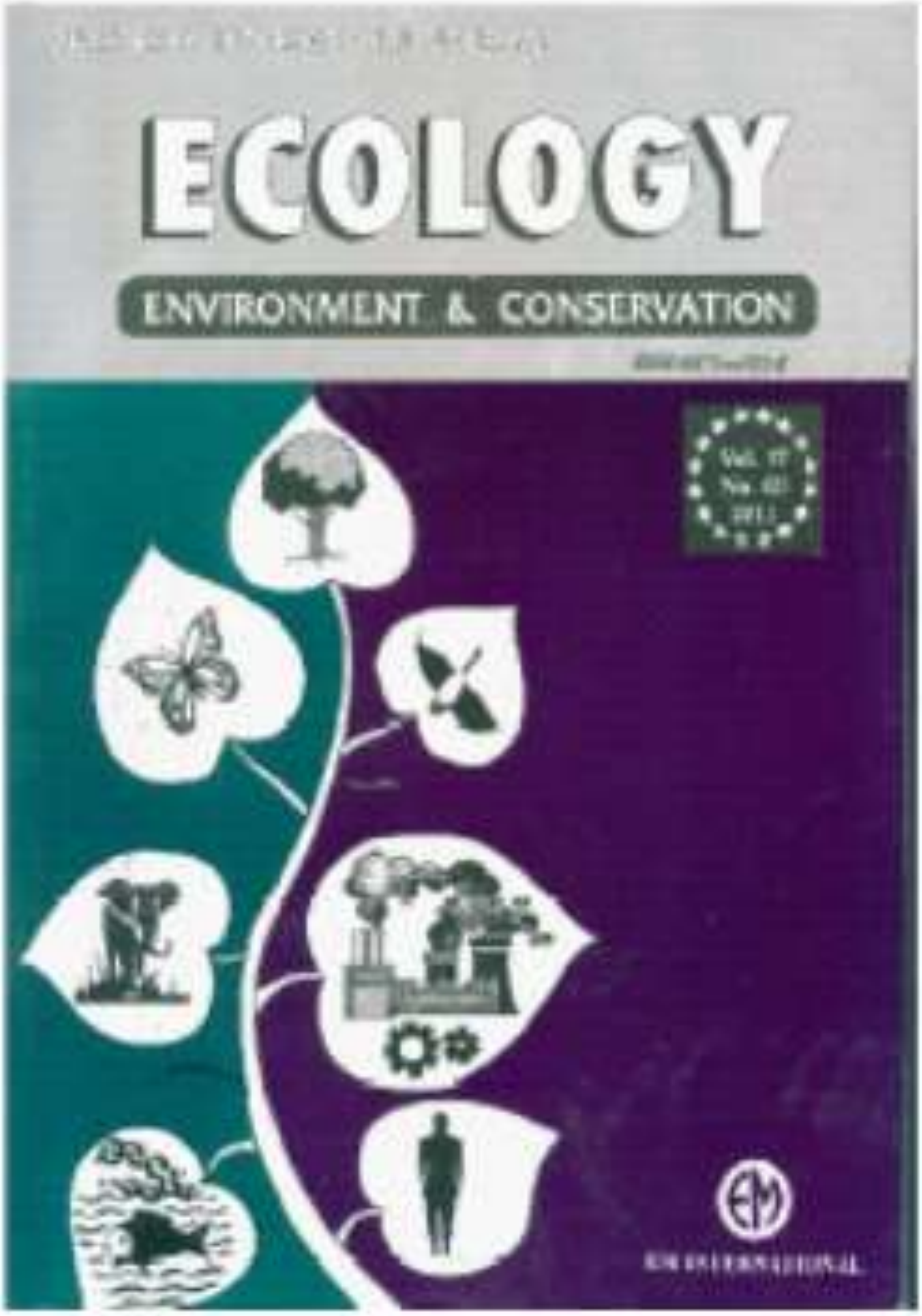
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Bioprospecting thermostable enzymes-producing thermophiles from Indonesia

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ABSTRACT

Thermostable enzymes-producing thermophilic microorganisms have been of industrial interest due to their ability to work optimally in high temperature which ensures high reaction efficiency and productivity, as well as reduces contamination risks. Efforts on isolating thermophilic microorganism with industrial potential have long been conducted in Indonesia, where there is large number of hot springs and geothermal area. Bacterial strains with the ability to produce thermostable industrial enzymes such as amylases, chitinase, lipase, protease, and xylanase have been isolated from various regions in Indonesia. This review is the first endeavor to collectively look into the researches on thermophilic microorganisms with industrial potential in Indonesia, their outcomes and proposed future directions based on the limitation of current studies.

