Supporting information for

Elucidating the role and regulation of a lactate permease as lactate transporter in *Bacillus coagulans* DSM1

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TABLE S1. Amino acid sequence identity of lactate permease from *B. coagulans* DSM1 with those from other strains.

Strains[#]	Accession No.[$]	Identity (%)	Strain property
Pseudomonas aeruginosa	SKA44056.1	52	Lactate utilizer
Pseudomonas putida	SKC12999.1	53	Lactate utilizer
Escherichia coli	KIG64568	35	Lactate utilizer
Pseudomonas fluorescens	KJH86543.1	42	Lactate utilizer
Bacillus subtilis	CAB12100.2	45	Lactate utilizer
Saccharomyces cerevisiae	CAA82062.1	67	Lactate utilizer
Bacillus coagulans 2-6	AEH52532	99	Lactate producer
Bacillus coagulans 36D1	AEO99927	99	Lactate producer
Bacillus coagulans P38	KGT37394	99	Lactate producer

[#] Strains are same with those mentioned in Fig. S1.

[$] Protein sequences are from GenBank.

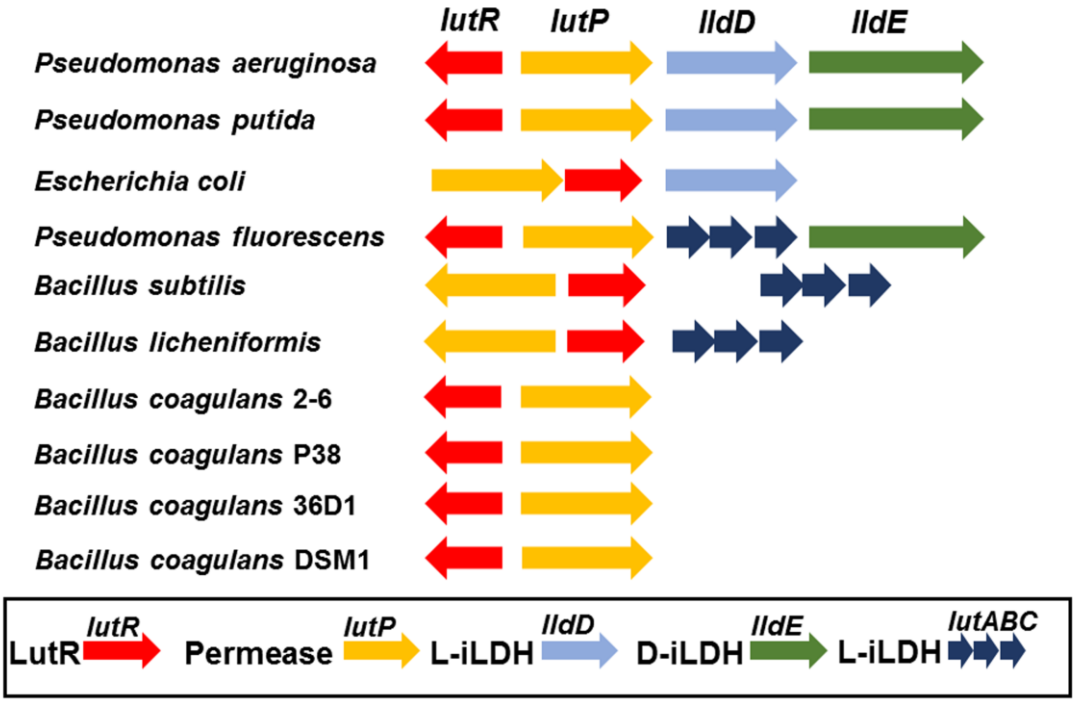

Fig. S1. Organizations of lactate utilization genes in representative members of sequenced bacterial species. Arrows indicate the direction of gene translation. The lactate utilization gene distributions of *Pseudomonas aeruginosa* and *Pseudomonas putida* are from reference (1). The lactate utilization gene distributions of *Escherichia coli*, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Bacillus licheniformis* are from reference (2). *Bacillus coagulans* DSM1 (NCBI Reference Sequence: NZ_CP009709.1). *Bacillus coagulans* 2-6 (NCBI Reference Sequence: NC_015634.1). *Bacillus coagulans* 36D1 (NCBI Reference Sequence: NC_015634.1). *Bacillus coagulans* P38 (NCBI Reference Sequence: NZ_JSVI00000000.1).

