

## 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.

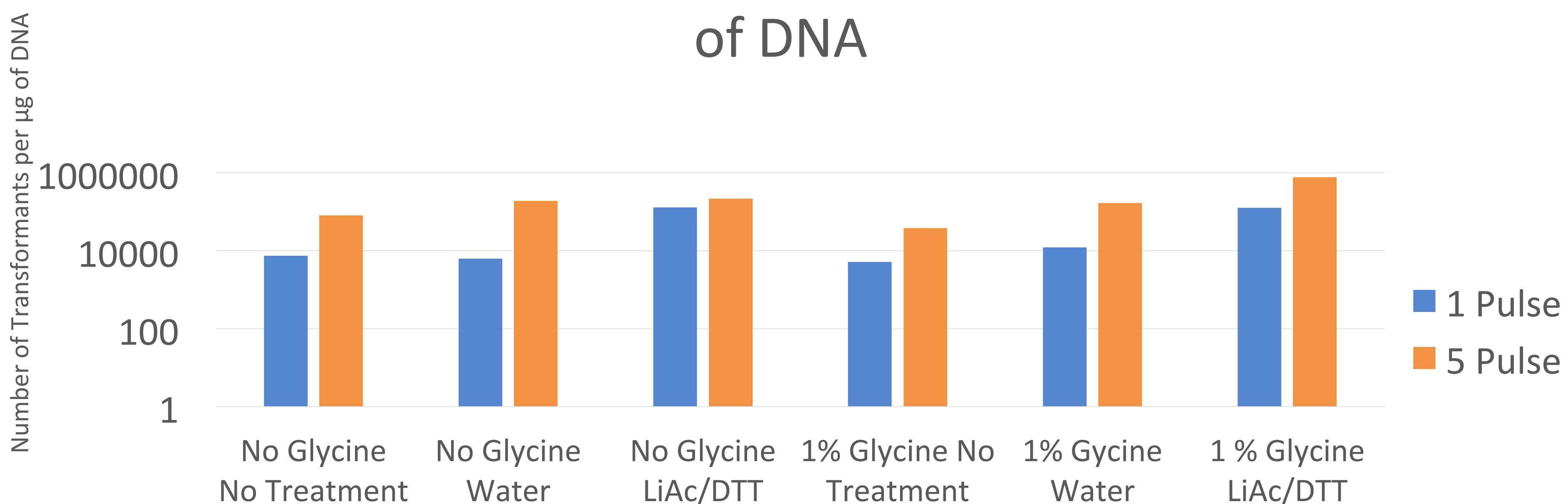
## Hypothesis

Increasing the number of electroporation pulses will increase transformation efficiency.

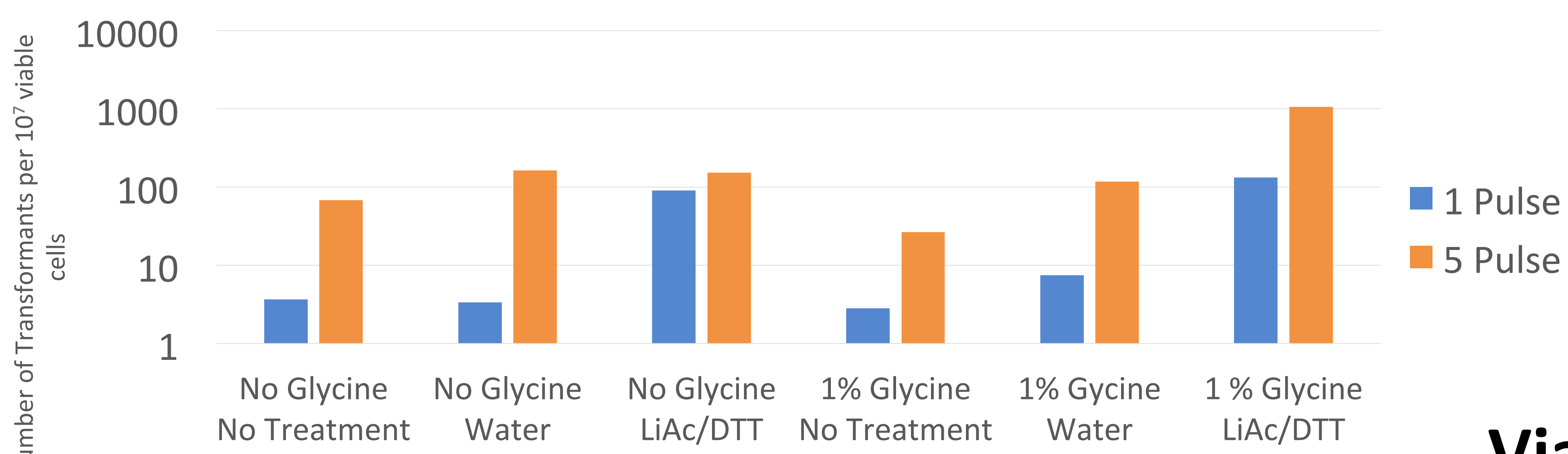
## Transformation Efficiency is Influenced by Growth Conditions and Pre-Treatment of Cells

