

## Supplementary Table 1

### Gene mutations identified in the efflux-deficient mutant, the LcS-s2 strain, compared with the LcS-wt strain

Presumed CF-efflux-associated genes, which were used for the gene complementation analysis, are highlighted with yellow.

Variant ID	Amino acid change <sup>1</sup>	ORF ID (Gene symbol <sup>2</sup> )	Estimated product <sup>2</sup>
1	D488N	ORF0031	hypothetical protein
2	W547[FS]	ORF0390	hypothetical protein
3	G39D	ORF0762	hypothetical protein
4	E140K	ORF0942	UPF0210 protein
5	A124V	ORF0980	phosphate-binding protein
6	D295N	ORF1083	ABC transporter substrate-binding protein
7	G106D	ORF1085	sugar ABC transporter permease
8	T441I	ORF1247 ( <i>atpA</i> )	ATP synthase subunit alpha
9	D172N	ORF1258	succinyl-diaminopimelate desuccinylase
10	S162F	ORF1318	hypothetical protein
11	G254D	ORF1442 ( <i>pfkA</i> )	ATP-dependent 6-phosphofructokinase
12	G107D	ORF1744	23S rRNA methyltransferase
13	G12E	ORF1924	NADP oxidoreductase
14	V142I	ORF2017	glutamine ABC transporter permease
15	H394Y	ORF2155	threonine synthase
16	P729L	ORF2425	hypothetical protein
17	V59A	ORF2598	ABC transporter ATP-binding protein
18	R29L	ORF2720	PTS N-acetylglucosamine transporter subunit IIABC
19	E206[FS]	ORF2864	signal transduction histidine kinase
20	P38S	ORF2896	PTS mannitol transporter subunit IIA
21	G127D	ORF2910	membrane protein

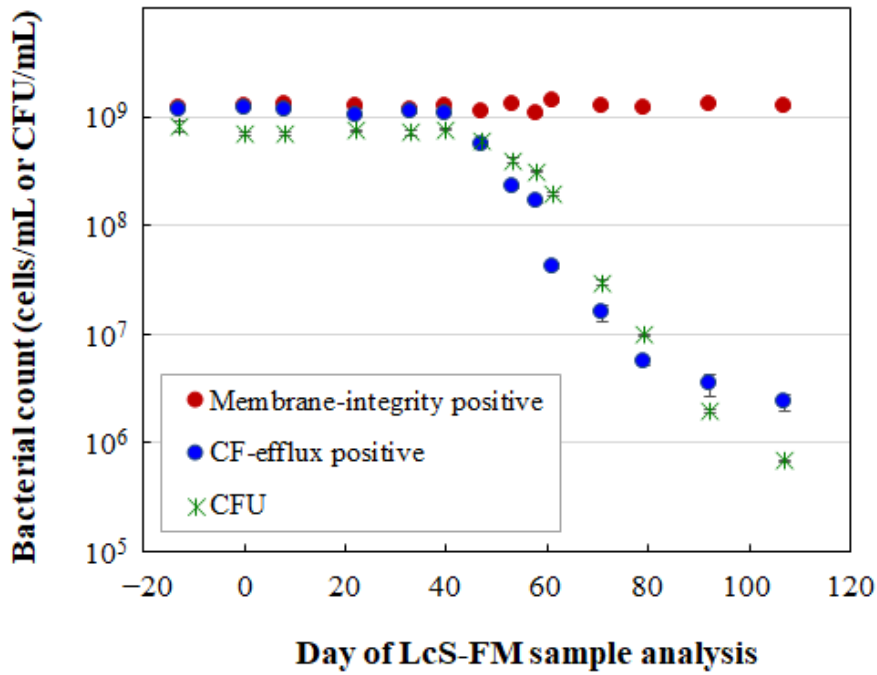
<sup>1</sup> FS indicates frame-shift mutation.

<sup>2</sup> Gene symbol and estimated product were assigned on the basis of a BLAST homology search ( $e < 0.00001$ , identity  $\geq 60\%$ , query coverage  $\geq 80\%$ ) using the DFAST default reference database v1.1.6 (DDBJ Fast Annotation and Submission Tool).

### Supplementary Figure 1

#### Comparison of FCM counts of CF-efflux-positive cells, those of membrane-integrity-positive cells, and CFU

LcS-FM samples stored at 4 °C for different periods were used for analysis. The FCM counts of CF-efflux-positive cells (L8+CF-PI-) and of membrane-integrity-positive cells (L8+Sytox-), as well as the numbers of colony forming units (CFUs), were compared. Day -14 means the day of production of LcS-FM and Day 0 means the best-before date of the product.



## Supplementary Figure 2

### CF-efflux activity in LAB strains

The CF-efflux activities of LAB strains were examined and the histograms of CF-fluorescence with (pink) and without (green) glucose were overlaid. When the median CF-fluorescence intensity with glucose was less than one-tenth of that without glucose, the strain was judged to be “efflux positive.”

