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Phytase Enzyme Characteristic of *Bacillus subtilis* to Increase the Quality of Poultry Feed

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Abstract

Phytic acid is one of the antinutrition factors that found in feed like cereals, it can reduce the digestibility of feed nutrients so it will decreasethe productivity of monogastrik. Decreasing in productivity is due to the limitations of enzymes to detox phytic acid. The aim of this research is to know how is the activity of phytase enzyme derived from microbe (*Basillus subtilis*) that grown on solid media. The 4 solid media were corn, rice bran, corn bran and brown rice. Observations were made between pH 4 - 7 range with 5 days incubation period at 25^{O}C room temperature on the *water bath shaker* at speed100 rpm. The results showed that the activity of phytase from Basillus subtilis had a long pH range of 4 – 7. The activity of the solid media did not show any significant difference so that all media can be used as a medium growth for bacteria to produce phytase enzymes. The phytase enzyme which produced on corn media was 0.313 µg/ml, rice bran medium was 0.317 µg/ml, corn bran media was 0.313 µg/ml and brown rice medium was 0.0315 µg/ml. The conclusion of this research is Basillus subtilis bacteria can be used to produce phytase enzyme and able to decrease phytic acid content in feedstuff.

Keywords: Cereal, Phytase, Phytic Acid, Solid Media Fermentation

Introduction

Grains in feed ration are up to 60% from total ration. One of the reason to make limit used in feed stuff is the antinutrition which can be decrease the nutrien value and digestibility. This case can affect the animal productivity. Phytic acid is one of the antinutrion factor in grains which can disturb the avian productivity. Decreasing productivity is caused by unable enzyme to degrade phytic acid.

One persen of phytic acid ore more in feed stuff are causing mineral deficiency in animal. Phytic acid in corn is 1,6 – 2,6 g/kg and in soy is 2,8 – 4,0 g/kg (Kornegay. 1996). High composition of phytic acid in avian feed ration needs inovation to make phytic acid lower in feed ration. Adding phytase enzyme can hydrolize phytic acid in feed ration. Phytase is enzyme which can hydrolize phytic acid to free phosphat ion and mio-inositol, so the P element and others nutrients can be used optimally by the animal and phosphat residue is decrease. Phytase enzyme can be found in plants, microorganism, and animal body tissue (Sajidan, 2009).

There are many mehtods which can be used to decrease the loss caused by physically, chemically, and bilogically phytic acid, for example processing, heating, germination, and fermentation. However, the effective and applicable alternative methods for producing phytase enzyme to decrease phytic acid in feed stuff still could not be explained. Microorganisms are individual unicelluler that produce enzyme potentially. Degradable microorganism could be actinomycetes and fungi. Production of phytase could use fermentation technic on solid media. The objective of this research is to test the microorganism capability (*Basillus subtilis*) to produce phytase enzyme when fermentation process order to hydrolize the phytic acid in avian feed stuff (corn, corn bran, rice bran, and brown rice). Phytases from *Bacillus* species have been reported to have the advantage of being naturally thermostable and being strictly specific for phytates (Farhat *et. al*., 2008).

Materials and Methods

Microbe isolat collection of PAU UGM *Basillus subtilis* FNCC 0061 rejuvenated in *Luria Berthani* media.

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Phytase enzyme collected from *Basillus subtilis* which is grown in corn fluor, rice bran, soy bran, and brown rice solid media in room temperature for 3 - 4 days on *water bath shaker* at speed 100 rpm. The result of microbe growth in solid media was extracted with 100 mM Sodium Acetate pH 5 which contains 100 mM NaCl, then filtered. Filtrat was sentrifuged for 15 minutes on 10.000 rpm and temperature 4˚C. The supernathan was contained phytase that activity and protein contain are measured.

Phytase enzyme activity is determined based on phospate ion that released from substrate along the hydrolize reaction according to Quan*et al* (2001) method. Chemicals 800 µl buffer acetate was made from 0,2 M buffer acetate pH 5,5 which contains 1 mM sodium acetate, 200 µl phytase enzyme added. Mixture was incubated in 37˚C temperature for 30 minutes, then the reaction is stopped with adding 1 ml 10% trichloroacetate (TCA). Phospate ion which ic released were measured by mix 100 µl hidrolize solution with 900 µl H_2O andcolour reagent. Colour reagent was made from 1 ml 0,6 M H_2SO_4 which contains 2% ascorbatacid and 0,5% ammonium molibdat. The mixture was reincubated in 50˚C temperature for 20 menit then measured by spectrofotometer 820 nm. One of phytase activity unit described as a number of enzyme that could release 1 µmol phospate ion in 1 minute.

