Transformation of Lactic Acid Bacteria

Bryan Coburn

Introduction
Genetic modification of lactic acid bacteria is important to many biotechnological and industrial applications. We have demonstrated how to increase transformation efficiency of Lactococcus lactis and Lactobacillus casei via electroporation.

Hypothesis
Increasing the number of electroporation pulses will increase transformation efficiency.

Transformation Efficiency is Influenced by Growth Conditions and Pre-Treatment of Cells
Lactococcus lactis (strain JB704) bacterial cells were transformed at 2500 Volts, 25 µF, and 200 Ω with the pTW8 vector. However, bacterial cells were grown and pre-treated differently. One culture of recipient cells was grown in MRS without 1% glycine and received no pre-treatment, were pre-treated for 30’ with water, or were pre-treated for 30’ with 100 mM lithium acetate and 10 mM dithiothreitol (DTT); a second culture of recipient cells was grown in MRS with 1% glycine and received no pre-treatment, were pretreated for 30’ with water, or were pre-treated for 30’ with 100 mM lithium acetate and 10 mM dithiothreitol (DTT).

Transformation Efficiency is Influenced by Electric Pulse Voltage
Lactobacillus casei (strain 32G) was grown in MRS with 1% glycine and pre-treated for 30’ with 100 mM lithium acetate and 10 mM dithiothreitol (DTT). These bacterial cells were transformed under different electroporation settings: the cells received 1 pulse or 5 pulse electroporation, and pulse voltage was set at 1500 V, 2000 V, or 2500 V. Resistance and capacitance were set at 200 Ω and 25µF respectively for all transformations. All cells were transformed with the pTRKH2 vector.

Conclusions
From these experiments we conclude that Lactococcus lactis and Lactobacillus casei will yield the higher number of transformants if given 5 electroporation pulses rather than 1 pulse. However, the viability of Lactobacillus casei cells is decreased at the higher electroporation voltages. Lactococcus lactis yielded the highest number of transformants when grown in MRS containing 1% glycine, pre-treated with 100 mM lithium acetate and 10 mM dithiothreitol (DTT), and exposed to 5 electroporation pulses. Lactobacillus casei yielded the highest number of transformants when electroporated at 2500 V with 1 pulse or at 1500 V when exposed to 5 electroporation pulses.