*Lactococcus lactis* (strain JB704) bacterial cells were transformed at 2500 Volts, 25  $\mu$ F, and 200  $\Omega$  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 10mM dithiothreitol (DTT).

### Average Number of Transformants per microgram of DNA



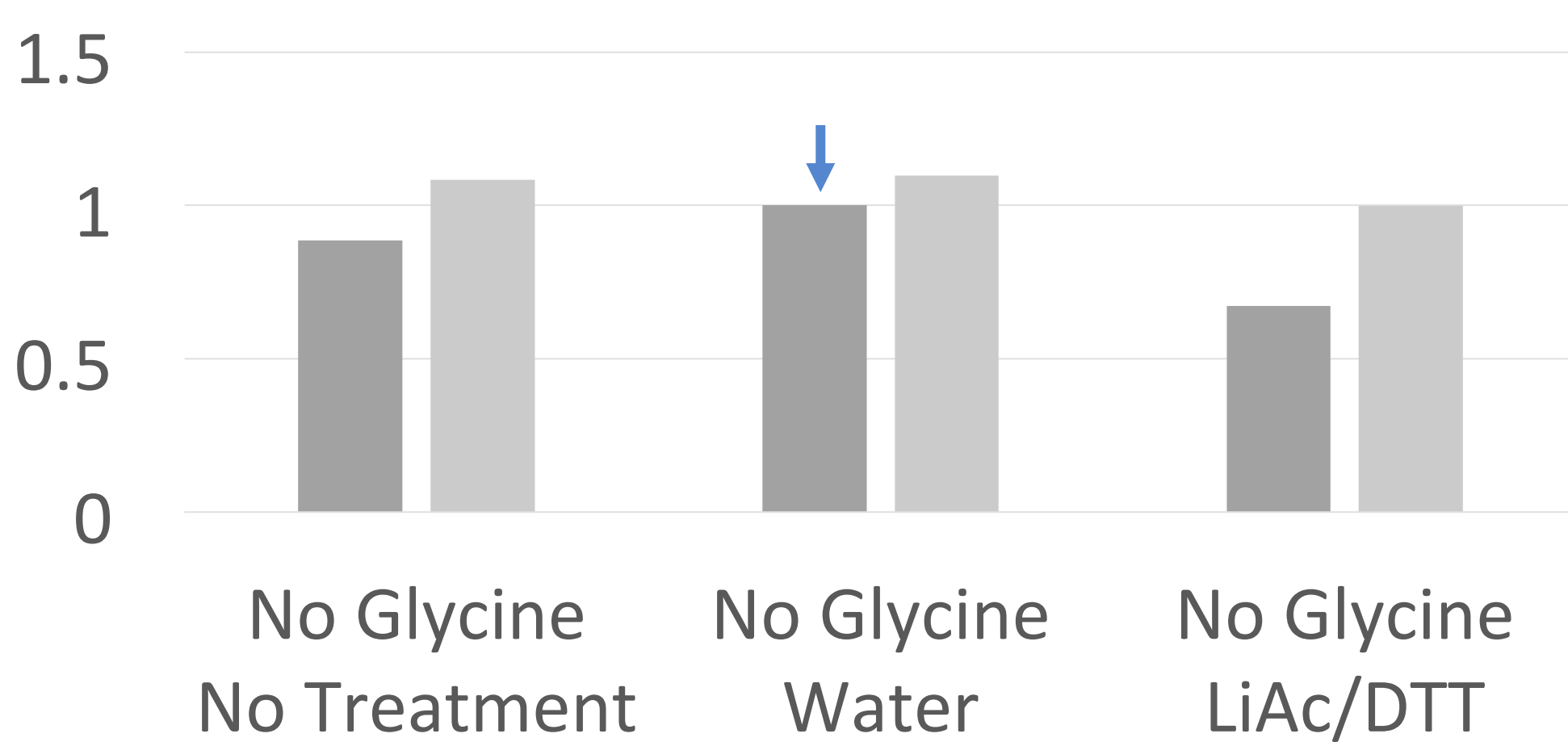
### Number of Transformants per 10 million Viable Cell



