**Improvement in D-lactic acid production in *Lactobacillus delbreuckii subsp. bulgaricus* mutants generated using random mutagenesis approach and optimization of fermentation conditions**

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**Abstract:**

The aim of this work is to generate an improved strain of *Lactobacillus delbreuckii subsp. bulgaricus* ATCC 11842 with enhanced D-lactic acid (DLA) production through random mutagenesis. The mutant strain with improved DLA production and high dry cell weight (DCW) was obtained using combined physical (Ultraviolet rays, UV rays) and chemical mutagenesis (N-methyl-N′-nitro-N-nitrosoguanidine, NTG) for several rounds. After multiple strain screening, Ldb\_NTG\_23, one positive mutant selected produced 1.96-fold increase in DLA production and 1.45-fold enhanced growth rate compared to wild strain ATCC 11842. The mutant strain showed stable DLA production for more than 5 generations and was further subjected to fermentation condition and media optimization using combined OFAT (One factor at a time) and RSM (Response surface methodology) studies. Optimal DLA production was obtained using sole carbon source as fructose at 30 g/L and casein hydrolysate as additional nitrogen source at 0.5 g/L in aerobic conditions. The utilization of various combinations of amino acids is currently under investigation for the purpose of augmenting the production of DLA. It is anticipated that under the optimized fermentation conditions acquired through Response Surface Methodology - Central Composite Design (RSM-CCD), the production of D-lactic acid (DLA) will escalate by over 50% in comparison to the wild strain with 99.08 % optical purity. Overall, mutant developed demonstrated faster growth rate, shorter lag phases, higher productivity, higher lactic acid yield, and could potentially have future industrial applications.

*Keywords: D-lactic acid, mutagenesis, Nitrosoguanidine, lactic acid bacteria, media optimization*