

RINGKASAN

Bakteri asam laktat (BAL) merupakan kelompok bakteri yang mampu memfermentasikan karbohidrat menjadi asam laktat dan diketahui memiliki manfaat di bidang pangan maupun kesehatan. Isolat BAL telah diisolasi dari lingkungan sedimen mangrove di Pantai Logending. Karakterisasi secara fenetik terhadap isolat BAL belum mampu mengidentifikasi sampai dengan tingkat spesies. Salah satu pendekatan identifikasi adalah menggunakan pendekatan genotipik yaitu analisis sekuen gen 16S rRNA. Identifikasi molekuler menggunakan 16S rRNA memiliki akurasi tinggi dalam menentukan taksonomi suatu bakteri. Sekuens rRNA khususnya 16S rRNA menjadi acuan utama dalam studi evolusi dan ekologi bakteri, termasuk determinasi hubungan filogenetik di antara taxa, eksplorasi keanekaragaman bakteri di lingkungan dan kuantifikasi kelimpahan relatif taksa dari berbagai tingkatan. Identifikasi bakteri berdasarkan marka 16S rRNA memerlukan teknik PCR (*Polymerase Chain Reaction*). Tujuan penelitian kali ini adalah untuk mengetahui identitas dari bakteri asam laktat yang diisolasi dari sedimen mangrove Pantai Logending dan hubungan filogenetiknya.

Penelitian dilakukan dengan metode survei. Tahapan kerja terdiri atas isolasi DNA, konfirmasi isolat BAL (penanaman pada medium MRSA+CaCO₃, pewarnaan gram, uji katalase-oksidadase dan pewarnaan endospora), amplifikasi gen 16S rRNA dengan metode *Polymerase Chain Reaction* (PCR) menggunakan primer universal 27F dan 1492R, sekuensing hasil PCR, analisis hasil sekuensing menggunakan *Basic Local Alignment Search Tool* (BLAST). Paramater yang diamati adalah ukuran dan urutan nukleotida sekuen gen 16S rRNA, persentase kemiripan urutan basa nukleotida pada bakteri. Data hasil BLAST dianalisis kemiripan dengan data yang ada di GenBank dan hubungan filogenetiknya menggunakan metode UPGMA, *Neighbour joining*, *Maximum Parsimony*, *Maximum Likelihood*.

Hasil penelitian didapatkan tiga isolat terpilih dan teridentifikasi hingga tingkat spesies yaitu LG 3 memiliki kemiripan dengan *Bacillus aerius strain 24 K* sebesar 100%, isolat LG-15 memiliki kemiripan dengan *Bacillus cereus* sebesar 99,80% isolat LG-71 memiliki kemiripan dengan *Bacillus tropicus strain MCCC 1A01406* sebesar 99,24% serta dua isolat lain hanya mampu teridentifikasi hingga tingkat spesies yaitu isolat LG-17 memiliki kemiripan dengan *Bacillus wiedmanni strain FSL W8-0169* sebesar 98,80%, isolat LG-114 memiliki kemiripan dengan *Bacillus tropicus strain MCCC 1A01406* sebesar 98,41%. Adapun kekerabatan masing-masing isolat *Bacillus tropicus strain MCC 1A01406*, *Bacillus cereus ATCC14579* dan *Bacillus tropicus strain MCCC* berada pada satu klade yang berarti menandakan bahwa spesies tersebut merupakan spesies yang sangat mirip. Bakteri *Bacillus aereus strain 24K* memiliki kekerabatan yang cukup jauh dengan dua isolat pembanding yaitu *Leunostoc lactis* dan *Ligilactobacillus salivarius*. Sedangkan *Bacillus wiedmannii strain FSL w8-0169* menjadi outgroup dari semua isolat sampel yang diuji

Kata kunci: *Bakteri Asam Laktat (BAL)*, *identifikasi genotipik*, *pohon filogenetik*, *sekuen gen 16S rRNA*

SUMMARY

Lactic Acid Bacteria (LAB) is a group of bacteria that can ferment carbohydrates into lactic acid and is known to have functions in food and health sectors. The isolates of LAB had been isolated from the environment of mangrove sediment in the Logending coast. The characterization of LAB's isolates genetically has not yet been able to identify them at the species level. One of the identification approaches is utilizing the genotypic approach which is the analysis of the gene 16S rRNA sequence. Molecular identification using 16S rRNA has a high accuracy to determine the taxonomy of a certain bacteria. Ribosome RNA sequences, specifically 16S rRNA, become the main reference in the study of bacteria evolution and ecology, including the determination of phylogenetic relation among taxa, exploration of the bacteria variety in the environment, and quantification of relative abundance of taxa from various levels. Identification of bacteria based on marker 16S rRNA requires the PCR (Polymerase Chain Reaction) technique. The purpose of the research is to know the identity of lactic acid bacteria which was isolated from mangrove sediment in the Logending coast and its phylogenetic relation.

The research was conducted using a survey method. The working steps consisted of DNA isolation, confirmation of LAB's isolates (cultivation in MRSA+CaCO₃ medium, Gram staining, catalase-oxidation test, and endospore staining), amplification of gene 16S rRNA with Polymerase Chain Reaction (PCR) method utilizing universal primer 27F and 1492R, sequencing of PCR product, analysis of sequencing result using Basic Local Alignment Search Tool (BLAST). The observed parameters are the size and nucleotide sequence of gene 16S rRNA sequence, and the similarity percentage of sequence of nucleotide bases in bacteria. The result data of BLAST were analyzed in terms of similarity to the data in the GeneBank and its phylogenetic relation using the UPGMA method, Neighbor Joining, Maximum Parsimony, and Maximum Likelihood.

The result of the research found there were 5 selected and identified isolates to the species level, which were LG3 isolate which had similarity to *Bacillus aerius strain 24K* of 100%, LG-15 isolate which had similarity to *Bacillus cereus* of 99,80%, LG-17 isolate which had similarity to *Bacillus wiedmanni strain FSL W8-0169* of 98,80%, LG-71 isolate which had similarity to *Bacillus tropicus strain MCCC 1A01406* of 99,24%, and LG-114 isolate which had similarity to *Bacillus tropicus strain MCCC 1A01406* of 98,41%. Regarding the genetic relationship of each *Bacillus tropicus strain MCCC 1A01406* isolate, *Bacillus cereus ATCC14579* and *Bacillus tropicus strain MCCC* were in one clade which gave a sign that those species are very similar. Bacteria *Bacillus aerius strain 24K* had a quite far similarity to the two compared isolates which were *Leunostoc lactis* and homofermentative. On the other hand, *Bacillus wiedmannii strain FSL w8-0169* became the outgroup of all isolate samples that were tested.

Keywords: *Gene 16S rRNA sequence, Genotypic identification, Lactic Acid Bacteria(LAB),Phylogenetic*