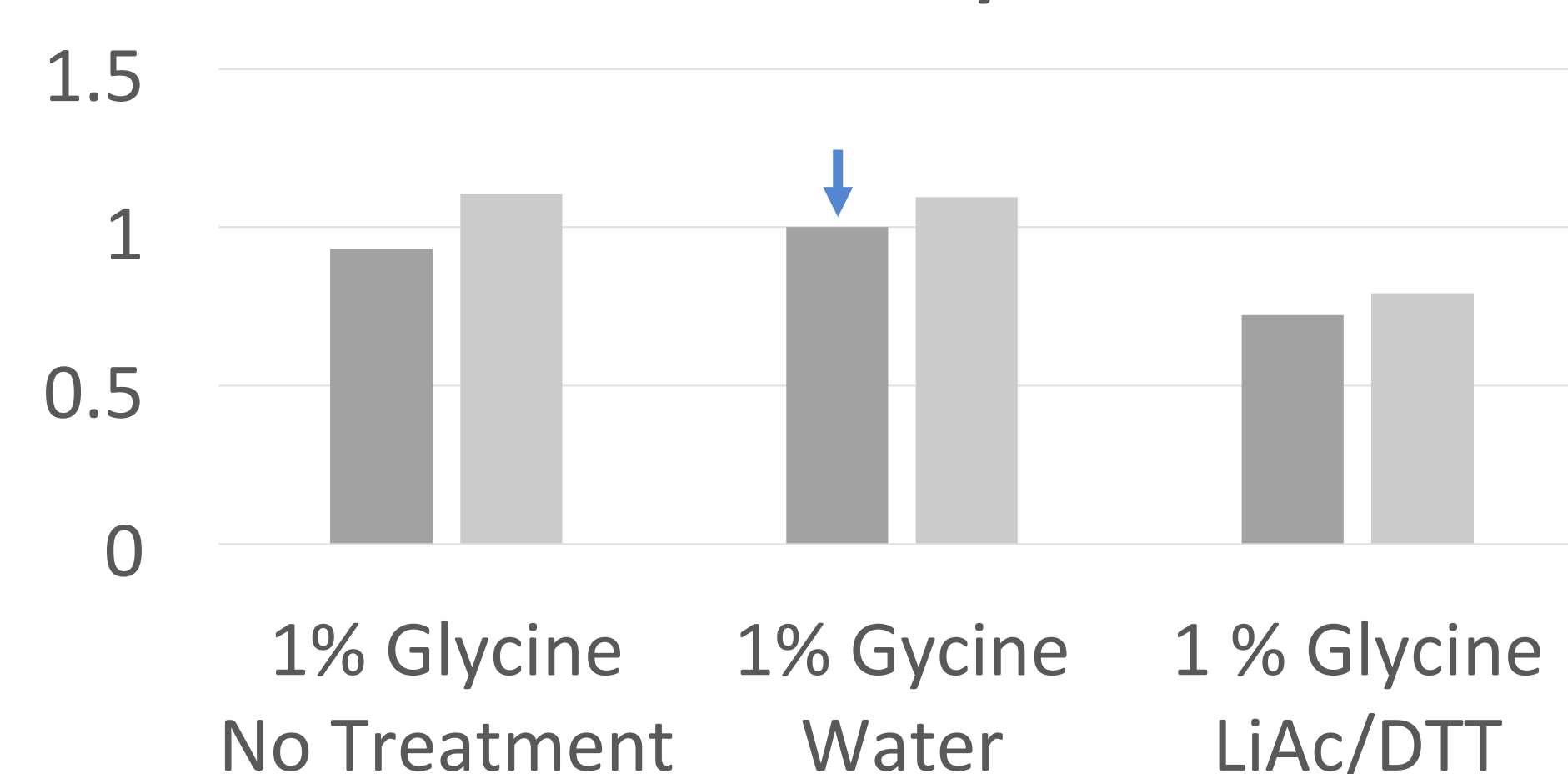
## Viability

Tranformant cultures generally contained about  $10^8$  cells per mL. Viability graphs have been normalized by dividing each CFU count by the CFU count for the tranformant sample indicated by the blue arrow in each graph.