The protein that dissolved measured by Bardford assay (Bradford, 1976), 10 µl phytase solution added to 1 ml bradford solution and incubated in room temperature for 15 minutes then the absorbance will be measured. Absorbancy were checked with spectrofotometer 595 nm. The results analyzed by regretion curve protein standart that is Bovine Serum Albumin (BSA) 1 µg/µl.

Results and Discussion

Corn and soy have antinutrition agent that is phytic acid which ic disturb nutrien degradable and digestibility of feed stuff, but it is main storage of phospor for grains (Bohn *et al*., 2008). Phytic acid in corn 1,6 – 2,6 g/kg and in soy 2,8 – 4,0 g/kg (Kornegay*.* 1996). The monogastric like avian have limit phytase enzyme so they could not absorb optimally nutrien in feed that bonded with phytic acid. Phospor element that bonded in phytic acid could not digest so they would be excreted with faeces and could cause the environment polution (Viveros *et al*., 2000). P accumulation on soil and water could make eutrofication or the condition that can make excessive growth for cyanobacteria, hypoxia, and the death of aquatic organism also Nitrous oxcide production which can cause green house effect.(Madingan*et al*., 1997). Complex formation would escaped through faeces and the deposition would make environment polution (Glick & Pasternak 2003).

First stage in enzyme production was microbe replications with isolate bacteria PAU UGM collection *Basillus subtilis* FNCC 0061 on *Luria Berthani* media (Graphic 1). Phytase are found enzyme in plants, microorganism, and animal body tissue of animal (Sajidan, 2009). Specific phytase activity of microorganism was higher than phytase in plants or animals (Sajidan, 2009). Solid media for fermentation were corn fluor, rice bran, soy bran, and brown rice in room temperature for 3-4 days on *water bath shaker* at 100rpm.

Figure 1. Rejuvinating of *Basillus subtilis* FNCC 0061

Measurement of phospat standart checked in this stage with standart curve that can be found on Figure 2.

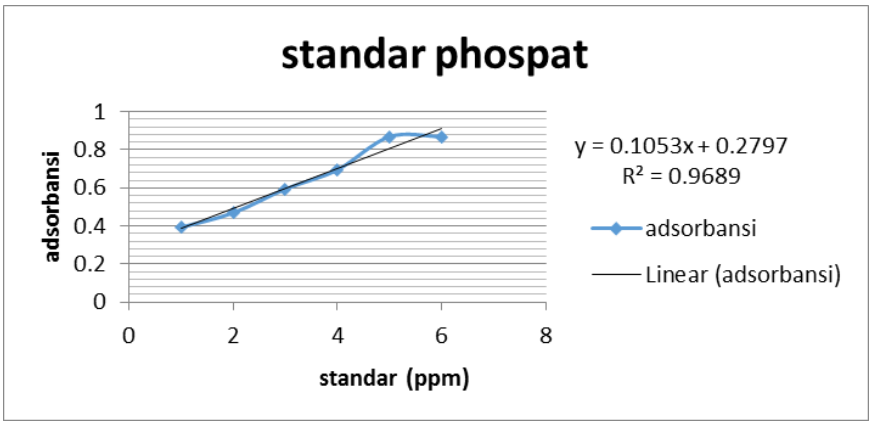

Figure 2. Standart Curve of Phosphat

Bateria inoculation on feed stuff media corn, corn bran, rice bran, and rice are conducted with pH treatments 4,5,6,and, 7. Purpose of this pH scale was to understand about which pH can produce high phytase activity. One of phytase activity unit described as a number of enzyme that could release 1 µmol phospate ion in 1 minute with Quan*et al* (2001) method. The results of enzyme activity could be found in Table 1.

Table 1. phytase enzym activity result in the differenced of pH and medium

pH	Medium	Enzyme activity (µg/ml)
4	Corn	0,300
	Corn bran	0,285
	Rice bran	0,283
	Brown rice	0,284
5	Corn	0,292
	Corn bran	0,284
	Rice bran	0,283
	Brown rice	0,284
6	Corn	0,287
	Corn bran	0,284
	Rice bran	0,283
	Brown rice	0,284
7	Corn	0,285
	Corn bran	0,285
	Rice bran	0,285
	Brown rice	0,284

The results of statistical analysis with factorial design patterns showed that the results were not significantly different at the level of P <0.05 so measurement of phytase activity showed that pH and media did not affect the production of phytase enzyme. *Basillus subtilis* could grow optimally on pH 4-7 and corn media, corn bran media, rice bran, and brown rice could produce phytse enzyme between 0,283-0,300 µg/ml. Extracelluler phytase enzyme can be collected from stable microbes on high temperature and pH 4-7(Gupta *et al.,* 2014). Purification of phytase enzyme can be found in Table 2.

