Isolation and Characterization of Phytase-Producing thermophilic Bacteria from Sulili Hot Springs in South Sulawesi

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Abstract—This study aimed to isolate, select and identify phytase-producing thermophilic bacteria from Sulili hot springs in the district Pinrang South Sulawesi, Indonesia. Water and sediment samples were obtained from Sulili hot springs the district Pinrang, South Sulawesi. Isolation was done using Luria Bertani (LB) medium at 60°C for 1 x 24 hours. Twelve isolates of thermophilic bacteria have been obtained, five of them have the ability to produce phytase. Indicated by the formation of a clear zone around the colonies grown on medium screening LB + Ca-phytate. Five isolates were then selected, three of whom are selected based isolates widest phytase activity qualitatively. Morphological characteristics and biochemical nature of the three selected isolates have been identified belong to genera Bacillus. Each has similarities with Bacillus licheniformis, Bacillus coagulans and Bacillus stearothermophilus.

Index Terms—Sulili hot springs, Phytase, thermophilic bacteria, Bacillus.

I. INTRODUCTION

The main ingredients that have been used as animal feed are generally derived from grains, seeds or legumes such as corn, soybeans, rice, wheat, sunflower, and its processing wastes such as wheat pollard and bran. The plant feed material contains phosphorus (P) in high levels and approximately 2/3 of the phosphorus content is in the form of phytate compounds. Not only strong binding of phosphorus but also binds to proteins and minerals (Mg, Fe, Zn, Mn, Ca) as well as protein enzymes that are very useful for the growth and production, so-called anti-nutritional substances [7].

If the amount of undigested phytate increases, resulting in a negative effect on the absorption of minerals and because it lowers solubility, digestibility also the absorption of protein and minerals and causes a decrease in the activity of digestive [7]. Phytate compounds cannot be digested by monogastric or non-ruminant animals due to absence of the enzyme phytase in the digestive tract, causing wasted phytate compounds along with feces into the environment. Sources of phosphorus-containing animals waste is a source of pollution. Phosphorus content of residual animals waste will be associated with the soil and will eventually interfere with water circulation system. Phosphorus undigested and excreted through the feces also caused
water pollution of rivers and lakes because of eutrophication is the excessive enrichment of waters that will nourish algae poisonous and can cause the death of fish and other biota [9].

Phytase application is one of the solution to overcome the high phytic acid in animal feed rations, because phytase has the ability to hydrolyze phytic acid contained in feed ingredients into the compound inositol and glucose and organic phosphorus compounds. These compounds very important role in the process of respiration to ATP formation. The addition of phytase enzyme of 750 FTU / kg resulted in a high phosphorus digestibility compared to the addition of below 500 FTU / kg of ration [6]. The high phytic acid in corn and bran will lead to disrupted metabolic processes nutrients in the digestive organs so that the digestive organs have to work extra to carry out its function in the process of digestion and metabolism of food. Anti-nutritive substances like phytic acid cause digestive organs work longer and will cause physiological disorders to these organs. Utilization of phytase can be applied to forage for example bran which is still very limited use, despite the high nutritional value. One of the problems the presence of anti-nutritive substances such as phytic acid which binds several important minerals forming complex compounds with Ca$^{2+}$, Zn$^{2+}$, Mg$^{2+}$ and Fe$^{3+}$. This has resulted in reduced digestibility and bioavailability [1].

Phytase can be classified as a phosphatase that has the ability to hydrolyze phytate compounds in the form 3-phytase and 6-phytase. Naming is based on the nature of the start of hydrolysis, which can hydrolyze phosphate (phosphate molecule / $\text{H}_2\text{PO}_4^-$) in phytic acid at the C atom for 3-phytase third and sixth C atom for 6-phytase. Phytase does have certain specific active group. To break the bonds of complex P in particular C atoms [5].

Phytase can be found in plants, some body tissues of animals and microorganisms like fungi and bacteria. Phytase from microorganisms has been studied intensively both extracellular and intracellular type. Phytase from plants have been isolated from wheat, soy, corn, grasses, lilies, grains, and nuts [3]. Phytase can be obtained from various sources, current has been obtained from plants, fungi, bacteria and rumen of ruminants, but the application of this enzyme are often constrained in production often requires a relatively high production costs if such a low environmental stability during storage and not resistance to heating during feed pelleting process. Phytase derived from plants, fungi and bacteria non-thermophilic has limitations on some properties like resistance to proteolysis, the catalytic efficiency, substrate specificity, stability at high temperatures and low acidity conditions. Phytase as phytic solvers are used as feed quality improvement efforts should be effective in hydrolyzing phytate in the digestive tract, stable against heating from feed production and storage, and the cost is cheap to produce it. By this fact, a very profitable alternative is if it can be obtained a superior source of the enzyme [1].

II. MATERIALS AND METHODS

Materials used in this study were Luria Bertani medium, ca-phytate, and reagents biochemical tests. Water and sediment samples from hot springs Sulili were taken aseptically and placed in sterile bottles that have been prepared and then put in a box of ice and immediately taken to the laboratory. As many as 10 ml of a suspension of sediment and water samples heat inoculated into 90 ml of liquid LB medium and incubated in a shaker incubator at 60°C for 24 hours at 100 rpm. Hereinafter cultures were inoculated on LB solid medium and bacteria that grow well purified on solid LB medium and then incubated at 60°C for 24 hours. Of colonies grown conducted repeated streaking on a new solid LB medium with the purpose to obtain isolates were truly pure. Pure culture isolates stored in agar slant storage at 4°C.

