Evolved Lactococcus lactis Strains for Enhanced Expression of Recombinant Membrane Proteins

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Supplementary Information

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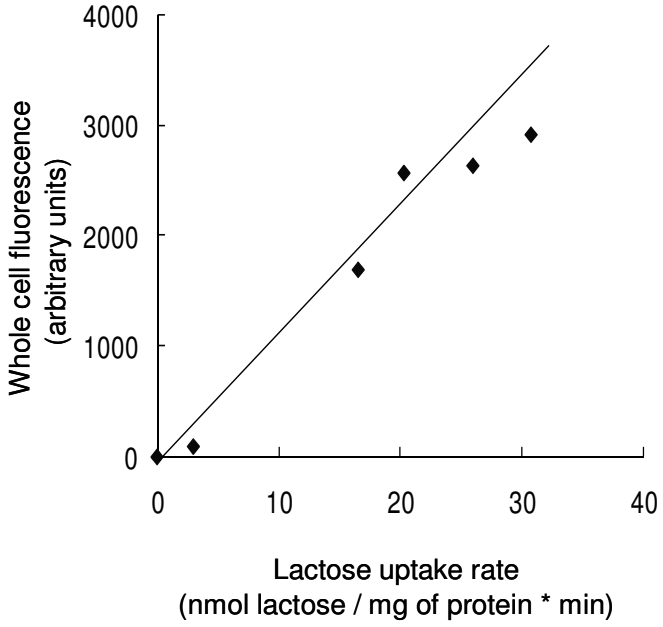
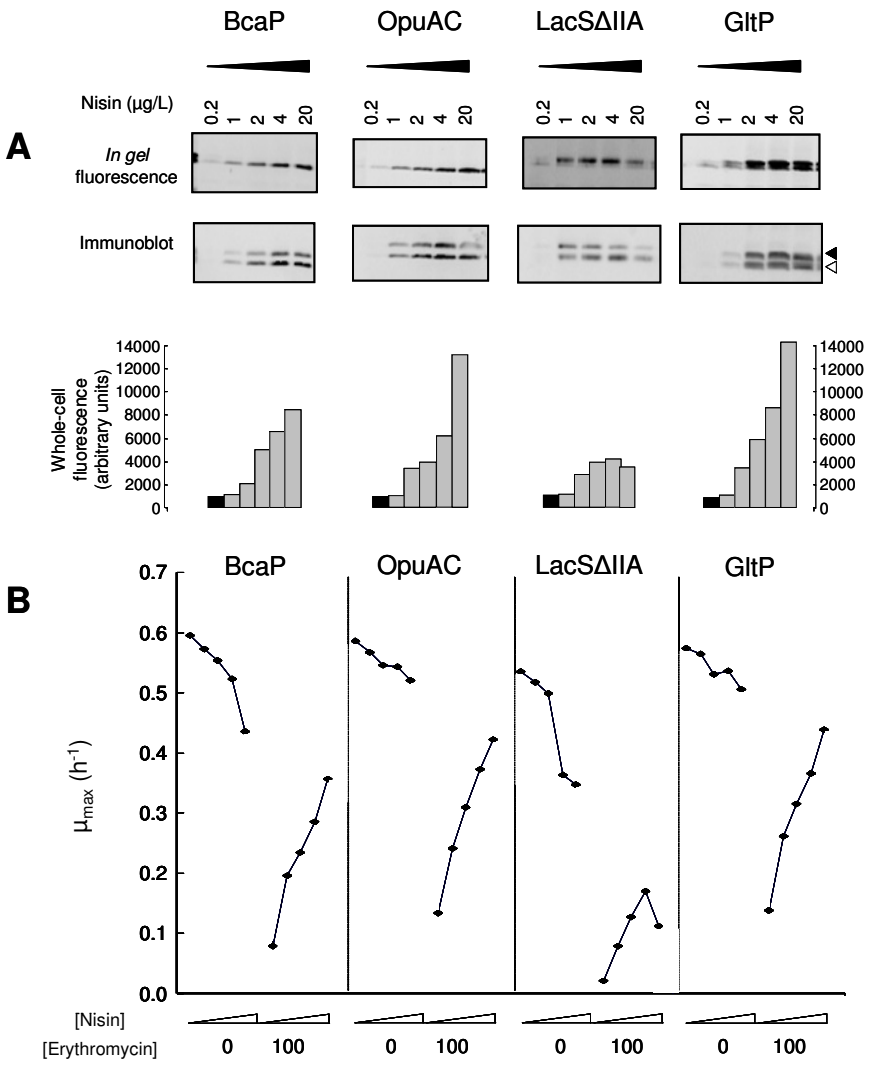
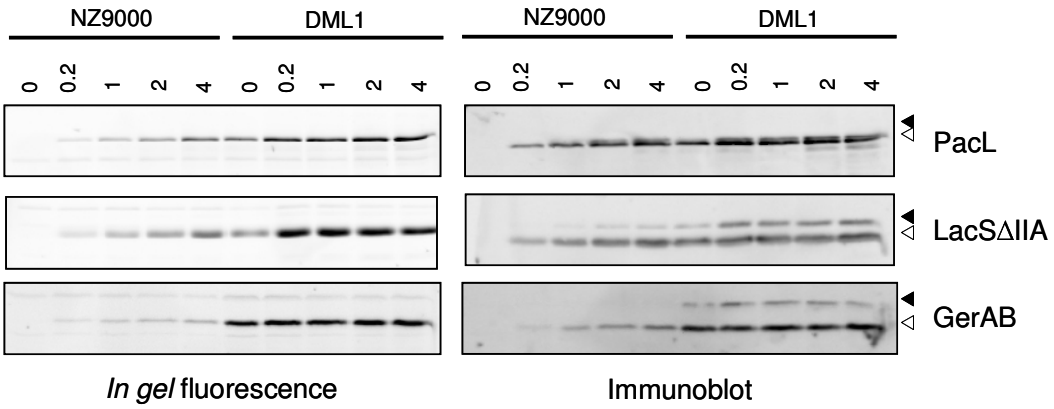