Table 2. Precipitate results of raw phytase extract

Sample	Enzyme activity (µg/ml)
CE phytase rice bran	0,318
CE phytase brown rice	0,321
CE phytase corn rice	0,305
CE phytase corn	0,291

Note: CE : Crude Extract

Phytat degradation is the termination process between the myoinositol group bond and the phosphoric acid group by the enzyme phytase produced by rumen microbes (Bedford and Partridge, 2001). Test results of crude extract phytase concentration that have been purified showed that Basillus subtilis was able to produce enzyme activity of 0.291 µg / ml for corn, 0.305 µg / ml of corn bran, rice bran 0.318 µg / ml and 0.321 µg / ml for . *Basillus subtilis* bacteria can ferment ingredients to produce phytase enzymes in a long pH range and in various media that support the life of these bacteria. So that the results obtained do not have a significant effect on phytase enzyme production. According to Liebert et al. (1993), phytase is mostly active in the crop and proventriculus of broilers. In the crop, pH ranges between 4.0 and 5.0, and between 2.5 and 3.5 in the proventriculus. Furthermore, phytic acid phosphate groups are protonated at low pH, allowing their hydrolysis by phytases (Maenz, 2001). Therefore, higher in-vitro phytase activity at low pH (lower than 5.0) indicates higher phytase activity at those segments of the digestive tract of broilers.

Measurement of Dissolved Protein

The dissolved protein content was measured by the Bardford assay method (Bradford, 1976). The results of the experiment were calculated by the standard curve curve protein equation (Figure 3) using the BSA standard (Bovine Serum Albumin) 1 µg / µl.

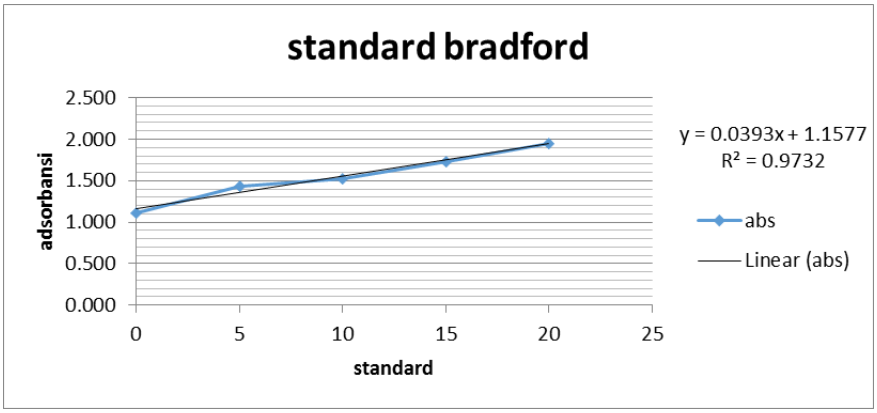

Figure 3. Bradford Standart for dissolved protein

The results of protein dissolved can be found in Table 3.

Table 3. Measurement of protein in the crude extract of the enzyme phytase

Sample	Enzyme activity (µg/ml)
Crude extract of corn enzyme	1,170
Crude extract of rice bran enzyme	1,171
Crude extract of corn bran enzyme	1,170
Crude extract of enzyme	1,170

Table 4. Measurement of protein in poultry feed media

Sample	Enzyme activity (µg/ml)
Corn	1,170
Rice bran	1,172
Corn bran	1,170
Brown rice	1,171

Protein measurement results showed that stable enzyme activity in all feed ingredients led to a reduction in biomass in all ingredients equal to all solid media 1,170 - 1,172 µg / ml. Basillus bacteria are facultative aerobic or anaerobic and resistant to extreme environments. Phytase enzyme activity from Bacillus increases in a phase of constant growth (Choi et al., 2001). Enzyme synthesis is related to certain growth phases of microbial cultures. Extracellular enzymes are widely synthesized in the exponential phase and the beginning of the stationary phase. Strain selection and environmental control (media and production process conditions) can affect the production of phytase enzymes. Factors that need to be controlled are temperature, pH, aeration conditions, composition of medium and fermentation medium (Indarwati, 2000).

Conclusion

This study concludes that Basillus subtilis in material fermentation can reduce phytic acid in animal feed ingredients so that the quality of animal feed ingredients can be improved. The results of measurements of phytase activity showed that Basillus subtilis bacteria could be used to produce phytase enzymes in several mediums of solid feed ingredients such as corn, rice bran, corn bran and brown rice. The results of phytase activity from *Basillus subtilis were* able to produce phytase enzymes at room temperature and in a fast incubation time of 5 days and in a long pH range 4 - 7. This study needs further research until the stage of making feed and direct application to livestock in vivo the results obtained are more real and maximum.

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