

## ISOLASI, SELEKSI, DAN IDENTIFIKASI BAKTERI ASAM LAKTAT PROTEOLITIK BERPOTENSI SEBAGAI PROBIOTIK DARI KOLOSTRUM SAPI PERAH

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### INTISARI

Penelitian ini bertujuan untuk memperoleh strain Bakteri Asam Laktat (BAL) baru dengan aktivitas proteolitik hasil isolasi, seleksi, dan identifikasi dari kolostrum sapi perah. Tahapan penelitian meliputi lima tahap yaitu pengkayaan sumber BAL (kolostrum), isolasi, seleksi, identifikasi, dan kajian BAL proteolitik sebagai kandidat probiotik. Prosedur seleksi meliputi uji aktivitas protease secara kualitatif dan kuantitatif. Prosedur identifikasi secara molekuler dengan menggunakan metode amplifikasi sekuens gen 16S rRNA menggunakan *primer universal* 27F (AGAGTTTGATCCTGGCT CAG) dan 1429 R (TAGGGTTACCTTGTTACGACTT). Isolat yang telah teridentifikasi kemudian diuji lebih lanjut sebagai kandidat probiotik meliputi uji ketahanan terhadap pH rendah (pH 2,5), ketahanan terhadap garam empedu (0,3%) dan aktivitas antimikrobia terhadap bakteri patogen. Hasil uji aktivitas protease, ketahanan pH rendah, ketahanan terhadap garam empedu, dan zona hambat antimikrobia kemudian dianalisis menggunakan Rancangan Acak Lengkap Pola Searah dan bila terdapat variabel signifikan karena perlakuan dilanjutkan dengan uji DMRT, sedangkan hasil isolasi dan identifikasi dianalisis secara deskriptif. Hasil penelitian isolasi diperoleh 87 total koloni dengan 25 koloni sebagai bakteri asam laktat karena menghasilkan zona bening. Hasil penelitian seleksi diperoleh enam isolat bakteri terbaik yaitu Bal Pro 3, Bal Pro 4, Bal Pro 8, Bal Pro 9, Bal Pro 20 dan Bal Pro 21 yang memberikan respon secara nyata terhadap aktivitas protease ( $P < 0,05$ ) dengan nilai 0,346-0,849 U/ml. Hasil identifikasi secara molekuler menggunakan gen 16s rRNA menunjukkan semua isolat teridentifikasi sebagai *Limosilactobacillus fermentum*. Keenam strain yang diperoleh dapat menjadi kandidat probiotik karena mampu memberikan respon nyata ( $P < 0,05$ ) untuk tumbuh dan bertahan pada pH rendah 2,5, garam empedu 0,3% serta dan memiliki aktivitas antagonistik terhadap bakteri patogen *E. coli* dan *S. aureus*. Kesimpulan yang dapat diambil dari penelitian ini yaitu diperoleh enam isolat BAL proteolitik dari sumber kolostrum sapi perah yang teridentifikasi sebagai *Limosilactobacillus fermentum* serta memenuhi syarat sebagai kandidat probiotik berdasarkan hasil uji ketahanan pH, ketahanan terhadap garam empedu dan aktivitas antimikrobia.

Kata kunci: bakteri asam laktat, kolostrum, aktivitas proteolitik, probiotik, gen 16s rRNA

## ISOLATION, SELECTION, AND IDENTIFICATION OF PROTEOLYTIC LACTIC ACID BACTERIA AS PROBIOTIC POTENTIAL FROM COLOSTRUM OF DAIRY CATTLE

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### ABSTRACT

The purpose of the study was to obtain new strains of Lactic Acid Bacteria (LAB) which has proteolytic activity by isolation, selection, and identification from the colostrum of dairy cows. The research had five stages, namely enrichment of LAB (colostrum) sources, isolation, selection, identification, and study of LAB as a candidate for probiotics. The selection procedure based on the qualitative and quantitative protease activity analysis. Molecular identification procedure using the 16S rRNA gene sequence amplification method used universal primary 27F (AGAGTTTGATCCTGGCTCAG) and 1429 R (TAGGGTTACCTTGTTACG ACTT). Isolates that had been identified were then further studied as probiotic candidates, including low pH resistance test (pH 2.5), resistance to bile salts (0.3%) and antimicrobial activity against pathogenic bacteria. The test results of protease activity, low pH resistance, resistance to bile salts, and antimicrobial bland zones were then analyzed using a Completely Randomized Design (CRD) of one way and if there were significant differences between variables due to the treatment, it continued with the DMRT test, while the results of isolation and identification was analyzed by description. The results of the isolation study obtained 87 total colonies with 25 colonies as lactic acid bacteria because they produced a clear zone. The test results of the selection study obtained six best bacterial isolates, namely Bal Pro 3, Bal Pro 4, Bal Pro 8, Bal Pro 9, Bal Pro 20 and Bal Pro 21 which gave a real response to protease activity ( $P < 0.05$ ) with a value of 0.346-0.849 U/ml. The results of molecular identification using the rRNA 16s gene showed all isolates identified as *Limosilactobacillus fermentum*. The six isolate strains obtained can be probiotic candidates because they were able to provide a real response ( $P < 0.05$ ) to grow and survive at a low pH of 2.5, bile salts of 0.3% as well as and had antagonistic activity against pathogenic bacteria *E. coli* and *S. aureus*. The conclusion that can be drawn from this study is that six proteolytic LAB isolates from dairy cow colostrum were identified as *Limosilactobacillus fermentum* and qualified as probiotic candidates based on the results of pH resistance tests, bile salt resistance and antimicrobial activity.

Keywords: lactic acid bacteria, colostrum, proteolytic activity, probiotics,  
16s rRNA gene