Supplementary materials for

Galactitol transport factor GatA relieves ATP supply restriction to enhance acid tolerance of *Escherichia coli* in the two-stage fermentation production of D-Lactate

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Table S1 Strains and plasmids used in this study.

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Figure S1 Effect of different biomass during the transition from the growth phase to the acid production phase.

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Table S1 Strains and plasmids used in this study.

Strains or plasmids	Relevant property[a]	Source
Strains		
E. coli K12 MG1655	[33]	Laboratory storage
E. coli LBBE317	[10]	Laboratory storage
E. coli (Vector)	MG1655 containing Vector, Amp^r	This study
E. coli (GatA)	MG1655 containing Vector/GatA, Amp^r	This study
E. coli LBBE317P	*E. coli* LBBE317 containing Vector, Amp^r	This study
E. coli LBBE317PGA	*E. coli* LBBE317 containing Vector/GatA, Amp^r	This study
Plasmids		This study
pTrc99a	Amp^r; inducible expression vector with *lacZ* promoter	This study
Vector/GatA	Amp^r; pTrc99a derivative containing a *gatA* gene	This study

[a] Amp^r, ampicillin resistant

Table S2 Bioreactor experimental fermentation conditions.

Fermentation mode	**Grow phase**		**D-lactate production phase**		**Cultivation temperature (°C)**	**Glucose added (g)**
	Aeration ($L \cdot min^{-1}$)	Agitation (rpm)	Aeration ($L \cdot min^{-1}$)	Agitation (rpm)		
Single-phase fermentation[a]	-	-	0	100	37	300
Two-phase fermentation[b]	3	400-600	0-1	100-200		

[a] No air was pumped throughout the fermentation process, and the interior of the container was kept in a microaerobic state by stirring at 100 rpm. All glucose is added at the beginning.

[b] Air is pumped into the bioreactor at a rate of 3 $L \cdot min^{-1}$ to initiate growth. The dissolved O_2 (DO) concentration is maintained above 30 % by stirring at 400-600 rpm. Glucose was added in three batches to keep the residual glucose concentration above 10 $g \cdot L^{-1}$.

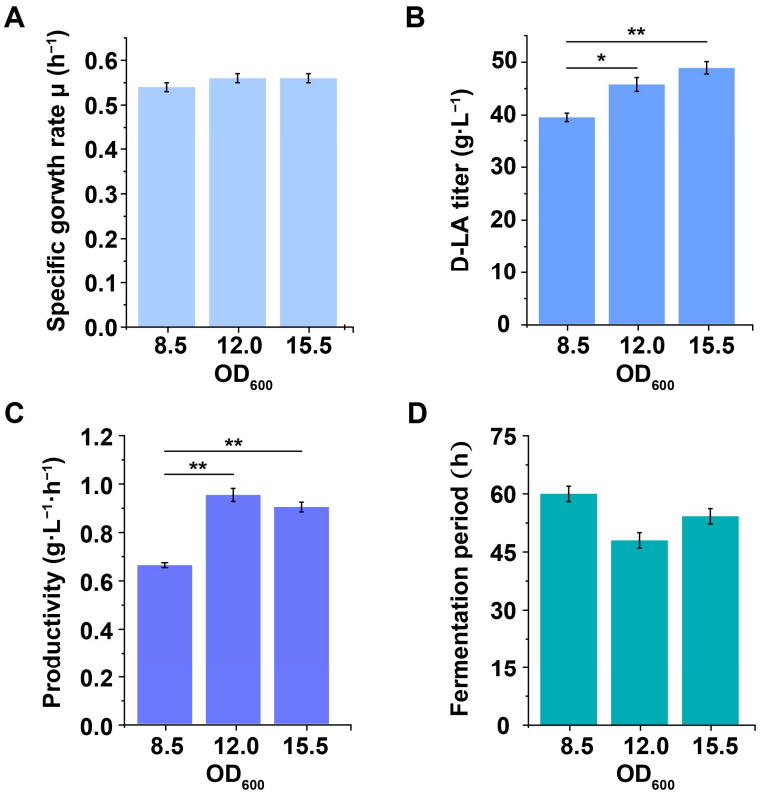

Figure S1. Effect of different biomass during the transition from the growth phase to the acid production phase. (A) Aerobic specific growth rate. (B) Titer of D-LA accumulation. (C) D-LA productivity. (D) Fermentation period. *, $p < 0.05$; **, $p < 0.01$.

