

Optimized production of D-lactic acid from whey permeate using metabolically engineered *Lactobacillus delbreuckii subsp. bulgaricus*

Construction of a metabolically engineered *Lactobacillus delbreuckii subsp. bulgaricus* strain for enhanced production of D-lactic acid (DLA) from whey-lactose.

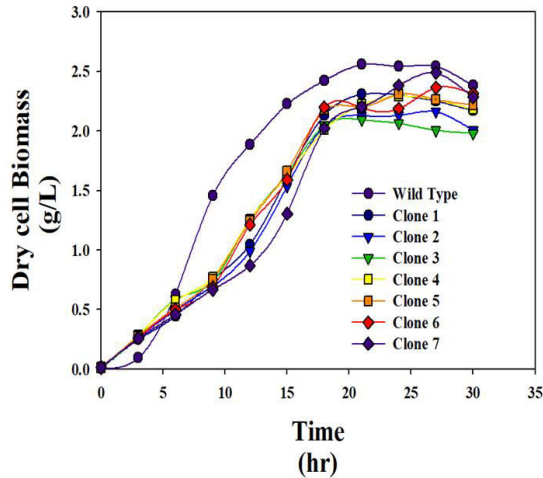
Evaluating sensor-regulator based dynamic metabolic engineering tool for regulating D-lactic acid production using DLA – inducible promoter repressor system in *Lactobacillus*.

Process optimization and separation of DLA from whey based media using metabolically engineered *Lactobacillus delbreuckii subsp. bulgaricus*.

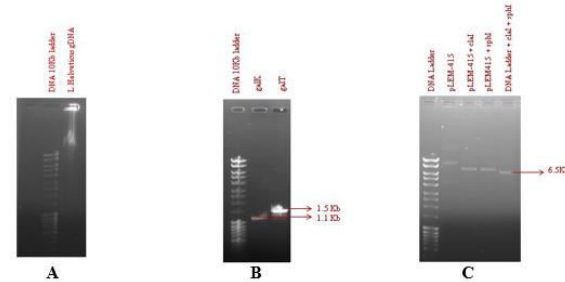
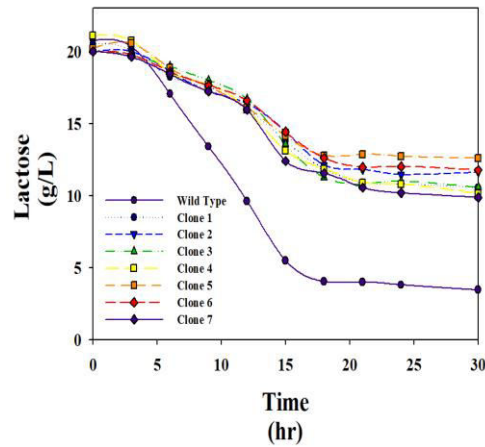
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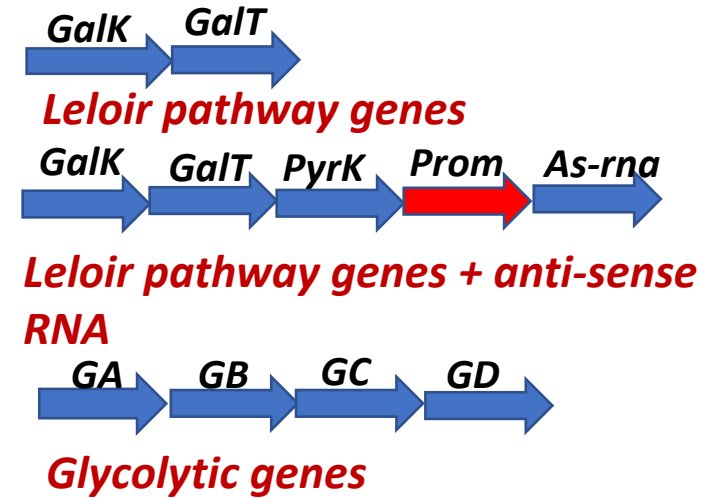
Growth curve of various clones



Substrate Profiling



A - Genomic DNA isolation of *Lactobacillus helveticus* CNRZ32.
 B - PCR Amplification of *galK* and *galT*.
 C - Restriction digestion of pLEM415 with *SphI* and *ClaI*.



Metabolic engineering strategies for enhanced DLA production

Creating Leloir pathway

ATP balance and gene silencing through RNAi mechanism

Overexpression of glycolytic genes

Microorganism	Growth rate (1/hr)	Lactose consumption rate (g/L hr)
Wild Type	0.152	33.07
Clone 1	0.123	18.88
Clone 2	0.126	16.74
Clone 3	0.116	18.86
Clone 4	0.114	19.68
Clone 5	0.131	14.82
Clone 6	0.128	16.44
Clone 7	0.112	20.34