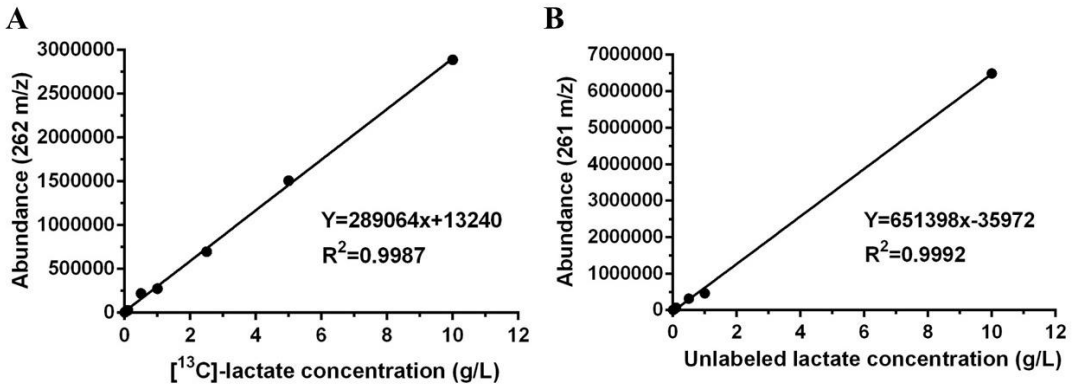

Fig. S2. Calibration curves of [^{13}C]-lactate concentration (A) and unlabeled lactate concentration (B) by GC-MS. Increasing amounts of [^{13}C]-lactate concentration (0.01, 0.05, 0.10, 0.50, 1.00, 2.50, 5.00 and 10.00 g/L) and unlabeled lactate concentration (0.01, 0.05, 0.10, 0.50, 1.00, and 10.00 g/L) were used.

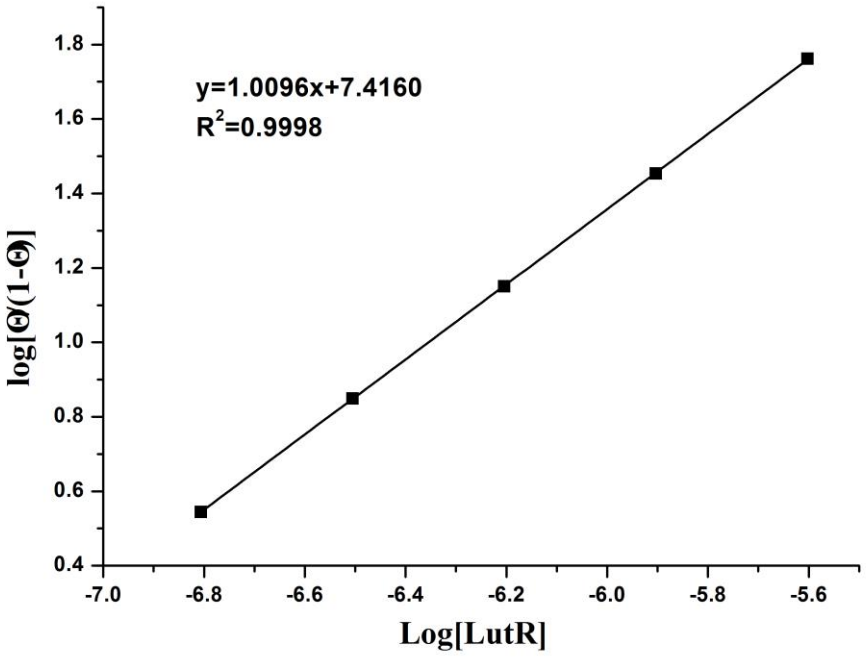

Fig. S3. Hill plot for LutR binding to fragment F2. The fractional saturation (Θ) was calculated as $\Theta = R_{eq} / R_{max}$. For each concentration, R_{eq} is the signal at equilibrium. R_{max} represents the maximum binding response when all the immobilized DNA was bound. Log $[\Theta/(1 - \Theta)]$ as a function of log [LutR] was used to fit with the Hill equation, $\log [\Theta/(1 - \Theta)] = K_H + n_H \log [\text{LutR}]$. K_H and n_H represent the Hill constant and Hill coefficient respectively.

