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Detection and sequence analysis of the gene encoding L-Lactate Dehydrogenase from starch-utilizing bacteria

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Lactate dehydrogenase is a key enzyme in lactic acid fermentation by most lactic acid bacteria. Most bacterial species have been reported to possess only one lactate dehydrogenase gene. The DNA sequence analysis of the gene encoding the enzyme is an important prerequisite to the genetic manipulation and improvement of lactate-producing strains. The lactate dehydrogenase gene polymorphism is also useful for lactococcal classifications. The gene encoding lactate dehydrogenase of two starch-utilizing and homofermentative bacterium isolates (SUT-1 and SUT-5) as well as *Lactococcus lactis* IO-1; a homolactic fermenting bacterium which is able to convert glucose to L-lactate with a conversion rate greater than 90%, and has been given permission to be used as the reference strain in this study; was detected. The detection was performed by amplifying bacterial DNA in polymerase chain reactions using ten primer pairs designed from sequences encoded bacterial lactate dehydrogenase to obtain suitable amplification primers for the isolates of interest. The most successful amplification was obtained when using three sets of primers designed from sequences complementary to conserved sequences at the extreme ends of the coding region of *Lactococcus lactis* L-lactate dehydrogenase gene. All of the bacterial strains (isolates SUT-1 and SUT-5, and *Lactococcus lactis* IO-1) produced results of approximate 1,000-bp, 670-bp, and 400-bp amplification products with the three sets of specific primers. The sequence analysis of 670-bp and 400-bp amplification fragments was performed. When these amplified fragment sequences were compared with each other, the comparisons of the approximate sequences of 670 nucleotides showed that isolates SUT-1 and SUT-5 had 39% and 47% homology respectively with *Lactococcus lactis* IO-1. The SUT-1 sequence had 52% homology with the SUT-5 sequence. And the comparisons of the sequences of about 400 nucleotides showed that isolates SUT-1 and SUT-5 had 80% and 75% homology respectively with *Lactococcus lactis* IO-1. The SUT-1 sequence had 84% homology with the SUT-5 sequence. These results reveal that isolates SUT-1 and SUT-5 have rather high similarity of L-lactate dehydrogenase gene sequence to *Lactococcus lactis* IO-1.