Isolation And Identification Of Penicillin Acylase Producing Bacteria Originated From Cow Intestine

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***Abstract*:** Bacterial resistance to penicillin antibiotics can be overcome by making semisynthetic penicillin derived from the 6-amino penicillanic acid (6-APA) raw material. 6-APA is the result of penicillin hydrolysis by the activity of penicillin acylase. Penicillin acylase is an enzyme that catalyzes the hydrolysis of penicillin into 6-amino penicillanic acid (6-APA). The use of penicillin acylase has reached 85% in the pharmaceutical industry in the world. The presence or absence of enzyme penicillin acylase produced by bacteria depends on the type and habitat of bacteria. This research was conducted with several stages, sample isolation, srceening of bacteria, and bacteria identification. The purpose of this study was to isolate and identify bacteria producing penicillin acylase derived from cow intestine. Results of bacterial screening obtained 14 bacterial isolates and 3 isolates of penicillin acylase producing bacteria with the visible clear zone around of the bacterial growth. Biochemical identification of USS.13 bacterial isolates using several biochemical tests showed that bacterial isolates of USS.13 were classified into the genus *Bacillus* sp.

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Keywords: isolation of bacteria, penicillin acylase, identification, *Bacillus* sp.

I. INTRODUCTION

Bacterial resistance to penicillin antibiotics can be overcome by making semisynthetic penicillin derived from the 6-amino penicillanic acid (6-APA) raw material. 6-APA is the result of penicillin hydrolysis by the activity of penicillin acylase. According to Torres-Bacete *et al*, (2015) penicillin acylase is an enzyme that catalyzes the hydrolysis of penicillin into 6-amino penicillanic acid (6-APA). Penicillin acylase is produced by various microorganisms, such as fungi and bacteria (Nandy *et al*., 2014). The use of penicillin acylase has reached 85% in the pharmaceutical industry in the world (Varshney *et al*., 2013). The presence or absence of enzyme penicillin acylase produced by bacteria depends on the type and habitat of bacteria. Different habitats will cause genetic and phenotypic bacterial differences (Sabarly *et al*., 2011). As well as the production of penicillin acylase, different habitats produce different amounts of penicillin acylase. Bacteria can be found in two different habitats, namely primary habitat (host) and secondary habitat (external environment) (Gordon *et al*., 2002). The primary habitat of bacteria is in the vertebrate animal's intestines (cows), while the secondary habitats of bacteria are on soil and water. The number of bacterial isolates producing penicillin acylase is currently very limited as well as bacterial identification. It is therefore necessary to isolate the bacteria producing penicillin acylase from the cow intestine and bacterial identification. The purpose of this study was to isolate and identify bacteria producing penicillin acylase derived from cow intestine.

II. METHODS

This research was conducted with several stages, namely sample isolation, screening of bacteria, and bacteria identification.

Sample isolation

Samples of bacteria taken from the cow's intestines and put into sterile sample bottles are then taken to the laboratory. 1 g sample is inserted into a 10^{-1} dilution tube containing sterile 9 ml aquades and then diluting dilution to 10^{-8}. 0.1 ml samples from the last three

dilutions to be planted spread plate on Nutrient Agar (NA) medium and incubated at 37°C for 24 hours. The growing colony was purified to a new NA medium with quadrant streak technique and incubated 24 hours at 37°C.

Screening of bacteria

1 ml of a bacterial culture of the sample and *Serratia marcessens* were planted into NA medium and incubated at 37°C and for 24 hours. Observations were made by looking at the presence or absence of clear zones around the growth of bacterial samples. If a clear zone is formed then the bacterial sample is tested positive to produce penicillin acylase (Meevootisoom *et al*., 1983). One bacterium with the largest clear zone was further identified by bacterial staining and biochemically.

Identification of bacteria

Bacterial identification was performed by bacterial staining and biochemical tests, such as Methyl Red and Voges Proskauer, SIM (Sulfide Indol Motility), Urea, Citrate, Sugar (glucose, lactose, sucrose, and maltose), Fermentation, Oxidation, and Nitrate test (Cowan, 1974).

