jurnal Akta Kimia Indonesia (Indonesia Chimica Acta)*, 10(2), pp.25-41. doi: 10.20956/ica.v10i2.6652.

Rafiee, Z. et al., 2024. Unveiling Antibacterial Potential and Physiological Characteristics of Thermophilic Bacteria Isolated from a Hot Spring in Iran. *Microorganisms*, 12(4), 834. doi: 10.3390/microorganisms12040834.

Raj, A. et al., 2018. Production and purification of xylanase from alkaliphilic *Bacillus licheniformis* and its pretreatment of eucalyptus kraft pulp. B*iocatalysis and Agricultural Biotechnology*, 15, pp.199-209. doi: 10.1016/j.bcab.2018.06.018.

Rakhmawati, A., Wahyuni, E.T. & Yuwono, T., 2021. Thermophilic bacteria isolated from Mount Merapi, Java, Indonesia as a potential lead bioremediation agent. *Biodiversitas Journal of Biological Diversity*, 22(6), pp.3101-3110. doi: 10.13057/biodiv/d220612

Ramadhan, R.F., Montesqrit, M. & Marlida, Y., 2020. Production of Thermostable Cellulase Enzyme by NG2 Bacteria Using Various Cellulose Sources from the Agriculture Waste. *Jurnal Ilmu dan Teknologi Peternakan*, 8(2), pp.64-72. doi: 10.20956/jitp.v8i2.8171.

Rollof, J., HedstrÖM, S.Å. & Nilsson-Ehle, P., 1987. Lipolytic activity of Staphylococcus aureus strains from disseminated and localized infections. *Acta Pathologica Microbiologica Scandinavica Series B: Microbiology*, 95(1-6), pp.109-113. doi: 10.1111/j.1699-0463.1987.tb03096.x.

Saadati, M. et al., 2024. Cloning and Production of Protease Enzyme from *Aeribacillus pallidus* P18 Strain. *Journal of Pure & Applied Microbiology*, 18(2), pp.1326-1335. doi: 10.22207/JPAM.18.2.56.

Sabaria, E. et al., 2024. Characterization of thermophilic bacteria from Ie Seum Hot Springs, Aceh Besar, Indonesia as producers of protease enzyme. *Biodiversitas Journal of Biological Diversity*, 25(5), pp.1867-1874. doi: 10.13057/biodiv/d250502

Sadeepa, D., Sirisena, K. & Manage, P.M., 2022. Diversity of microbial communities in hot springs of Sri Lanka as revealed by 16S rRNA gene high-throughput sequencing analysis. *Gene*, 812, 146103. doi: 10.1016/j.gene.2021.146103.

Sadettin, S. & Dönmez, G., 2006. Bioaccumulation of reactive dyes by thermophilic cyanobacteria. *Process Biochemistry*, 41(4), pp.836-841. doi: 10.1016/j.procbio.2005.10.031.

Sahay, S., 1999. The use of psyllium (isubgol) as an alternative gelling agent for microbial culture media. *World Journal of Microbiology and Biotechnology*, 15, pp.733–735. doi: 10.1023/A:1008954128637.

Sako, Y., et al., 1996. *Rhodothermus obamensis* sp. nov., a modern lineage of extremely thermophilic marine bacteria. *International Journal of Systematic and Evolutionary Microbiology*, 46(4), pp.1099-1104.

Saksono, B. & Sukmarini, L., 2010. Structural analysis of xylanase from marine Thermophilic *Geobacillus stearothermophilus* in Tanjung Api, Poso, Indonesia. *Hayati Journal of Biosciences*, 17(4), pp.189-195. doi: 10.4308/hjb.17.4.189.

Sari, D.C. et al., 2020. Aerial mycelium formation in rare thermophilic Actinobacteria on media solidified with agar and gellan gum. *IOP Conference Series: Earth and Environmental Science*, 483(1), 012017. doi: 10.1088/1755-1315/483/1/012017.

