

1 TABLE S1. Histidine kinase and response regulators identified in *L. casei* genomes

| System | Gene tag    |           | Family | Most similar sequence <sup>1</sup>          |
|--------|-------------|-----------|--------|---|
|        | BL23        | ATCC334   |        |   |
| TC01   | LCABL_02090 | LSEI_0220 | IIIA   | LSA0278 ( <i>L. sakei</i> )                 |
|        | LCABL_02080 | LSEI_0219 | OmpR   | LSA0277 ( <i>L. sakei</i> )                 |
| TC02   | LCABL_05270 | LSEI_0461 | IIIA   | RBAM_005810 ( <i>B. amyloliquefaciens</i> ) |
|        | LCABL_05260 | LSEI_0460 | OmpR   | CD0486 ( <i>C. difficile</i> )              |
| TC03   | LCABL_07770 | LSEI_0712 | IIIA   | LVIS_0355 ( <i>L. brevis</i> )              |
|        | LCABL_07760 | LSEI_0711 | OmpR   | llrF ( <i>Lc. lactis</i> )                  |
| TC04   | LCABL_10490 | LSEI_0935 | IIIA   | LSA0501 ( <i>L. sakei</i> )                 |
|        | LCABL_10480 | LSEI_0934 | OmpR   | LSA0500 ( <i>L. sakei</i> )                 |
| TC05   | LCABL_10650 | LSEI_0951 | IIIA   | CLI_0968 ( <i>C. botulinum</i> )            |
|        | LCABL_10640 | LSEI_0950 | OmpR   | CLI_0967 ( <i>C. botulinum</i> )            |
| TC06   | LCABL_12060 | LSEI_1042 | IIIA   | LSA1214 ( <i>L. sakei</i> )                 |
|        | LCABL_12050 | LSEI_1041 | ompR   | LVIS_1316 ( <i>L. brevis</i> )              |
| TC07   | LCABL_14270 | LSEI_1208 | IIIA   | CTC00159 ( <i>C. tetani</i> )               |
|        | LCABL_14280 | LSEI_1209 | OmpR   | SMU.1038c ( <i>S. mutans</i> )              |
| TC08   | LCABL_14440 | LSEI_1223 | II     | Lp_1943 ( <i>L. plantarum</i> )             |
|        | LCABL_14430 | LSEI_1222 | NarL   | Lp_1942 ( <i>L. plantarum</i> )             |
| TC09   | LCABL_16420 | LSEI_1419 | IIIA   | STER_1309 ( <i>S. thermophilus</i> )        |
|        | LCABL_16430 | LSEI_1420 | OmpR   | SAG0976 ( <i>S. agalactiae</i> )            |
| TC10   | LCABL_18840 | LSEI_1666 | II     | LSA1370 ( <i>L. sakei</i> )                 |
|        | LCABL_18830 | LSEI_1665 | NarL   | EF2911 ( <i>E. faecalis</i> )               |
| TC11   | LCABL_18970 | LSEI_1678 | IIIA   | Hpk5 ( <i>L. plantarum</i> )                |
|        | LCABL_18980 | LSEI_1679 | OmpR   | LSA1384 ( <i>L. sakei</i> )                 |
| TC12   | LCABL_19610 | LSEI_1741 | IIIA   | LSA1455 ( <i>L. sakei</i> )                 |
|        | LCABL_19600 | LSEI_1740 | OmpR   | LSA1454 ( <i>L. sakei</i> )                 |

|      |             |            |           |                                      |
|------|-------------|------------|-----------|--------------------------------------|
| TC13 | Pseudogene  | Pseudogene |           |                                      |
|      | LCABL_25620 | LSEI_2389  | lytT      | <i>pltR</i> ( <i>L. plantarum</i> )  |
| TC14 | LCABL_27660 | LSEI_2600  |           | TDE0032 ( <i>T. denticola</i> )      |
|      | LCABL_27650 | LSEI_2599  | lytT      | CAC1581 ( <i>C. acetobutylicum</i> ) |
| TC15 | LCABL_28710 | LSEI_2680  | IIIA      | OEOE_0489 ( <i>O. oeni</i> )         |
|      | LCABL_28720 | LSEI_2681  | OmpR      | LP_0283 ( <i>L. plantarum</i> )      |
| TC16 | LCABL_30120 | LSEI_2807  | IIIA      | LSA0078 ( <i>L. sakei</i> )          |
|      | LCABL_30130 | LSEI_2808  | OmpR      | LSA0077 ( <i>L. sakei</i> )          |
| TC17 | LCABL_30710 | LSEI_2868  | CitA      | EF1209 ( <i>E. faecalis</i> )        |
|      | LCABL_30720 | LSEI_2869  | CitB (IV) | <i>dpiA</i> ( <i>S. pyogenes</i> )   |

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1 <sup>1</sup> Sequences of *L. casei* or *Lactobacillus rhamnosus* are not considered.