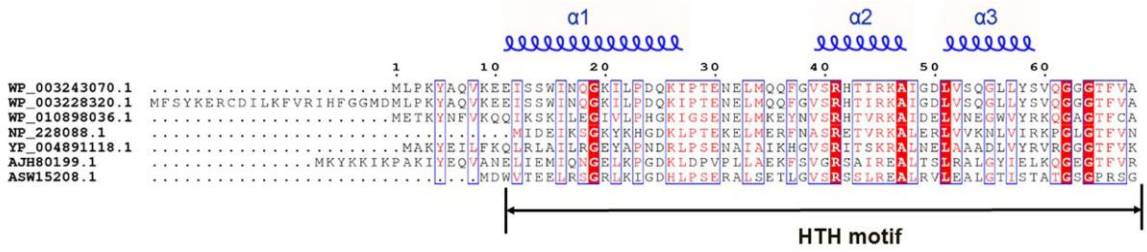

Fig. S4. Sequence alignment of *B. coagulans* DSM1 LutR with the 6 members of the GntR family. HTH motif and alpha-helices of the DNA binding domain are labeled. The residues with similar physico-chemical properties are colored in red. The homologues are: ASW15208.1 (*Corynebacterium glutamicum*), AJH80199.1 (*Bacillus coagulans* DSM 1), WP_003243070.1 (*Bacillus subtilis*), WP_010898036.1 (*Bacillus halodurans*), WP_003228320.1 (*Bacillus* sp.), YP_004891118.1 (*Lactobacillus plantarum* WCFS1), NP_228088.1 (*Thermotoga maritima* MSB8).

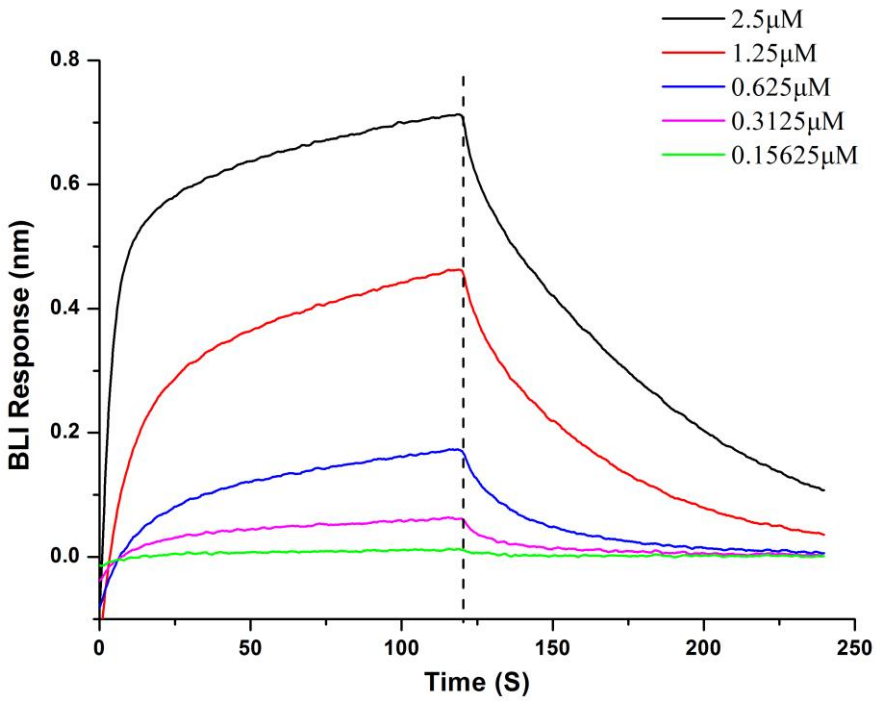

Fig. S5. Effects of glucose on the interaction between LutR and fragment F2 measured by BLI. Glucose (120 mM) was added in the binding buffer for 15 min before the binding. ForteBio Octet RED 96 was used for real-time analysis of interactions between fragment F2 and increasing concentrations of LutR (2.5, 1.25, 0.625, 0.3125 and 0.15625 μM).

REFERENCES

1. Gao C, Hu C, Zheng Z, Ma C, Jiang T, Dou P, Zhang W, Che B, Wang Y, Lv M, Xu P. 2012. Lactate utilization is regulated by the FadR-type regulator LldR in *Pseudomonas aeruginosa*. *J Bacteriol* **194**: 2687–2692. https://doi.org/10.1128/JB.06579-11.
2. Pinchuk GE, Rodionov DA, Yang C, Li X, Osterman AL, Dervyn E, Geydebrekht OV, Reed SB, Romine MF, Collart FR, Scott JH, Fredrickson JK, Beliaev AS. 2009. Genomic reconstruction of *Shewanella oneidensis* MR-1 metabolism reveals a previously uncharacterized machinery for lactate utilization. *Proc Natl Acad Sci U S A* **106**: 2874–2879. https://doi.org/10.1073/pnas.0806798106.