Satarzadeh, N. et al., 2024. Purification, Characterization, and Assessment of Anticancer Activity of Iron Oxide Nanoparticles Biosynthesized by Novel Thermophilic Bacillus tequilensis ASFS1. *Journal of Basic Microbiology*, 64(9), e2400153. doi: 10.1002/jobm.202400153

Schultz, J. et al., 2022. Unraveling the genomic potential of the thermophilic bacterium *Anoxybacillus flavithermus* from an Antarctic geothermal environment. *Microorganisms*, 10(8), 1673. doi: 10.3390/microorganisms10081673.

Sembiring, A., 2019. Isolasi dan uji aktivitas bakteri penghasil selulase asal tanah kandang sapi. *BIOSEL (Biology Science and Education): Jurnal Penelitian Science dan Pendidikan*, 8(1), pp.21-28. doi: 10.33477/bs.v8i1.843.

Shaeer, A. et al., 2022. Looking into a highly thermostable and efficient recombinant manganese-catalase from Geobacillus thermopakistaniensis. *Journal of bioscience and bioengineering*, 133(1), pp.25-32. doi: 10.1016/j.jbiosc.2021.09.012

Sharif, S. et al., 2023. Optimization of amylase production using response surface methodology from newly isolated thermophilic bacteria. *Heliyon*, 9 (1), e12901. doi: 10.1016/j.heliyon.2023.e12901

Sharma, A.K. et al., 2015. Isolation and screening of extracellular protease enzyme from bacterial and fungal isolates of soil. *International Journal of Scientific Research in Environmental Sciences*, 3(9), pp.0334-0340. doi: 10.12983/ijsres-2015-p0334-0340

Sharma, N. & Leung, I. K., 2021. Novel thermophilic bacterial laccase for the degradation of aromatic organic pollutants. *Frontiers in Chemistry*, 9, 711345. doi: 10.3389/fchem.2021.711345

Sharma, N. et al., 2013. Comparative study of potential cellulolytic and xylanolytic bacteria isolated from compost and their optimization for industrial use. *Journal of Agroalimentary Processes and Technologies*, 19(3), pp.284-297.

Sharma, V., Ayothiraman, S. & Dhakshinamoorthy, V., 2019. Production of highly thermo-tolerant laccase from novel thermophilic bacterium *Bacillus* sp. PC-3 and its application in functionalization of chitosan film. *Journal of Bioscience and Bioengineering*, 127(6), pp.672-678. doi: 10.1016/j.jbiosc.2018.11.008

Shimomura, K. & Kamada, H., 1986. Roles of gelling agents in plant tissue culture. *Plant Tissue Culture Letters*, 3(1), pp.38-41. doi: 10.5511/plantbiotechnology1984.3.38.

Shirling, E.B. & Gottlieb, D., 1966. Methods for characterization of Streptomyces species. *International Journal of Systematic and Evvolutionary Microbiology.*, 16(3), pp.313–340. doi: 10.1099/00207713-16-3-313.

Shukla, R.J. & Singh, S.P., 2015. Production optimization, purification and characterization of α-amylase from thermophilic *Bacillus licheniformis* TSI-14. *Starch-Stärke*, 67(7-8), pp.629-639. doi: 10.1002/star.201500046

Siangbood, H. & Ramanujam, P., 2011. A report on thermophilic cyanophyta (cyanobacteria) from Jakrem Hotspring, Meghalaya. *International Journal on Algae*, 13(2), pp.178-185. doi: 10.1615/InterJAlgae.v13.i2.70.

Silaban, S., Sihotang, N.I.Y. & Gurning, K., 2021. Isolation and Characterization of Thermophilic Bacteria as Amylase Enzyme Produced by Hots Spring in Rianiate Samosir, Indonesia. *Rasayan Journal of Chemistry*, 14 (2), pp.99-109.

Silva, V. et al., 2022. Optimization of key factors affecting hydrogen and ethanol production from xylose by Thermoanaerobacterium calidifontis VCS1 isolated from vinasse treatment sludge. *Waste and Biomass Valorization*, 13, pp.1897–1912. doi: 10.1007/s12649-021-01635-3

Simair, A.A. et al., 2017. Production and partial characterization of α-amylase enzyme from *Bacillus* sp. BCC 01-50 and potential applications. *BioMed Research International*, 2017, 9173040. doi: 10.1155/2017/9173040.