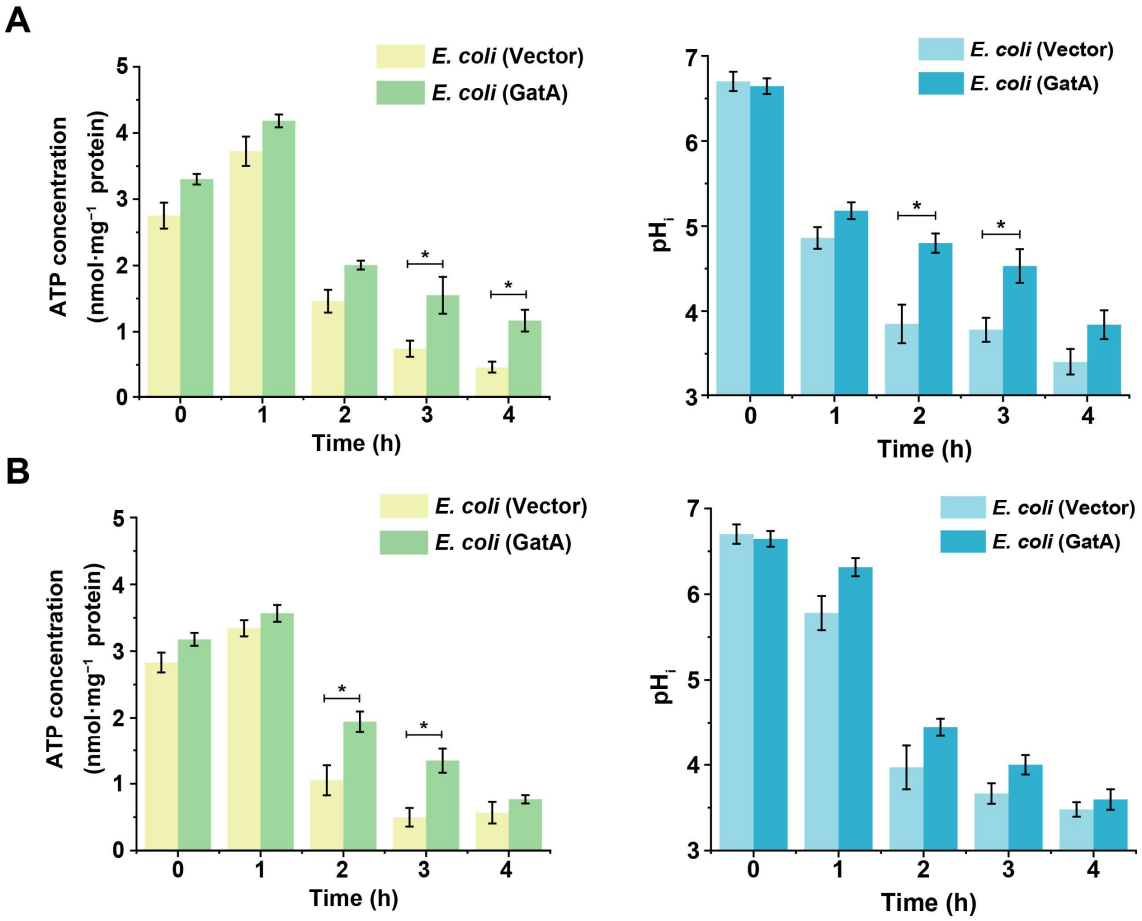

Figure S2. Intracellular ATP concentration and pH under itaconic and succinic acid lethal stress. (A) Changes in intracellular ATP concentration and pH with incubation time of the strain under itaconic acid lethal stress conditions (pH 4.2). (B) Changes in intracellular ATP concentration and pH with incubation time of the strain undersuccinic acid lethal stress conditions (pH 4.3). *, $p < 0.05$.