Phytase activity qualitatively testing done touched with a sterile swab simultaneously on medium plates LB + Ca-phytate and incubated at 60°C for 24 hours. Bacteria that have shown activity of phytase showed a clear zone around the colony. Index of Phytase is indicated by the ratio between the diameters of the clear zone of the colony diameters. Three isolates with the highest Index of Phytase (as superior isolates) were selected and stored at 4°C as stock isolates for subsequent purposes.

Three isolates were Hereinafter characterized and selected superior identified using observation procedure manually and supported with the use of microbial kit. Observation procedures manual includes observation of morphology, physiology, and biochemistry of bacteria. Identification steps to follow guide Bergey's Manual of Determinative Bacteriology [10]. Biochemical tests were performed among other Gram staining, spore staining, motility test, catalase, $\text{H}_2\text{S}$ production, starch hydrolysis, fermentation of glucose, gelatin test and Voges-Proskauer test and the nature of growth.

III. RESULT

As an effort to get a bacterial strain producing thermostable phytase enzyme in Indonesia, the isolation of bacteria from hot springs in the district Sulili Pirang South Sulawesi has been conducted. Twelve isolates of thermophilic bacteria were then selected for phytase-producing bacteria, five isolates showed proteolytic activity that marked the formation of a clear zone around the colonies grown on Luria farming medium supplemented with Ca-Phytate. Three of the five isolates were isolated with phytase index selected as the highest superior isolates. Isolates B has phytase index of 3.57; isolates F 3.06; and K 2.39. All three isolates were chosen each identified based on manual observation that includes observation of morphology, physiology and biochemistry of bacteria (Table 1).

| TABLE 1. CHARACTERISTICS OF ISOLATES PRODUCING PHYTASE FROM SULILI HOT SPRINGS | www.scirj.org |
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IV. DISCUSSION

Isolation was conducted by using a minimal medium containing components meet the basic needs of the bacteria, which is to produce cells or metabolisms, provide energy for biosynthesis and for the maintenance of the cell. The addition of Ca-phytate intended to induce and increase the speed of synthesis of phytase by isolated bacteria. Cultivation conducted at pH 7 because it is a natural pH habitat isolates the source location. The whole process of incubation at the stage of isolation and selection is carried out at high temperature (60°C). Incubation at high temperatures is intended that the bacteria is truly netted bacteria phytase enzyme whose activity with high stability at high temperatures [2].

The results of the characterization of phytase hydrolysis ability qualitatively indicate the formation of a clear zone on the test medium (LB medium + Ca-phytate), namely the loss of white medium around the colony isolates. This indicates the presence of the enzyme phytase produced by each isolate. The formation of this clear zone can be explained that the phytate is added to the LB medium plates in the form of ca-phytate comprising heksakis mio-inositol phosphate and bound to each other through hydrogen bonds with relatively large molecular size, so it cannot be used directly by the bacteria as source of nutrients because the large molecules cannot be transferred into the bacterial cell. Large molecules that can be used as a carbon or energy source for protein synthesis only when the first hydrolyzed first into smaller molecules outside the cell. For the purposes bacteria synthesize and secrete extracellular enzymes called extracellular phytase that can break down phytic with large molecules which are outside the cell into inorganic orthophosphate and phosphorus esters mio-inositol lower, even in certain circumstances be phosphate and mio-inositol free [3].

Bacterial isolates have been obtained from this study is the result of using the media isolation plate. The success of this method because most bacteria easily grow on solid media, so it will be easily isolated by spreading the cells on to the cup, so that it will grow colonies separate. Isolation conducted with pouring method, intended to spread evenly microorganisms in isolation media, to get a single isolates faster and to optimize the media, so that the isolation does not require a lot of time and cost. Casting method also allows isolates scattered over the surface of the media, so as to facilitate retrieval isolates were grown for work the next stage.

Prior to pouring the medium, hot water and sediment samples were homogenized and then inoculated into 90 ml of liquid LB medium and incubated in a shaker incubator at 60 ° C for 24 hours at 100 rpm. It is intended to prepare microorganisms ready to be planted and does not require a long time to adapt its growth on solid media. The final stage of this isolation is a characterization for the sake of identification of the isolated bacteria. Three isolates that have the highest activity of the five isolates were isolated, chosen to be identified. Series of biochemical and physiological tests include motolitas test, catalase, Voges-Proskauer, starch hydrolysis, hydrolysis of proteins, sugar test, test H2S formation, as well as gelatin test.
Based on observations of Gram reaction, cell morphology, and catalase tests, the third is a bacteria isolates of Bacillus because it has a rod-shaped vegetative cells, forming endospores, and are catalase positive. Further testing for identification to species level conducted and concluded as follows: isolates B as *Bacillus licheniformis*, isolate F as *Bacillus stearothermophilus* and isolates K as *Bacillus coagulans*. Identification was based on Bergey's manual of Determinative Bacteriology manual. Observation procedures manual includes observation of morphology, physiology and biochemistry of bacteria [10].

V. CONCLUSIONS

Based on the research results, it was concluded that from a hot spring Sulili it was obtained five isolates of thermophilic bacteria which have the ability to produce phytase and three of which were selected bacteria based on qualitative phytase activity. Three isolates were identified as *Bacillus licheniformis*, *Bacillus coagulans* and *Bacillus stearothermophilus*.

REFERENCES