III. RESULTS AND DISCUSSION

Results of bacterial screening obtained 14 bacterial isolates and 3 isolates of penicillin acylase producing bacteria with visible clear zones around bacterial growth samples (Table 1). The USS.13 bacterial isolates had the largest cleft zone of 6.33 mm and the bacterial isolate USS.12 had the smallest cleft zone of 3.55. The clear zone around the bacterial growth is formed because *Serratia marcescens* is sensitive to penicillin acylase produced by bacterial isolates.

Table 1. Clear zone of bacteria isolates producing penicillin acylase

No	Isolate code	Clear zone (mm)
1	USS.1	4.79
2	USS.2	-
3	USS.3	-
4	USS.4	-
5	USS.5	-
6	USS.6	-
7	USS.7	-
8	USS.8	-
9	USS.9	-
10	USS.10	-
11	USS.11	-
12	USS.12	3.55
13	USS.13	6.33
14	USS.14	-

Isolation of penicillin acylase producing bacteria has been done, namely *Acinetobacter* sp. AP24 originating from Lake Loktak, India (Philem *et al*., 2015) and *Streptomyces lavendulae* (Torres-Bacete *et al*., 2015). The difference of the clear zone produced by the three bacterial isolates is closely related to the type and habitat of the bacteria. Environmental and genetic variations have an effect on bacterial physiology. Different bacterial habitats will cause differences in genetic variation (genetic drift) and phenotype (Sabarly *et al*., 2011). The phenotype of an organism is influenced by genetic drift and the environment. Genetic drift found in the secondary environment is more variable than that of the primary environment (Gordon *et al*., 2002). The staining results on bacterial isolate USS.13 showed the sample bacteria belonging to Gram-positive bacteria (Figure).

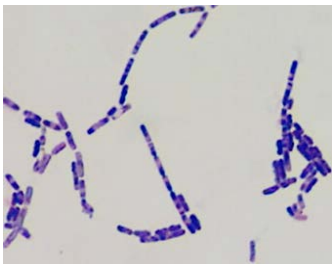

Figure. Result of staining of bacterial isolate USS.13

Gram-positive bacteria characterized by purple cells, stem cell shape, and have endospores. According to Hadioetomo (1993), Gram-positive bacteria can withstand the primary dyes of violet crystals violet so that the cells appear purple, whereas Gram-negative bacteria cannot withstand the primary dyes of violet crystals and therefore, stained by safranin so that the cells appear red. Gram staining or differential staining is very important in its use in bacteriology. It can divide the true bacteria into two physiological groups, thereby greatly facilitating the identification of its kind (Volk & Wheeler 1993).

Biochemical identification of bacteria isolates USS.13 using several biochemical tests showed that bacterial isolate of USS.13 was classified into *Bacillus* sp. genus (Table 2).

Table 2. Biochemical test results of bacterial isolate USS.13

No.	Biochemical assay	Result
1	SIM	+
2	Urea	+
3	Citrate	+
4	Lactose	-
5	Glucose	+
6	Sucrose	+
7	Maltose	-
8	MR	-
9	VP	+
10	Oxidase	+
11	Fermentasion	+
12	Nitrate	-

Bacillus sp. capable secretion of proteins directly into the medium so widely used in industry (Lakowizt *et al*., 2017). The results of Torres-Becete *et al*. (2015) showed that the penicillin acylase produced by *Streptomyces lavendulae* ATCC 13664 was able to be secreted into the medium. In *E.coli* penicillin acylase is not secreted to the medium but to the periplasmic space so it is necessary to solve bacterial cells to obtain penicillin acylase. *Bacillus* is motile (occasional nonmotil reaction), producing spores that are usually resistant to heat, aerobes (some facultative anaerobes), positive catalase, varying oxidation, and are Gram positive. The acid is produced from the hydrolysis of sucrose, lactose and mannose, but the gas is produced through positive tests on glucose, citrate, catalase and Voges-Proskauer.

IV. CONCLUSION

Three isolates of the bacteria producing penicillin acylase from the cow intestine belonging to the genus *Bacillus* sp.

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