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Diversity of moderately thermo-alkaliphile bacteria producing amylase originated from Pariangan hot Spring, West Sumatra, Indonesia

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ABSTRACT

Moderately thermo-alkaliphile bacteria are bacteria originating from hot springs that have a pH above 8. Moderately thermo-alkaliphile bacteria are currently used as a source of various enzymes, such as lipases, proteases, and amylase. Amylase is an enzyme that can hydrolyze polysaccharides into simple sugar. Amylase produced by moderately thermo-alkaliphile bacteria plays a role in various industrial fields, such as in the leather tanning industry, biscuits, production, textiles, and as detergent additives. In the detergent industry, amylase is needed which is resistant to high temperatures and pH. The world's need for amylase continues to increase to 65% of the needs of enzymes worldwide. Isolation of moderately thermo-alkaliphile bacteria from Pariangan hot springs of West Sumatra has been successfully carried out and 17 bacterial isolates were obtained. From 17 isolates, there were 3 isolates of bacteria that produced amylase, namely PR 1.2, PR 2.3, and PR 3.1. The characterization and analysis of the biochemical properties of the three isolates are not known. The aims of the study were to the identification of moderately thermo-alkaliphile bacteria producing amylase originated from Pariangan hot spring, West Sumatra, Indonesia. The method used is characterization of isolates (color, shape, edges, and elevation), Gram staining (morphology and spores), biochemical properties of isolates (TSIA, Catalase, Oxidase, Indole, Urea Citrate, Lactose, Glucose, Sucrose, Mannitol, MR, VP, OF, Arabinose, Nitrate, and Gelatin. The results of this study showed that isolates of PR 1.2 bacteria, PR 2.3 belonging to *Bacillus* sp and isolates of PR 3.1 bacteria belonging to *Enterobacter* sp.

Key words: *A diversity, Moderately thermo-alkaliphile bacteria, Amylase, Biochemical, Pariangan hot spring*

Introduction

Bacteria are widely used as sources of enzyme production because bacteria are able to produce enzymes in a fast time and in large quantities. Production of amylases from bacteria is beneficial for human population as their starch degrading ability can be exploited for preparation of special food items, easily digestible for infants, patients, and elderly people. Enzymes derived from moderately thermo-alkaliphile bacteria are very much needed in industry because they are resistant to high temperatures and pH during industrial processes (Khedr *et al*., 2017). Moderately thermo-alkaliphile bacteria are bacteria originating from hot springs that have a pH above 8. Moderately thermo-alkaliphile bacteria are currently used as a source of various enzymes, such as lipases, proteases, and amylase. Amylase produced by bacteria or bacteria amylase is an enzyme that can hydrolyze polysaccharides to simple sugar (Megahati *et al*., 2017). Amylase produced by moderately thermo-alkaliphile bacteria plays a role in

various industrial fields, such as in the leather tanning industry, biscuits, textiles, and as detergent additives (Indriati *et al*., 2018). In the detergent industry, amylase is needed which is resistant to high temperatures and pH. The world's need for amylase continues to increase to 65% of the needs of enzymes worldwide (Abdullah *et al*., 2014).

Isolation of moderately thermo-alkaliphile bacteria from Pariangan hot springs of West Sumatra has been successfully carried out and 17 bacterial isolates were obtained. From 17 isolates, there were 3 isolates of bacteria that produced amylase, namely PR 1.2, PR 2.3, and PR 3.1. The characterization and analysis of the biochemical properties of the three isolates are not known. The aims of the study were to the identification of moderately thermo-alkaliphile bacteria producing amylase originated from Pariangan hot spring, West Sumatra, Indonesia.

Materials and Methods

Characterization of bacteria isolate PR 1.2, PR 2.3, and PR 3.1

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 form, spore staining and reaction to Gram staining (Hadioetomo, 1993).

Biochemical properties of bacteria isolate PR 1.2, PR 2.3, and PR 3.1

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

Results and Discussion

The process of observing bacterial morphological features or screening morphology may help to group two or more bacteria suspected of having the same species, especially if the bacteria to be identified are present in large quantities (Indriati *et al*., 2018).