Singh, R. et al., 2025. Development of a process for enhanced biogas production from lignocellulosic feedstocks using an efficient thermophilic inoculum and its metagenomic study. *Biocatalysis and Agricultural Biotechnology*, 64, 103519. doi: 10.1016/j.bcab.2025.103519

Sittipol, D. et al., 2019. Cloning, expression, purification and characterization of a thermo-and surfactant-stable protease from Thermomonospora curvata. *Biocatalysis and Agricultural Biotechnology*, 19, 101111. doi: 10.1016/j.bcab.2019.101111

Soy, S., Nigam, V.K. & Sharma, S.R., 2021. Enhanced production and biochemical characterization of a thermostable amylase from thermophilic bacterium Geobacillus icigianus BITSNS038. *Journal of Taibah University for Science*, 15(1), pp.730-745. doi: 10.1080/16583655.2021.2002549

Soy, S. et al., 2023. Exploring microbial diversity in hot springs of Surajkund, India through 16S rRNA analysis and thermozyme characterization from endogenous isolates. *Scientific Reports*, 13(1), 14221. doi: 10.1038/s41598-023-41515-5

Stetter, K.O., 1996. Hyperthermophilic procaryotes. *FEMS microbiology reviews*, 18(2-3), pp.149-158. doi: 10.1111/j.1574-6976.1996.tb00233.x

Suddin, S. et al., 2019. Molecular barcoding based 16S rRNA gene of Thermophilic bacteria from vulcanic sites, Linow Lake, Tomohon. *Materials Science Forum*, 967, pp.83-92. doi: 10.4028/www.scientific.net/MSF.967.83.

Sui, B. et al., 2023. Recent advances in the biodegradation of polyethylene terephthalate with cutinase-like enzymes. *Frontiers in Microbiology*, 14, 1265139. doi: 10.3389/fmicb.2023.1265139.

Sürmeli, Y., Tekedar, H.C. & Şanlı-Mohamed, G., 2024. Sequence identification and in silico characterization of novel thermophilic lipases from Geobacillus species. *Biotechnology and Applied Biochemistry*, 71(1), pp.162-175. doi: 10.1002/bab.2529

Susilowati, P.E. et al., 2012. Produksi Xilanase dari Isolat Sumber Air Panas Sonai, Sulawesi Tenggara, menggunakan Limbah Pertanian. *Jurnal Natur Indonesia*, 14(3), pp.199-204.

Takami, H. et al., 2004. Thermoadaptation trait revealed by the genome sequence of thermophilic *Geobacillus kaustophilus. Nucleic Acids Research*, 32 (21), pp.6292-6303. doi: 10.1093/nar/gkh970.

Tang, Y. et al., 2022. Oral therapy of recombinant Subtilisin QK-2 potentiates thrombolytic effect in a carrageenan-induced thrombosis animal model. *Journal of Functional Foods*, 88, 104896. doi: 10.1016/j.jff.2021.104896

Tarrahimofrad, H. et al., 2020. Structural and biochemical characterization of a novel thermophilic Coh01147 protease. *PLoS One*, 15(6), e0234958. doi: 10.1371/journal.pone.0234958.

Thakur, N., Singh, S.P. & Zhang, C., 2022. Microorganisms under extreme environments and their applications. *Current Research in Microbial Sciences*, 3, 100141. doi: 10.1016/j.crmicr.2022.100141.

Tkáčová, J. et al., 2015. Screening of carotenoid-producing strains isolated from natural sources. *Acta Chimica Slovaca*, 8(1), pp.34-38. doi: 10.1515/acs-2015-0007.

