

**Physiological Properties and Medium Optimization of MGPR3.1 Thermo-Alkaliphile Isolate**<sup>[1]</sup>Indriati, G., <sup>[1]</sup>Megahati, R. R. P., and <sup>[2]</sup>Putri, D. W.<sup>[1]</sup>Biology Education Department STKIP PGRI Sumatera Barat, Padang, West Sumatra, Indonesia<sup>[2]</sup>Biology Department, Padang State University, Padang, West Sumatra, Indonesia

**Abstract.** Amylase produced by bacteria or bacteria amylase is an enzyme that can hydrolyze starch to sugar. Amylase-producing bacteria can be found in seawater, volcanic craters, compost, and hot springs. Hot springs are a potential place as a source of amylase-producing bacteria. Indonesia has many hot springs such as Pariangan hot springs. The temperature of Pariangan hot spring between 47-50°C and pH ranged between 8.4-9.0. This caused Pariangan hot springs have a high level of bacterial diversity. Isolation and screening of thermo-alkaliphile amylase producing bacteria originating from Pariangan hot spring have been successfully performed. There were 4 isolates of 1 bacterium isolate with the highest activity, i.e. isolate MGPR3.1. Physiological properties and optimization medium of amylase activity are unknown. The aim of the study was to determine the physiological properties and increase the amylase activity produced by MGPR3.1 isolates. The results showed that MGPR3.1 isolates were Gram-positive and belonged to *Bacillus* sp.

**Keywords:** physiological properties, optimization, thermo-alkaliphile, hot spring

**Introduction**

Amylase produced by bacteria or bacteria amylase is an enzyme that can hydrolyze starch to sugar (Megahati et al., 2017). Amylase-producing bacteria can be found in seawater, volcanic craters, compost, and hot springs. Hot springs are a potential place as a source of amylase-producing bacteria. Indonesia has many hot springs, such as Semurup hot springs, Ciater hot springs, Rimbo Panti hot springs, Sumedang hot springs, and Pariangan hot springs. The temperature of Pariangan hot spring between 47-50 ° C and pH ranged between 8.4-9.0. This caused Pariangan hot springs have a high level of bacterial diversity (Indriati et al., 2018). Therefore, bacteria from the Pariangan hot spring are known as thermo-alkaliphile bacteria. Thermo-alkaliphile bacteria have the potential in the food and non-food industries.

In the field of food industry, amylase plays a role in the manufacture of glucose syrup, bread making, and baby food. In the field of non-food industry, amylase plays a role in the paper industry, leather tanning, pharmaceuticals, textiles and detergent additives (Indriati and Megahati 2018). Amylase is one of the three largest industrial groups enzymes and accounts for approximately 65% of the world enzyme (Abdullah et al., 2014). Isolation and screening of amylase producing-bacteria originating from Pariangan hot spring, West Sumatra province has been successfully performed. There were 4 isolates of 1 bacterium isolate with the highest activity, i.e. isolate MGPR3.1. Physiological properties and optimization results of the medium to amylase activity are unknown. The aim of the study was to determine the physiological properties and increase the amylase activity produced by MGPR3.1 isolate.

**Methods****1. Characterization of isolate MGPR3.1**

Characterization of bacteria is done through macroscopic observation, ie observation of colony form from bacteria, colony edge, elevation, surface, and colony pigmentation.

Microscopic observation was done through observation of bacterial cell forms, spore staining and reaction to Gram staining (Hadioetomo, 1993).

## 2. Physiology properties of isolate MGPR3.1

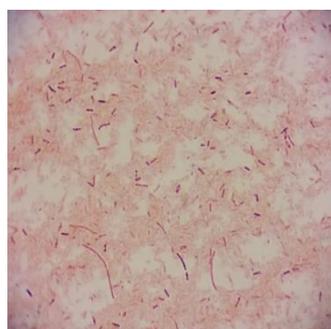
Physiology properties of bacteria were performed by biochemical tests, such as TSIA, Catalase, Oxidase, Indole, Citrate Urea, Lactose, Glucose, Sucrose, Mannitol, MR, VP, OF, Arabinose, Nitrate, and Gelatin (Cowan, 1977).

## Results and Discussion

Isolation of thermo-alkaliphile bacteria originating from Pariangan hot springs has been successfully carried out. 4 isolates and 1 isolate with the highest activity were obtained namely MGPR3.1 isolates.

### 1. Characterization of isolate MGPR3.1

Characterization results show that MGPR3.1 isolates have white colonies, round, flat elevation, slippery edges. Characterization of CS3.1 bacterial isolates derived from compost showed that irregular shapes, grooved edges, and elevated elevations (Megahati, 2018). The result of Gram staining and biochemical properties showed that MGPR3.1 isolates were classified to Gram-positive and have spores (Figure 1).