Characterization of bacteria isolate PR 1.2, PR 2.3, and PR 3.1

The result of this study showed that the bacteria isolate PR 1.2, PR 2.3 had cream colonies, rounded shape, flat surface, and flat elevation. Bacteria isolate PR 3.1 has yellowness, colonies, rounded shape, flat smooth, and flat elevation. Characterization of bacterial morphology derived from compost has also been done by Megahati, 2018, the results obtained are SEM11 bacterial isolates, irregular shapes, grooved edges, and elevated elevations. The result of Gram staining and biochemical properties showed that PR 1.2 and PR 2.3 isolate classified to Gram-positive and has spores. PR 3.1 classified to Gram negative and has not spores (Figure 1).

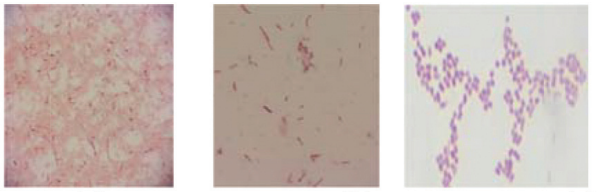

Fig. 1. The result of Gram staining (a) isolate PR 1.2, (b) PR 2.3 and (c) PR3.1.

The endospore produced by *Bacillus* aims protecting bacteria from a state that is not profitable such as drought, nutrient deficiency, freezing, as well as chemicals. The bacterial endospore types this is resistant to environmental changes, resistant to heat, and chemical disinfectants certain in a long time (Panda *et al.,* 2013). If the environment is good, then endospores will experience sporogenesis and be forming a vegetative cell (Mohammad *et al*., 2017).

The results of microscopic observations made by Hastuti (2012) show that amylase-producing bacteria are Gram negative bacteria in the form of stem cells and do not have endospores.

Biochemical properties of bacteria

Based on the results of the test biochemical properties of bacterial isolate PR 1.2 and PR 2.3 showed that isolates belong to *Bacillus* sp. Bacterial isolate PR 3.1 belong to *Enterobacter* sp The results of characterization and test of biochemical properties of bacterial isolates originating from hot springs in Jordan showed that isolates were grey, creamy, and white, opaque or translucent, rough or smooth, with regular or irregular edges. Colonies might appear finely wrinkled and adherent to the agar surface. Based on Gram staining, the isolates were found mostly to be Gram-positive and microscopic observation revealed spore-forming rod-shaped bacte-

rium arranged in the chain (Mohammad *et al*., 2017).

The genus Bacillus was isolated from all explored sites, the presence of Bacillus in all sampled locations could be due to the ability of this genus to move at high rates and their resistance to harsh environmental conditions (Connor *et al*., 2010, in addition to its adaptation for hot surroundings (Aanniz *et al*., 2012; Indriati and Megahati, 2018)

Bacillus is known to produce a variety of extracellular enzymes and can be used in various industries (Annamalai *et al*., 2011). The same study by Kawasaki *et al*., 2012 reported that Bacillus was found in the Odishi hot springs, India that could produce various thermostable enzymes (thermozyme). So it is with Khalil, 2011 reported that Bacillus sp was found in hot springs in Saudi Arabia that could produce amylases with high activity.

According to Cowan, 1974 Enterobacter is a group of Gram-negative rod-shaped bacteria that are short, aerobic and facultative anaerobic, capable of producing gas, on positive motility tests, able to ferment certain types of carbohydrates, able to use citrate as the only source of carbon, and able yields 2,3-butanediol in the VP (Voges-Proskauer) test. Enterobacter bacteria are called coliform bacteria that live in the digestive tract and are widespread in soils and waters (Schlegel, 1994).

Table 1. The result of a test of biochemical properties

No	Test of biochemical properties	Result		
		Isolate PR 1.2	Isolate PR 2.3	Isolate PR 3.1
1	TSIA	Red/yellow	Red/yellow	Red/yellow
2	Catalase	+	+	+
3	Oxidase	-	-	+
4	Indole	-	-	+
5	Urea	+	+	-
6	Citrate	-	-	+
7	Lactose	-	-	+
8	Glucose	+	+	-
9	Sucrose	-	+	-
10	Mannitol	+	+	+
11	MR	+	+	+
12	VP	+	-	+
13	OF	-	-	-
14	Arabinose	-	-	+
15	Nitrate	+	+	-
16	Gelatine	+	+	-
17	Xylose	-	+	-

Conclusion

The results of this study showed that isolates of PR 1.2 bacteria, PR 2.3 belonging to *Bacillus* sp and isolates of PR 3.1 bacteria belonging to *Enterobacter* sp.

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