Torreblanca, M. et al., 1986. Classification of non-alkaliphilic halobacteria based on numerical taxonomy and polar lipid composition, and description of *Haloarcula* gen. nov. and *Haloferax* gen. nov. *Systematic and Applied Microbiology*, 8(1-2), pp.89-99. doi: 10.1016/S0723-2020(86)80155-2

Uddin, M.E. et al., 2014. Isolation and characterization of proteases enzyme from locally isolated Bacillus sp. *American Journal of Life Sciences*, 2(6), pp.338-344. doi: 10.11648/j.ajls.20140206.12

Ullah, I. et al., 2021. Identification and characterization of thermophilic amylase producing bacterial isolates from the brick kiln soil. *Saudi Journal of Biological Sciences*, 28(1), pp.970–979. doi: 10.1016/j.sjbs.2020.11.017

Umezawa, H. et al., 1965. A new antibiotic, kasugamycin. *The Journal of Antibiotics, Series A*, 18(2), pp.101-103. doi: 10.11554/antibioticsa.18.2_101

Usman, N.J. et al., 2023. Characterization of recombinant cutinase from Thermobifida cellulosilytica and its application in tomato cutin degradation. *Biocatalysis and Agricultural Biotechnology*, 47, 102603. doi: 10.1016/j.bcab.2023.102603

Vaidya, A. et al., 2018. Comparative analysis of thermophilic proteases. *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*, 4, pp.65-91. doi: 10.26479/2018.0406.06

Verma, A. et al., 2020. *Halocatena pleomorpha* gen. nov. sp. nov., an extremely halophilic archaeon of family Halobacteriaceae isolated from saltpan soil. *International Journal Systematic and Evolutinary Microbiolology*, 70(6), pp.3693–3700. doi: 10.1099/ijsem.0.004222.

Verma, J.P. et al., 2018. Characterization and screening of thermophilic Bacillus strains for developing plant growth promoting consortium from hot spring of Leh and Ladakh region of India. *Frontiers in Microbiology*, 9, 1293. doi: 10.3389/fmicb.2018.01293

von Hegner, I., 2020. Extremophiles: a special or general case in the search for extra-terrestrial life?. *Extremophiles*, 24(1), pp.167-175. doi: 10.1007/s00792-019-01144-1

Vora, J.U., Jain, N.K. & Modi, H.A., 2014. Extraction, Characterization and Application studies of red pigment of halophile *Serratia marcescens* KH1R KM035849 isolated from Kharaghoda soil. *International Journal of Pure & Applied Bioscience*, 2(6), pp.160-168.

Wahyuna, D., Agustien, A. & Periadnadi., 2012. Isolasi Dan Karakterisasi Bakteri Termo-Proteolitik Sumber Air Panas Sungai Medang, Sungai Penuh, Jambi. *Jurnal Biologi Universitas Andalas*, 1(2), pp.93-98. doi: 10.25077/jbioua.1.2.%25p.2012.

Wang, G. et al., 2022. Thermophilic Nucleic Acid Polymerases and Their Application in Xenobiology. *International Journal of Molecular Sciences*, 23 (23), 14969. doi: 10.3390/ijms232314969.

Weber, G., Bornscheuer, U.T. & Wei, R., 2021. *Enzymatic Plastic Degradation*, Cambridge, M.A: Academic Press.

Welday, G.T., Abera, S. & Baye, K., 2014. Isolation and characterization of thermo-stable fungal alpha amylase from geothermal sites of Afar, Ethiopia. *International Journal of Advances in Pharmaceutical Research*, 3(1), pp.102-127.

Wezyah, A., 2013. *Produksi Enzim Selulase dari Aspergillus niger dan Kemampuannya Menghidrolisis Jerami Padi.* Universitas Andalas, Padang.

Widiana, D. R. et al., 2022. Purification and characterization of thermostable alpha-amylase from Geobacillus sp. DS3 from Sikidang Crater, Central Java, Indonesia. *Indonesian Journal of Biotechnology*, 27(4), pp.212-218. doi: 10.22146/ijbiotech.71643

Wirajana, I. N., et al., 2024. Prokaryotic communities profiling of Indonesian hot springs using long-read Oxford Nanopore sequencing. *BMC Research Notes*, *17*(1), 286. doi: 10.1186/s13104-024-06941-2

Witasari, L.D. et al., 2010. Cloning of thermostable DNA polymerase gene from a thermophilic *Brevibacillus* sp. isolated from Sikidang Crater, Dieng Plateu, Central Java. *Indonesian Journal of Biotechnology*, 15(2), pp.72-78.