SFigure 1: Relationship between transport activity, measured as lactose counterflow, and whole cell fluorescence observed in *L. lactis* NZ9000 cells overexpressing LacSΔIIA-GFP-ErmC. The error in the measurements of transport in independently grown cells is in the order of 20%. Given this error and based on multiple datasets, a linear relationship between whole cell fluorescence and lactose uptake rate is suggested.



SFigure 2: Correlation between the amount of membrane protein-GFP-ErmC fusions expressed and sensitivity to erythromycin. **(A)** Levels of folded protein assessed by *in-gel* fluorescence, immunoblot and whole cell fluorescence. Black and white arrows on the right of the immunoblots indicate the positions of nonfluorescent (misfolded) and fluorescent (folded) protein species, respectively. The tandemly fused proteins were expressed to different levels by induction with increasing amounts of nisin A (in µg/L, final concentration) as indicated above the lanes. Mean values of triplicate whole-cell fluorescence assays are shown. Black columns represent uninduced samples. The doublet bands of GltP-GFP (top panel) are attributed to partial unfolding of GltP in SDS, as observed previously. **(B)** Growth rate (μ_{max}) of *Lactococcus lactis* expressing the corresponding MP-GFP-ErmC fusion under progressively higher induction stress in the absence and presence of erythromycin (100 µg/ml). For each line, the five points represent the different induction levels (0.2, 1, 2, 4 and 20 µg/L of nisin A); increasing nisin levels are indicated by the triangular symbol below the figure. No growth was observed in uninduced cultures in the presence of 100 µg/ml of erythromycin.



SFigure 3: Nisin A concentration-dependence of the expression of PacL-GFP, LacSΔIIA-GFP and GerAB-GFP in NZ9000 (wild type) and DML1 strains, as assessed by *in-gel* fluorescence and immunoblot analysis. Nisin A concentrations are indicated above the panels (in µg/L, final concentration). Black and white arrows on the right of the immunoblots indicate the positions of nonfluorescent (misfolded) and fluorescent (folded) protein species, respectively.