TABLE S2. Bacterial strains and plasmids used in this study

| Strain or plasmid             | Relevant characteristics <sup>a</sup>  | Ref. or source         |
|-------------------------------|--|------------------------|
| <i>E. coli</i> DH5 $\alpha$   | F <sup>-</sup> <i>endA1 hsdR17 gyrA96 thi-1 recA1 relA1 supE44 <math>\Delta</math>lacU169</i><br>( $\Phi$ 80 <i>lacZ</i> $\Delta$ M15) | Stratagene             |
| <i>L. casei</i> BL23          | Wild type  | B. Chassy, U. Illinois |
| <i>L. casei</i> TC01          | LCABL_02080 mutant; pRV02080 Ery <sup>r</sup>  | This study             |
| <i>L. casei</i> TC02          | LCABL_05260 mutant; pRV05260 Ery <sup>r</sup>  | This study             |
| <i>L. casei</i> TC03          | LCABL_07760 mutant; pRV07760 Ery <sup>r</sup>  | This study             |
| <i>L. casei</i> TC04          | LCABL_10480 mutant; pRV10480 Ery <sup>r</sup>  | This study             |
| <i>L. casei</i> TC05          | LCABL_10640 mutant; pRV10640 Ery <sup>r</sup>  | This study             |
| <i>L. casei</i> TC06          | LCABL_12050 mutant; pRV12050 Ery <sup>r</sup>  | This study             |
| <i>L. casei</i> TC07          | LCABL_14280 mutant; pRV14280 Ery <sup>r</sup>  | This study             |
| <i>L. casei</i> TC08          | LCABL_14430 mutant; pRV14430Ery <sup>r</sup>   | This study             |
| <i>L. casei</i> TC09          | LCABL_16430 mutant; pRV16430 Ery <sup>r</sup>  | This study             |
| <i>L. casei</i> TC10          | LCABL_18830 mutant; pRV18830 Ery <sup>r</sup>  | This study             |
| <i>L. casei</i> TC11          | LCABL_18980 mutant; pRV18980 Ery <sup>r</sup>  | This study             |
| <i>L. casei</i> TC12          | LCABL_19600 mutant; pRV19600 Ery <sup>r</sup>  | This study             |
| <i>L. casei</i> TC13          | LCABL_25620 mutant; pRV25620 Ery <sup>r</sup>  | This study             |
| <i>L. casei</i> TC14          | LCABL_27650 mutant; pRV27650 Ery <sup>r</sup>  | This study             |
| <i>L. casei</i> TC15          | LCABL_28720 mutant; pRV28720 Ery <sup>r</sup>  | This study             |
| <i>L. casei</i> TC16          | LCABL_30130 mutant; pRV30130 Ery <sup>r</sup>  | This study             |
| <i>L. casei</i> TC17          | BL23 $\Delta$ <i>maeR</i>  | BL315 (1)              |
| <i>L. casei</i> $\Delta$ RR01 | BL23 $\Delta$ <i>rrp11</i> (LCABL_2080)  | This study             |

