

## ISOLASI DAN KARAKTERISASI BAKTERI ASAM LAKTAT PENGHASIL FITASE DARI SALURAN PENCERNAAN UNGGAS SERTA KARAKTERISASI FITASENYA

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### INTISARI

Pakan unggas asal biji-bijian mengandung anti nutrisi asam fitat yang mengganggu penyerapan mineral, menurunkan tingkat pencernaan, dan menyebabkan polusi. Asam fitat dapat dikurangi dengan pemberian fitase. Eksplorasi dan pemanfaatan bakteri asam laktat penghasil fitase asal unggas lokal belum banyak dilakukan sehingga perlu dilakukan penelitian untuk mengisolasi BAL penghasil fitase dari saluran pencernaan unggas lokal, menapis isolat BAL yang menghasilkan fitase tertinggi, mengidentifikasi isolat BAL, mengetahui potensi BAL penghasil fitase sebagai probiotik, mengetahui pengaruh pH dan suhu terhadap aktivitas fitase, dan menentukan nilai parameter kinetika enzim ( $K_m$  dan  $V_{maks}$ ). Seleksi BAL penghasil fitase dilakukan pada media MRS-MOPS. Uji kemampuan BAL sebagai kandidat probiotik meliputi kemampuan bertahan pada pH asam, getah lambung, dan garam empedu. Pemurnian fitase dilakukan secara parsial dan didialisis. Fitase tersebut dikarakterisasi aktivitasnya pada suhu dan pH yang berbeda dan ditentukan kinetika enzimnya. Hasil menunjukkan bahwa dari dua puluh empat isolat BAL (ayam kampung dan bebek) yang memiliki aktivitas fitase, terdapat dua isolat dengan aktivitas fitase tertinggi yaitu isolat A1-E (41,9 U/ml) dan A2-11 (28,7 U/ml) dengan aktivitas tertinggi dicapai pada pertumbuhan BAL pada jam ke-24. Kedua isolat merupakan bakteri Gram positif, sel berbentuk batang, katalase negatif, tumbuh optimum pada suhu 37 °C dengan agitasi 100 rpm, dan kondisi minim  $O_2$ , serta memiliki homologi dengan *Lactobacillus plantarum* (99,9 %). Hasil uji kemampuan BAL terhadap pH asam, getah lambung, dan garam empedu menunjukkan bahwa waktu inkubasi yang lebih lama dibandingkan waktu retensi pakan di saluran pencernaan dapat menurunkan viabilitasnya. Pemurnian fitase secara parsial menunjukkan bahwa aktivitas spesifik asal isolat A1-E dan A2-11 lebih tinggi dibandingkan ekstrak kasar. Fitase dari *L. plantarum* A1-E mempunyai aktivitas optimum enzim pada pH 4,5 dan suhu 37 °C, nilai  $K_m$  0,70  $\mu M$  dan  $V_{maks}$  1,24  $\mu M$ /menit, sedangkan *L. plantarum* A2-11 mempunyai aktivitas optimum enzim pada pH 5,5 dan suhu 40 °C, nilai  $K_m$  0,33  $\mu M$  dan  $V_{maks}$  0,98  $\mu M$ /menit. Dapat disimpulkan bahwa BAL asal saluran pencernaan unggas menghasilkan fitase ekstraseluler yang berpotensi sebagai kandidat probiotik dengan karakteristik enzim yang spesifik.

**Kata kunci:** asam fitat, fitase, probiotik, bakteri asam laktat, unggas

## ISOLATION AND CHARACTERISATION OF LACTIC ACID BACTERIA AS PHYTASE PRODUCER FROM DIGESTIVE TRACT OF POULTRY AND THE PHYTASE CHARACTERISATION

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### ABSTRACT

Poultry feed were dominated with seeds contained anti-nutrient called phytic acid that disturb mineral absorption, decreased digestibility, and caused pollution. The phytic acid could be reduced by the addition of phytase. Exploration and usage of lactic acid bacteria has not been conducted yet. Therefore it needs to conducted a study of isolate LAB as phytase producer from digestive tract of local poultry, to screen the LAB isolate that produce high phytase activity, to identify the LAB isolate, to determine the LAB potency as probiotic, to determine the effect of pH and temperature on phytase activity, and to determine the enzyme kinetic values ( $K_m$  and  $V_{max}$ ). The selection of phytase producing LAB selection was conducted in MRS-MOPS medium. The assays of LAB ability as probiotic candidates consisted of resistance to acidic conditions, gastric juice, and bile salt. Purification of phytase was performed partially then dialyzed. The phytase was then characterized for its activities under different temperature and pH. The phytase kinetic properties were also determined. The results showed that among 24 LAB isolates (native chicken and ducks) that had phytase activity, there were two isolates had the highest phytase activity which were A1-E isolate (41.9 U/mL) and A2-11 (28.7 U/mL) with highest phytase activity at 24 hours growth. Both isolates were Gram-positive bacteria, bacil-shape of cell, catalase negative, optimum growth at temperature 37 °C with agitation 100 rpm, and minimal condition of  $O_2$ , and also had homology with *Lactobacillus plantarum* (99.9 %). The assay results of LAB viability in acidic pH, gastric juice, and bile salt showed that longer incubation time than feed retention time in gastrointestinal tract could decrease the viability. Partial purification of phytase showed that the specific activity of isolate A1-E and A2-11 were higher than the crude extract. Phytase of *L. plantarum* A1-E had optimum enzyme activity at temperature 37 °C and pH 4.5, the  $K_m$  value was 0.70  $\mu M$  and  $V_{max}$  was 1.24  $\mu M$ /minute, whereas *L. plantarum* A2-11 had optimum enzyme activity at temperature 40 °C and pH 5.5, the  $K_m$  value was 0.33  $\mu M$  and  $V_{max}$  was 0.98  $\mu M$ /minute. It could be concluded that LAB from digestive tract of poultry produced the extracellular phytase and potential as probiotic candidate and had specific enzyme characteristics.

**Keywords:** phytic acid, phytase, probiotic, lactic acid bacteria, poultry