***Key words** : Thermophilic Microorganisms, Thermostable Enzymes, Hot Springs, Indonesia, Bioindustry*

Introduction

Thermophilic microorganisms which grow optimally at temperature above 50 ^{o}C, have been of great interest in bioindustry. Thermostable enzymes produced by those microorganisms can be utilized in bioprocess involving high temperature (60-105 ^{o}C) such as starch liquefaction and lignocellulosic materials saccharification (Elleuche *et al.*, 2014). Furthermore, thermophilic microorganisms are promising hosts for fermentation offering a number potential of advantage over mesophilic hosts. Thermophilic hosts work optimally at high temperature comparable to chemical refineries ensuring high efficiency and productivity as shown in the production of fructose, methane, and hydrogen (Zeldes *et al.*, 2015). Moreover, high-temperature bioprocessing offers reduced risks of contamination from unwanted microorganisms and phage infection, reduced cooling costs of the fermenter, as well as reduced water and energy consumption for sterilization (Chen and Jiang, 2018).

Due to the numerous advantages on bioindustry, the diversity and potential of thermophilic microorganisms have been widely explored. Survey and exploration to hot springs, geothermal fields, hydrothermal vents, desert, and even man-made environments such as compost facilities resulted in the discovery of novel thermophilic microorganism strains and species with industrial potential (Urbieta *et al.*, 2015).

One of the best areas to explore the biodiversity thermophilic microorganisms with industrial potential is Indonesia. As one of the most tectonically active areas in the world, with over 70 volcanoes, Indonesia has substantial number of hot springs and geothermal regions which harborlarge communities of thermophilic bacteria (Yohandini *et al.*, 2015). Efforts to isolate novel strain and species of thermophilic microorganisms from those ecosystems and

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screen the ability of isolated microorganisms to produce thermostable industrial enzymes have long been conducted.

In this review,the recent discovery of thermophilic microorganisms with the ability to produce thermostable industrial enzymes in Indonesia will be discussed. This review is the first that focusing the current status of exploration of thermophilic microorganisms with industrial potential in Indonesia.

Area and methods of exploration

Based on Table 1, most of thermophilic microorganism explorations in the last 10 years in Indonesia were conducted in Java, Sumatera, and Sulawesi islands. Interestingly some of the studied environments are suitable for hyperthermophiles where temperature reached >80°C and thermophiles-acidophiles where pH was below 4 (Urbieta *et al.*, 2015). The biodiversity of thermophiles in those ecosystems were mainly explored using culture-dependent methods such as traditional microorganism culture and culture-independent methods using molecular biology techniques.

Although, traditional microorganism culture methods have been important in characterizing the biochemical and physiological properties, as well as potential application of thermophiles isolates, however, this method is only able to reveal limited number of thermophiles.This is due to the commonly understood fact that only small fraction of microorganism in a given environment is culturable (Boteva and Kambourova, 2018; Chaudhary *et al.*, 2019). Most of the isolates obtained using this method are from order Bacilliales family Paenibacillaceae and Bacillaceae. The member of family Paenibacillaceae were isolated from Tanjung Sakti (*Brevibacillusthermoruber*) and Cisolok (*Paenibacilluscisolokensis*) (Yohandini *et al.*, 2015; Yokota *et al.*, 2016) While the isolated member of Bacillaceae were *Bacillus* sp from West Sumatera, as well as *Anoxybacillusrupiensis*, *Anoxybacillus flavithermus*, and *Geobacilluspallidus*, from South Sumatera (Harnentis *et al.*, 2013; Yohandini *et al.*, 2015). Even isolates from the same genus (*Bacillus*spp) were obtained from the explorationsin Sumatera (Jambi) and Sulawesi (North Sulawesi) islands,where the sampling area have different characteristics and are distantly apart (more than 4000 km) (Arzita *et al.*, 2017; Simanjuntak and Samuel, 2018).

A more inclusive approach on studying biodiversity of thermophiles was represented by the use of molecular biology techniques such as 16S rRNA gene sequencing of environmental DNA and denaturing gradient gel electrophoresis (DGGE). The use of those methods successfully revealed the abundance of unique and previously unculturable microorganisms such as members of genus *Ralstonia,Delftia*, and *Thermus* in Gedongsongo and members of phylum Crenarchaeota in Kamojang (Aditiawati *et al*., 2009; Aminin *et al*., 2008).