|                         |   |            |
|-------------------------|---|------------|
| <i>L. casei</i> ΔRR01-c | BL23 Δ <i>rrp11</i> harbouring plasmid pT1-RR01                                   | This study |
| <i>L. casei</i> ΔRR06   | BL23 Δ <i>rrp7</i> (LCABL_12050)  | This study |
| <i>L. casei</i> ΔRR06-c | BL23 Δ <i>rrp7</i> harbouring plasmid pT1-RR06                                    | This study |
| <i>L. casei</i> ΔRR12   | BL23 Δ <i>rrp1</i> (LCABL_19600)  | This study |
| pRV300                  | Insertional vector for <i>Lactobacillus</i> , Amp <sup>r</sup> , Ery <sup>r</sup> | (2)        |
| pRV02080                | pRV300 containing a 372-pb internal fragment of LCABL_02080                       | This study |
| pRV05260                | pRV300 containing a 368-pb internal fragment of LCABL_05260                       | This study |
| pRV07760                | pRV300 containing a 369-pb internal fragment of LCABL_07760                       | This study |
| pRV10480                | pRV300 containing a 399-pb internal fragment of LCABL_10480                       | This study |
| pRV10640                | pRV300 containing a 367-pb internal fragment of LCABL_10640                       | This study |
| pRV12050                | pRV300 containing a 422-pb internal fragment of LCABL_12050                       | This study |
| pRV14280                | pRV300 containing a 406-pb internal fragment of LCABL_14280                       | This study |
| pRV14430                | pRV300 containing a 347-pb internal fragment of LCABL_14430                       | This study |
| pRV16430                | pRV300 containing a 354-pb internal fragment of LCABL_16430                       | This study |
| pRV18830                | pRV300 containing a 349-pb internal fragment of LCABL_18830                       | This study |
| pRV18980                | pRV300 containing a 378-pb internal fragment of LCABL_18980                       | This study |
| pRV19600                | pRV300 containing a 382-pb internal fragment of LCABL_19600                       | This study |
| pRV25620                | pRV300 containing a 373-pb internal fragment of LCABL_25620                       | This study |
| pRV27650                | pRV300 containing a 365-pb internal fragment of LCABL_27650                       | This study |
| pRV28720                | pRV300 containing a 383-pb internal fragment of LCABL_28720                       | This study |
| pRV30130                | pRV300 containing a 364-pb internal fragment of LCABL_30130                       | This study |
| pRVRR01                 | pRV300 containing fused flanking fragments upstream and downstream of LCABL_02080 | This study |
| pRVRR06                 | pRV300 containing fused flanking fragments upstream and downstream of LCABL_12050 | This study |

|          |   |            |
|----------|---|------------|
| pRVRR12  | pRV300 containing fused flanking fragments upstream and downstream of LCABL_19600                     | This study |
| pT1NX    | Expression vector for Gram-positive bacteria harboring the constitutive P1 promoter, Ery <sup>r</sup> | (3)        |
| pT1-RR01 | pT1NX with cloned LCABL_02080 (RR01) expressed from the P1 promoter                                   | This study |
| pT1-RR06 | pT1NX with cloned LCABL_12050 (RR06) and its promoter region  | This study |
| pT1-RR12 | pT1NX with cloned LCABL_19600 (RR12) and its promoter region  | This study |

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1 <sup>a</sup> Amp<sup>r</sup>, ampicillin resistance; Ery<sup>r</sup>, erythromycin resistance.

1 TABLE S3. Oligonucleotides used in this study.

| Name       | Sequence (5'→3') <sup>a</sup>     | Application  |
|------------|-----------------------------------|--|
| Lsei219RF  | GATGCCTAATATGTCTGGGATG            | Amplification of <i>rrp11</i> internal fragment (RR01) |
| Lsei219RR  | CTCATCAGCCGAGAAAACAC              |  |
| Lsei460RF  | CAACTTACCTGATACTGACG              | Amplification of <i>spaR</i> internal fragment (RR02)  |
| Lsei460RR  | G TTCACGACTAAAGACTTGCC            |  |
| Lsei711RF  | GCCAGAATTAAATGGTTTTGACG           | Amplification of <i>llrF</i> internal fragment (RR03)  |
| Lsei711RR  | CCCACAAACGATCAAAAATCTG            |  |
| Lsei934RF  | GCTGCCGAGTCTGAGCGGC               | Amplification of <i>rrp2</i> internal fragment (RR04)  |
| Lsei934RR  | CCCCGTTCAATAAAGCATCTCG            |  |
| Lsei950RF  | CTGAGCATGCCCGATGGTG               | Amplification of LCABL_10640 internal fragment (RR05)  |
| Lsei950RR  | GCGTGTCCCACAAGGCTGC               |  |
| Lsei1041RF | TTTTGTGCGACCTCAATCTGCCAAAAATGGAC  | Amplification of <i>rrp7</i> internal fragment (RR06)  |
| Lsei1041RR | TTTTCTGCAGCCAATTGCAGTAATTG TTCACG |  |
| Lsei1209RF | GCTGCCAAACGTCACCGGTG              | Amplification of LCABL_14280 internal fragment (RR07)  |
| Lsei1209RR | GCGTTCATGAACGGCTCGCC              |  |
| Lsei1222RF | GAGATGCCCAAGTTAACCGG              | Amplification of <i>rrp6</i> internal fragment (RR08)  |
| Lsei1222RR | G TTCCTTCCGAGAGAAACAGC            |  |
| Lsei1420RF | CAGTCTGCCATATTTTAATGG             | Amplification of LCABL_16430 internal fragment (RR09)  |
| Lsei1420RR | CAGCGCTGAACAAAAGACTC              |  |
| Lsei1665RF | TTTTGTGCGACGGATCYCGTGATGCCGGTG    | Amplification of LCABL_18830 internal fragment (RR10)  |
| Lsei1665RR | TTTTCTGCAGCTGTCGCAATTTCTTGATTCG   |  |
| Lsei1679RF | GATGTTGCCAGAATTAACCGG             | Amplification of <i>rrp5</i> internal fragment (RR11)  |
| Lsei1679RR | CGTCACGAGCCAAAACAACG              |  |
| Lsei1740RF | CCAGTTTTTGACGGCTATTATTGG          | Amplification of <i>rrp1</i> internal fragment (RR12)  |
| Lsei1740RR | GCCATAAGTCCTTTAATAACC             |  |
| Lsei2389RF | CCCTGGATCTTCTGTAAGTGG             | Amplification of <i>pltR</i> internal fragment (RR13)  |
| Lsei2389RR | GCAAAATGCTTCGATGACAGC             |  |
| Lsei2599RF | CAAGTTAGGTT CAGGCATGG             | Amplification of LCABL_27650 internal fragment (RR14)  |
| Lsei2599RR | CGTCGAGTTGAGGAAGAAAC              |  |
| Lsei2681RF | GGATGCTGCCTAAACTAGACG             | Amplification of <i>rrp2</i> internal fragment (RR15)  |
| Lsei2681RR | CAAGTCATCCCTCGAACAAGC             |  |
| Lsei2808RF | GATGTTACCAAAAATTGATGGG            | Amplification of <i>rrp3</i> internal fragment         |
| Lsei2808RR | CAAGGTGTCGAGCAAGGTAG              |  |

