BTX Applications

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Genetic transformation of a potential bioproduction Host, *Bacillus subtilis,* by BTX electroporation

Development of butanol-tolerant Bacillus subtilis strain GRSW2-B1 as a potential bioproduction Host

Electroporation was successfully applied for *Bacillus subtilis* (strain GRSW2-B1) transformation by optimization of several parameters including the growth phase, cell density, plasmid DNA concentration, recovery medium, recovery period and optimizing the electroporation buffer. High efficiency transformation of pHY300PLK plasmid DNA uptake by electro-transformation was achieved.

Significantly, it was found that the composition of the electroporation buffer was a critical factor affecting electro-transformation efficiency. In this case, it exhibited a significant influence on cell competency and transformation efficiency of GRSW2-B1. The presence of sucrose and Mg2+ in HSMG buffer increased the transformation efficiency by 20%, 50% and 70% over those in HS buffer, glycerol solution, and water, respectively. Mg2+ and sucrose typically promote electro-transformation efficiency and cell viability because they stabilize the cell membrane from temporary distortion due to a high-voltage electric field, although they are not ascertainably advantageous for all bacteria (Wang and Griffiths 2009).

The highest transformation efficiency of butanol-tolerant GRSW2-B1 at 5.17 × 10^3 CFU (µg DNA) was achieved when the competent cells were prepared from cells grown in LB medium to late-exponential phase with OD_{600} of 0.6, and washed with ice-cold HSMG buffer. Plasmid DNA of pHY300PLK was then introduced at 200 ng to the competent cells, and chilled on ice for 20 min before electroporation was performed at 25 µF, 200 Ω, with the optimized field strength at 10.5 kV/cm, yielding a time constant of 4.7 ± 0.1 ms. Then, an osmotically well-balanced TSB-plus medium was immediately added to the pulsed cells and incubated for 3 hours to reseal the membrane permeability and for recovery of the transformants, before spreading on LB medium agar plates including an appropriate antibiotic (i.e. tetracycline at 10 µg/ml or kanamycin at 5 µg/ml).

Overall results indicated that *B. subtilis* GRSW2-B1 has the potential to be engineered and further established as a genetic host for bioproduction of butanol.

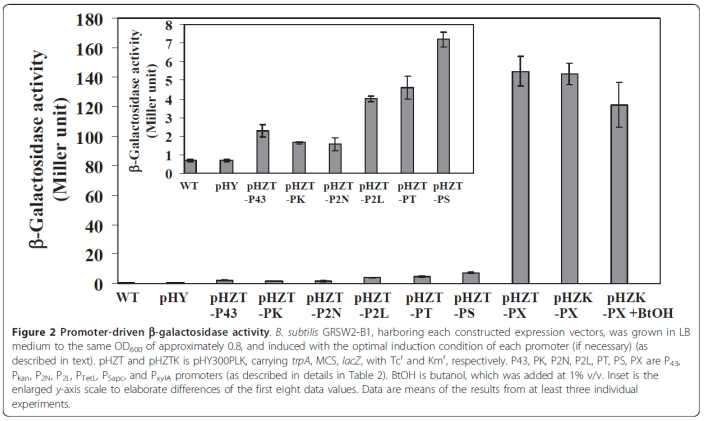

Figure 2 Promoter-driven β-galactosidase activity. *B. subtilis* GRSW2-B1, harboring each constructed expression vectors, was grown in LB medium to the same OD_{600} of approximately 0.8, and induced with the optimal induction condition of each promoter (if necessary) (as described in text). pHZT and pHZTK is pHY300PLK, carrying *trpA*, MCS, *lacZ*, with Tc^r and Km^r, respectively. P43, PK, P2N, P2L, PT, PS, PX are P_{43}, P_{kan}, P_{2N}, P_{2L}, P_{tetL}, P_{sapC}, and P_{xylA} promoters (as described in details in Table 2). BtOH is butanol, which was added at 1% v/v. Inset is the enlarged *y*-axis scale to elaborate differences of the first eight data values. Data are means of the results from at least three individual experiments.

ECM 630 Exponential Decay Wave Electroporation System is available with a Safety Stand for cuvette electroporation or with a Plate Handler for processing high throughput of 25 or 96 well plates.

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45-0423	ECM 630 Generator, 2 x 2mm gap 96-well plates, plate seals, and HT200 plate handler

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