Thermophilic microorganisms with industrial potential

Thermophilic microorganism explorations in Indonesia have been conducted mainly for these 3 purposes: [1]Bioprospectingthermophilic microorganisms with industrial potential, [2] Profiling the microbial communities in hot ecosystems, and [3] Dis-

Table 1. Selectedexploration area for thermophiles in Indonesia

Name	Area	Temperature (°C)	pH	References
Tanjung Sakti hot spring	South Sumatera	80-91	7-8	(Yohandini *et al.*, 2015)
Hot spring at South Solok District	West Sumatera	75-95	8	(Harnentis *et al.*, 2013)
Sungai Tutunghot spring at Kerinci Seblat National Park	Jambi (Sumatera)	70-85	8.4	(Arzita *et al.*, 2017)
Kalianda Island coastal hot springs	Lampung (Sumatera)	58.5-68.5	6.0-6.5	(Xu *et al.*, 2013)
Geyser at Cisolok	West Java	70-80	7	(Yokota *et al.*, 2016)
Kawah Hujan B hot spring, Kamojang	West Java	90-92	1.8-1.9	(Aditiawati *et al.*, 2009)
GS-2 hot spring at Gedongsongo field	Central Java	50	4	(Aminin *et al.*, 2008)
Lake Linow hot mud	North Sulawesi	90-110	7.08-8.35	(Simanjuntak and Samuel, 2018)

covering of novel. Among them, bioprospecting of thermophilic microorganisms with thermostable industrial enzymes activities has become the main goal of the most of the explorations.

As shown in table 2, a wide variety of industrially important enzymes were produced by thermophilic bacteria isolated from hot springs in Indonesia. Thermostable agarose is an important enzyme for agar hydrolysis in microbiological media industry and extract biological substances, such as unsaturated fatty acids, vitamins, and carotenoids from algae (Li *et al.*, 2014). Thermostable amylase can be utilized for starch gelatinization and liquefication in the food and bioethanol industries (Ibrahim *et al.*, 2013). Xylanase and mannase with high stability would increase the efficiency of biobleaching, food and feed processing and the solubilization of lignocellulose for the production of second generation bioethanol (Elleuche *et al.*, 2015), while thermostable protease and lipase mainly used in food and detergent industries (Rigoldi *et al.*, 2018).

Future Perspectives

The efforts on discovering thermophilic microorganisms with industrial potential in Indonesia have been conducted for around 20 years(Huber *et al.*, 1996; Lee *et al.*, 1999; Nam *et al.*, 2002). Since then, numerous explorations have been conducted in various hot ecosystems in Indonesia with a small number mentioned in table 2. However, although volcanic and geothermal area are spread across Indonesia, current explorations are still limited to Java, Sumatera, and Sulawesi islands. Future exploration efforts in another area (i.e. Lesser Sunda Is-

Table 2. Selected Thermostable Enzymes-producing Thermophilic Microorganisms isolated from various area in Indonesia

Enzymes	Source	Associated ecosystem	Properties	References
Agarase	*Bacillus* sp. BI-3	Kalianda Island coastal hot spring, Lampung	• Work optimally at 70°C, pH 6.4	(Li *et al.*, 2014)
Amylase	*Bacillus* sp. RSII-1b	Lejja hot sprin, South Sulawesi	• Work optimally at 55°C-60°C, pH 6.0 • Activity of 0.165U/mL crude extract	(Arfah *et al.*, 2015)
	Bacillus licheniformisBT5.9	Cangar hot spring, East Java	• α-amylase with optimum expression at 50°C, pH 5.0 • Activity of 0.327 U/ml crude extract	(Ibrahim *et al.*, 2013)
Chitinase	*Bacillus licheniformis*	Cangar hot spring, East Java	• Expressed at 52°C, pH 7.0	(Chrisnasari *et al.*, 2018)
Lipase	DMS-3 isolate (rod-shaped, Gram positive bacteria)	Domas hot spring, West Java	• Alkaline lipase with optimum expression at 70°C, pH 9.0	(Febriani *et al.*, 2010)
Mannase	*Bacillus* sp	Hot springs at South Solok District, West Sumatera	• Work optimally at 55°C-60°C, pH 6.0 • Activity of 0.165U/mL crude extract	(Harnentis *et al.*, 2013)
Protease	*Fictibacillusgelatini*	Sungai Tutung hot spring, Jambi	• Alkaline protease expressed at 60°C, pH 8.0 • Proteolytic index of 6.15	(Arzita *et al.*, 2017)
	Brevibacillus sp PLI-1	Kalianda Island coastal hot spring, Lampung	• Alkaline protease expressed at 70°C, pH 8.0-9.0 • Proteolytic index of 10.3	(Wang *et al.*, 2012)
Xylanase	*Bacillus*sp	Sapan Sungai Aro hot spring, West Sumatera	• Expressed at 60°C • Xylanolytic index of 0.74	(Irdawati *et al.*, 2018)
	*Paenibacillus*sp. XJ18	TNBD Forest, Jambi	• The highest activity showed at 90°C, pH 5.0.	(Kurrataa'yun and Meryandini, 2015)

lands and Molucca Archipelago) would provide valuable information regarding the biodiversity of beneficial thermophiles in Indonesia.

Furthermore, a major limitation of current study on thermophiles with industrial potential in Indonesia is that very few or even almost no attempts have been made towards development to commercial scale. Thus, efforts to bridge the gap between research on thermophilic microorganism potential and commercialization need to be carried out.

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