**Figure 1. Results of Gram staining**

### 2. Physiology properties of isolate MGPR3.1

The results of the physiological properties of MGPR3.1 isolates belonged to *Bacillus* sp (Table 1). *Bacillus* sp is a rod-shaped bacteria, has endospores, and is aerobic. The results of research by Al-Johani et al., (2017) showed that bacteria isolated from Al-Ain Alhara hot spring located at Gazan, KSA belonged to *Bacillus* sp and were rod-shaped, had endospores, and aerobic.

**Table 1: The results of a test of physiology properties**

No.	Test of biochemical properties	Result
1	TSIA	Red / yellow
2	Catalase	+
3	Oxidase	-
4	Indole	-
5	Urea	+
6	Citrate	-
7	Lactose	-
8	Glucose	+
9	Sucrose	-
10	Mannitol	+
11	MR	+

12	VP	+
14	OF	-
15	Arabinose	-
16	Nitrate	+
17	Gelatine	+
18	Xylose	-

The physiological properties of thermophile bacteria originating from the hot springs of Semurup, Kerinci, Indonesia shows that the bacteria belong to *Bacillus* sp (Megahati, 2018). *Bacillus* sp is a bacterium that can adapt to extreme environments because *Bacillus* requires simple nutrition (Khiyami et al., 2012). *Bacillus* was isolated from all explored sites, the presence of *Bacillus* in all cases was due to its ability to move high rates and their resistance to harsh environmental conditions (Connor et al., 2010). *Bacillus* is motile (nonmotile reactions sometimes occur), producing spores that are usually resistant to heat, aerobes (some facultative anaerobic species), positive catalase, varying oxidation, and classified as Gram-positive (Megahati, 2018). Most of *Bacillus* is a mesophyll bacterium that grows with an optimal temperature between 30-45 ° C, although there are some that belong to the thermophile group with optimal temperatures of 65°C. *Bacillus* is known to produce various extracellular enzymes and can be used in various industries (Annamalai et al., 2011).

### Conclusions

The results showed that thermo-alkaliphile MGPR3.1 bacterial isolates were Gram-positive bacteria and belonged to *Bacillus* sp.

### References

- Abdullah, J. R., Shaheen, N., Iqtedar, M., Naz, S. & Iftikhar, N. (2014). Optimization of cultural conditions for the production of alpha-amylase by *Aspergillus niger* (BTM-26) in solid state fermentation. *Pakistan Journal of Botany*, 46(3): 1071-1078.
- Al-Johani, N. B., Al-seeni, M. N. & Ahmed, Y.M. (2017). Optimization of Alkaline A-Amylase Production by Thermophilic *Bacillus Subtilis*. *Afr. J. Tradit Complement Altern Med*. 14(1): 288-301.
- Annamalai, N., Thavasi, R., Vijayalaksmi, S. & Balasubramanian, T. (2011). Extraction, Purification and Characterization of Thermostable, Alkaline Tolerant A-amylase from *Bacillus cereus*. *Indian Journal of Microbiology*, 5(14): 424-429.
- Connor, N., Sikorski, J. & Rooney, A.P. (2010). Ecology of speciation in the genus *Bacillus*. *Applied and Environmental Microbiology*, 76(5): 1349-1358.
- Cowan, S. T. & Steel, K. J. (1974). *Manual for the identification of medical bacteria*. London, New York, Melbourne: Cambridge University Press.
- Hadioetomo, S.R. (1993). *Basic Microbiology in Basic Laboratory Techniques and Procedures*. Jakarta: PT. Gramedia.
- Khiyami, M. A., Serour, E. A., Shehata, M. M. & Bahklia, A.H. (2012). Thermo-aerobic Bacteria from Geothermal Springs in Saudi Arabia. *African Journal of Biotechnology*, 11(17): 4053–4062.
- Megahati, R.R.P., Mansyurdin, Agustien, A. & Tjong, D.H. (2017). Optimization of Bacteria Amylase Activity from *Bacillus licheniformis* Strain SEM11. *Int. J. Curr. Microbiol. App. Sci*, 6(11): 2938-2946.
- Megahati, R.R.P. (2018). Isolation and Properties of Biochemical of Amylase Producing-Bacteria from Compost 2018. *International Journal of Innovative Science and Research Technology*, 3(5): 277-279.

- Megahati, R.R.P. (2018). *Molecular Identification (16S rRNA) Gene of Amylase Producing-bacteria*. Saarbrücken: Lambert Academic Publishing.
- Indriati, G. & Megahati, R.R.P. (2017). Optimization of Medium of Amylase Production by *Bacillus licheniformis* Strain MGI Originated from Pariangan Hot Spring, West Sumatra, Indonesia. *International Journal of Advanced Research*, 5(11): 660-664.
- Indriati, G., Megahati, R.R.P. & Rosba, E. (2018). Potency of Amylase-producing Bacteria and Optimization Amylase Activities. *IOP Conf. Series: Materials Science and Engineering* 335 (2018) 012023.