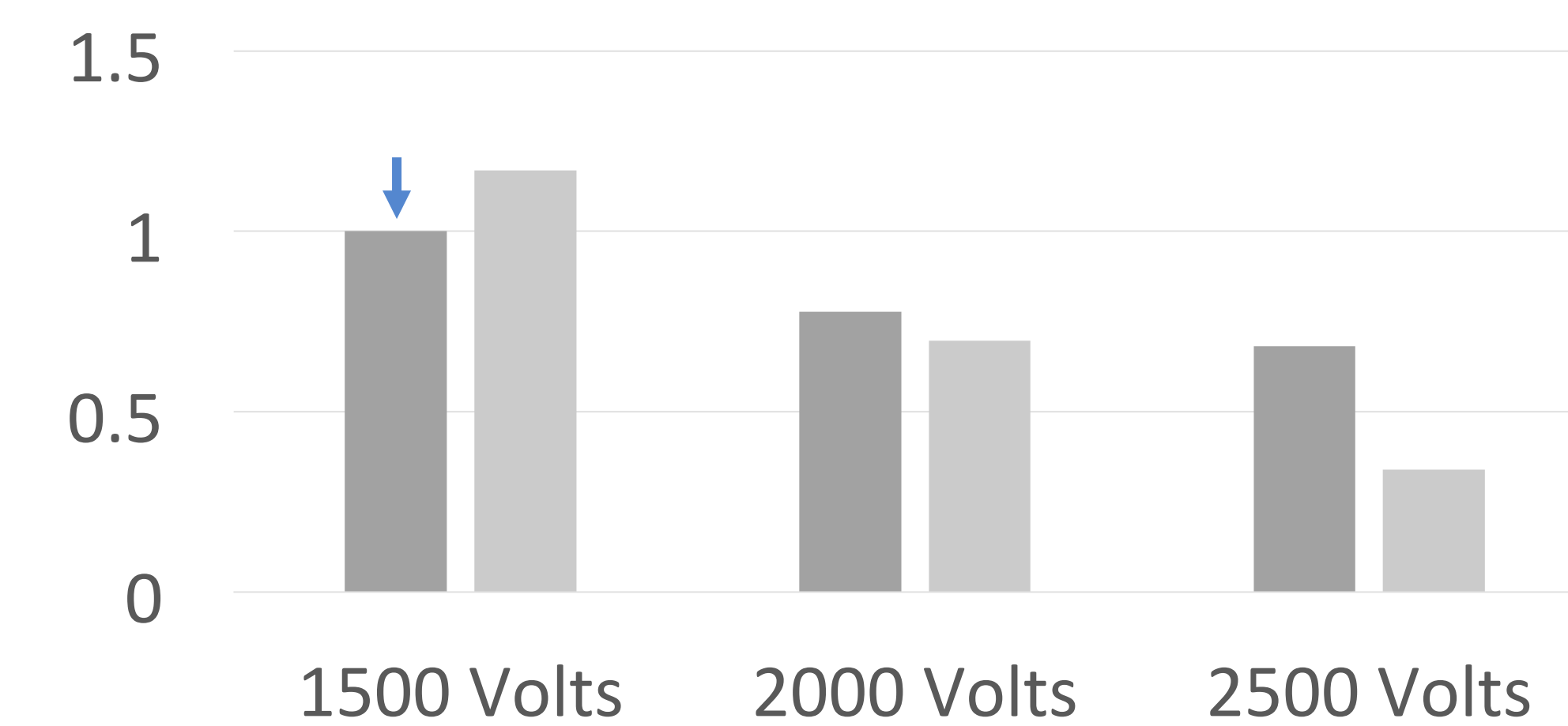
### Viability



### Viability



### Viability



## 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.

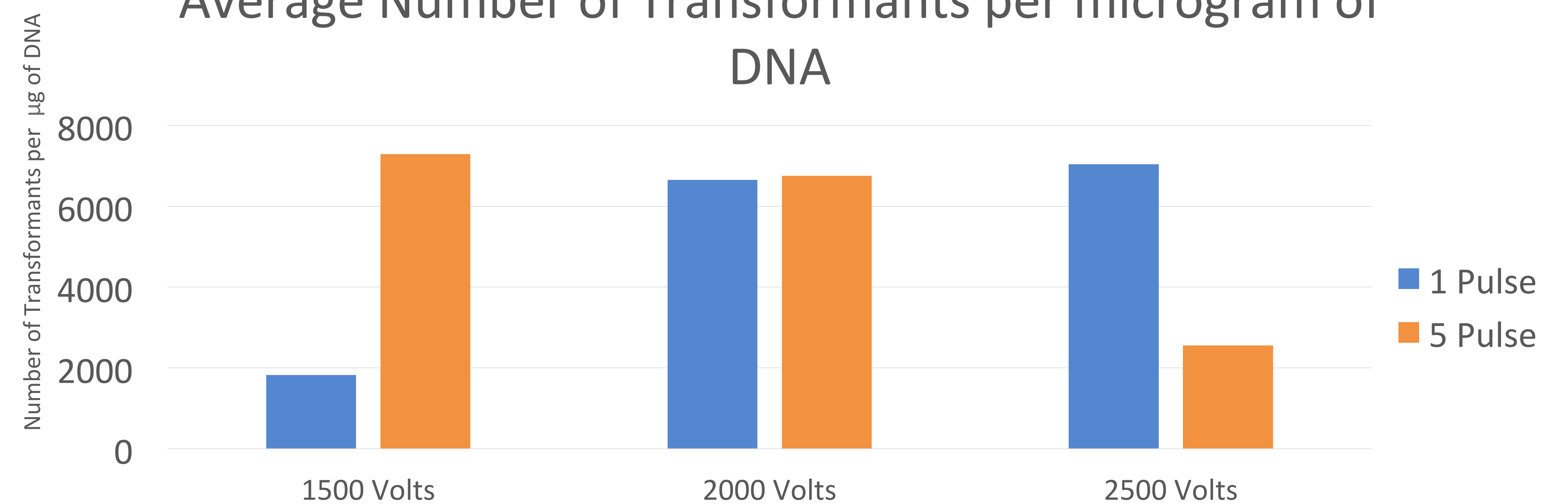
*Lactococcus lactis* yielded the highest number of transformants when grown in MRS containing 1 % glycine, pre-treated with 100 mM lithium acetate and 10 mM dithiothreitol (DTT), and exposed to 5 electroporation pulses.

*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.

## 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  $\Omega$  and 25 $\mu$ F respectively for all transformations. All cells were transformed with the pTRKH2 vector.

### Average Number of Transformants per microgram of DNA



### Average Number of Transformants per 10 Million Viable Cells

