

Penapisan dan Optimasi Produksi Enzim Protease Serupa Tripsin (PST) dari Beberapa *Lactobacillus plantarum* Untuk Enzim Pencernaan = Screening and Optimisation Tripsin-like Protease Enzymes Production from *Lactobacillus plantarum* for Digestive Enzymes

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Abstrak

Penelitian penapisan beberapa *Lactobacillus plantarum* dan optimasi produksi protease serupa tripsin (PST) dilanjutkan dengan pemekatan dan karakterisasi parsial telah dilakukan. Tripsin memiliki peran penting dalam pencernaan protein di usus kecil namun produksi tripsin komersial saat ini masih terbatas oleh masalah sertifikasi halal dan risiko penularan penyakit yang bersumber dari babi atau sapi. Penelitian bertujuan menyeleksi koleksi isolat *L. plantarum* yang menghasilkan aktivitas PST tertinggi dan menentukan kondisi optimum dalam produksi PST dari *L. plantarum* terpilih menggunakan Response Surface Methodology (RSM) diikuti dengan pemurnian dan karakterisasi parsialnya. Tujuh isolat *L. plantarum* yang diperoleh dari makanan tradisional Indonesia diseleksi secara kualitatif dan kuantitatif. Semua isolat *L. plantarum* menunjukkan aktivitas proteolitik, terlihat adanya zona bening di sekitar koloni. Zona bening menunjukkan adanya potensi *L. plantarum* sebagai sumber PST secara kualitatif. Hasil pengujian kuantitatif menunjukkan bahwa isolat dengan kode B6 (*L. plantarum* WBM-4) menghasilkan PST dengan aktivitas tripsin tertinggi sebesar 0,16 mU/mL. *Lactobacillus plantarum* WBM-4 diisolasi dari buah Menteng Banjarmasin paling berpotensi untuk menghasilkan PST. Selanjutnya, *L. plantarum* WBM-4 dioptimalisasi produksi menggunakan Respon Surface Methodology (RSM) dan karakterisasi PST. Kondisi optimal ditentukan pada komposisi medium dengan 1,96% glukosa, 0,39% yeast extract, 1,97% skim milk, dan pH 6,62, menghasilkan aktivitas PST sebesar 0,303 mU/mL. Pemekatan enzim kasar di bawah kondisi optimum menggunakan viva spin 5000 MWCO meningkatkan kemurnian hingga 11,08 kali lipat, dengan aktivitas sebesar 2,47 mU/mL. Karakterisasi parsial menunjukkan berat molekul PST sekitar ~19 kDa dan ~29 kDa, stabilitas dalam rentang suhu 30 - 40°C, dan aktivitas optimal pada pH 7,0 - 8,0. Penambahan ion logam EDTA, Ca²⁺, dan Zn²⁺ memengaruhi aktivitas PST. Penyimpanan PST selama 30 hari pada suhu 4°C aktivitas tersisa PST masih 65% sedang pada suhu 24-28°C aktivitas hanya tersisa 15%. Hasil penelitian ini memberikan gambaran tentang potensi PST yang berasal dari *L. plantarum* untuk aplikasi suplemen pencernaan dan memberikan alternatif sumber tripsin yang halal dan aman.

.....Research on screening of several *Lactobacillus plantarum* and optimization of trypsin-like protease production (TLP) followed by concentration and partial characterization has been carried out. Trypsin has an important role in protein digestion in the small intestine, but commercial trypsin production is currently limited by halal certification issues and the risk of transmission of diseases sourced from pigs or cattle. The study aimed to select a collection of *Lactobacillus plantarum* isolates that produced the highest TLP activity and determine the optimum conditions in TLP production from selected *L. plantarum* using Response Surface Methodology (RSM) followed by purification and partial characterization. Seven isolates of *L. plantarum* obtained from traditional Indonesian food were selected qualitatively and quantitatively. All *L. plantarum* isolates exhibited proteolytic activity, with clear zones around the colony. The clear zone shows the potential of *L. plantarum* as a qualitative source of TLP. The results of quantitative testing showed that

isolates with code B6 (*L. plantarum* WBM-4) produced TLP with the highest trypsin activity value 0.16 mU/mL. *L. plantarum* WBM-4 isolated from Banjarmasin Menteng fruit has the most potential to produce TLP. Furthermore, *L. plantarum* WBM-4 optimized production using Response Surface Methodology (RSM) and TLP characterization. Optimal conditions were determined in the composition of the medium with 1.96% glucose, 0.39% yeast extract, 1.97% skim milk, and pH 6.62, resulting in TLP activity of 0.303 mU/mL. Crude enzyme concentration under optimum conditions using viva spin 5000 MWCO increases purity up to 11.08-fold, with an activity of 2.47 mU/mL. Partial characterization shows TLP molecular weights of approximately ~29 kDa and ~19 kDa, stability in the temperature range of 30 - 40 °C, and optimal activity at pH 7.0 - 8.0. The addition of EDTA, Ca²⁺, and Zn²⁺ metal ions affect TLP activity. TLP storage for 30 days at 4°C the remaining activity of PST is still 65% while at 24-28°C the activity is only 15%. The results of this study provide an overview of the potential of PST derived from *L. plantarum* for digestive supplement applications and provide an alternative source of trypsin that is halal and safe.