Xie, G. et al., 2019. Heterologous expression and characterization of a novel subtilisin-like protease from a thermophilic Thermus thermophilus HB8. *International Journal of Biological Macromolecules*, 138, pp.528-535. doi: 10.1016/j.ijbiomac.2019.07.101

Yadav, A.N. et al., 2015. Haloarchaea endowed with phosphorus solubilization attribute implicated in phosphorus cycle. *Scientific Reports*, 5(1), 12293. doi: 10.1038/srep12293.

Yadav, S.C., Pande, M. & Jagannadham, M.V., 2006. Highly stable glycosylated serine protease from the medicinal plant Euphorbia milii. *Phytochemistry*, 67(14), pp.1414-1426. doi: 10.1016/j.phytochem.2006.06.002

Yang, X. et al., 2020. Facile one-pot immobilization of a novel thermostable carboxylesterase from Geobacillus uzenensis for continuous pesticide degradation in a packed-bed column reactor. *Catalysts*, 10(5), 518. doi: 10.3390/catal10050518

Yang, X. et al., 2021. Assessment of the production of Bacillus cereus protease and its effect on the quality of ultra-high temperature-sterilized whole milk. *Journal of Dairy Science*, 104(6), pp.6577-6587. doi: 10.3168/jds.2020-19818

Yildiz, S.Y., 2024. Exploring the hot springs of golan: a source of thermophilic bacteria and enzymes with industrial promise. *Current Microbiology*, 81(4), 101. doi: 10.1007/s00284-024-03617-9

Zafrida, S. et al., 2022. Optimization of crude protease production from Bacillus thuringiensis HSFI-12 and thrombolytic activity its enzyme dialysate. *Trends in Sciences*, 19(23), pp.1952-1952. doi: 10.48048/tis.2022.1952

Zalma, S.A. & El-Sharoud, W.M., 2021. Diverse thermophilic *Bacillus* species with multiple biotechnological activities are associated within the Egyptian soil and compost samples. *Science Progress*, 104(4), 00368504211055277. doi: 10.1177/00368504211055277

Zeldes, B.M. et al., 2015. Extremely thermophilic microorganisms as metabolic engineering platforms for production of fuels and industrial chemicals. *Frontiers in Microbiology*, 6, 1209. doi: 10.3389/fmicb.2015.01209.

Zhang, J. et al., 2024. Effects of thermophilic bacteria inoculation on maturity, gaseous emission and bacterial community succession in hyperthermophilic composting. *Science of The Total Environment*, 927, 172304. doi: 10.1016/j.scitotenv.2024.172304

Zhang, J. et al., 2025. Enzymatic anti-felting finishing of the dyed woolen textiles through the collaborative action of mild reduction and protease treatment. *International Journal of Biological Macromolecules*, 307, 142160. doi: 10.1016/j.ijbiomac.2025.142160

Zhang, M., et al. 2008. Expression, purification, and characterization of a thermophilic neutral protease from Bacillus stearothermophilus in Bacillus subtilis. *Science in China Series C: Life Sciences*, 51(1), pp.52-59. doi: 10.1007/s11427-008-0009-9

Zhang, X. et al., 2024. Study on activation strategy and effect of protease activity during the post-processing stage of dry-cured meat based on electrical stimulation. *Food Control*, 161, 110363. doi: 10.1016/j.foodcont.2024.110363

Zhu, D. et al., 2020. Recent development of extremophilic bacteria and their application in biorefinery. *Frontiers in Bioengineering and Biotechnology*, 8, 483. doi: 10.3389/fbioe.2020.00483

Zhu, N. et al., 2021. Thermal pretreatment enhances the degradation and humification of lignocellulose by stimulating thermophilic bacteria during dairy manure composting. *Bioresource Technology*, 319, 124149. doi: 10.1016/j.biortech.2020.124149