

SEQUENCE ANALYSIS AND CLONE OF LACTATE DEHYDROGENASE (LDH) GENE FROM VARIOUS LACTIC ACID BACTERIA ISOLATES

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ABSTRACT

The aims of this study are to determine the species of B25 lactic acid-producing bacteria isolates based on 16S rRNA gene marker, to get DNA clones which are amplified using specific primer for homologous LDHs genes from B25 and B26 lactic acid-producing bacteria isolate by PCR technique, to determine the homology level of DNA sequences that have been isolated with LDH gene sequences in the genebank.

*This research is an exploratory research. Identification of bacterial species was carried out based on the 16S rRNA gene marker using the PCR method followed by sequencing. The sequences obtained were then analyzed using the Basic Local Alignment Search Tool (BLAST). Species of isolate B25 were then determined based on sequence similarity (99%) and their relationship in the phylogenetic tree. Isolation of LDH homologous genes was carried out through gene amplification by PCR technique using specific primers for homologous LDH genes (ldb 1010, ldb 0101, ldb 1147, ldb 2021, ldb 0813). Sequencing of the amplified fragments was then carried out at 1st BASE Laboratories. Sequence analysis was performed using the MegaX and Clone 9 programs. Homology confirmation was carried out based on the homologous LDH gene sequences in the genebank (NCBI). Identification of B25 isolate based on 16S rRNA markers showed that the isolate had 99.90% similarity to *Bacillus proteolyticus*. Amplification with specific primers for homologous LDH genes resulted in fragments of varying sizes (500 - 2500 bp). The results of DNA sequence analysis produced by amplification using specific primers for the *ldb1010* gene (coding for 2-hydroxyacid dehydrogenase) from isolate B25 assisted by phylogenetic tree construction showed that fragments with a length of 1500 and 1700 bp had similarities with the gene encoding 2-hydroxyacid dehydrogenase in bacteria. *Bacillus clarus* with a confidence level of 100%. Confirmation in the 650 and 2500 bp long fragments were similar to the gene encoding D-lactate dehydrogenase (D-LDH) in *Bacillus clausii* bacteria with a phylogenetic tree level and confidence of 78%.*

Kata Kunci: *Lactic acid producing bacteria, 16S rRNA gene marker LDH homolog, 2-hydroxyacid dehydrogease*