|                  |  |   |
|------------------|--|---|
| DEL_219AF        | TTTTGTCGACGCGCAGTTCATTCAAATTACC                | (RR16)  |
| DEL_219A<br>R    | GATTTTTTCGCTCATGATCGTGTATTGGCTCCGTTTATG        | Amplification of <i>rr01up</i> fragment               |
| DEL_219BF        | CATAAACGGAGCCAATACACGATCATGAGCGAAAAAGTC        | Amplification of                                      |
| DEL_219B<br>R    | TTTTCTGCAGCCTCAACGCGATAAAAACG                  | <i>rr01down</i> fragment                              |
| PT219FOR         | TTTTAGATCTCATAAACGGAGCCAATACAATG               | Amplification of <i>rr01</i>                          |
| PT219REV         | TTTTACTAGTCGTTAGGCCTCAACCTTATAG                | gene  |
| DEL1041AF        | TTTTGTCGACGCTTTTTGTCTGCTAGGTGAC                | Amplification of                                      |
| DEL1041A<br>R    | GCTGGTAGAGCATTTTCATCGATGTACCCCTCATTTCACAG      | <i>rr06up</i> fragment                                |
| DEL1041B<br>R    | TTTTCTGCAGGATGTTATCAAGATCCGTGC                 |   |
| DEL1041BF        | CTGTGAAATGAGGGGTACATCGATGAAAATGCTCTACCAGC      | Amplification of                                      |
| PT1041FOR        | TTTTAGATCTGAAATGAGGGGTACATCATG                 | <i>rr06down</i> fragment                              |
| PT1041REV        | TTTTACTAGTGCATTTTCATCGATCAAGCC                 | Amplification of <i>rr06</i>                          |
| CP1041FOR        | TTTTGAATTCGATTAAGGTGCAAACGTTATG                | gene  |
| CP1041REV        | TTTTACTAGTATCGATCAAGCCCCGAATCATC               | Amplification of promoter region and <i>rr06</i> gene |
| DEL1740AF        | TTTTAAGCTTCTTCCATATCAATAATTCGGTC               | Amplification of                                      |
| DEL1740A<br>R    | GAAACCGCATCTGCCAGCCTCGATTTTAAACACGTGACCAA<br>C | <i>rr12up</i> fragment                                |
| DEL1740B<br>R    | TTTTACTAGTGTACCTTGATTGATTACCGAGG               |   |
| DEL1740BF        | GTTGGTCACGTGTTTAAAATCGAGGCTGGCAGATGCGGTTT<br>C | Amplification of                                      |
| PT1740FOR        | TTTTAGATCTGAAATGGAGTTGGTCACGTG                 | <i>rr12down</i> fragment                              |
| PT1740REV        | TTTTACTAGTCCAGCCTCCTAAGGAACG                   |   |
| CP1740FOR        | TTTTGAATTCATGCATGTGCTGCCAACTCC                 | Amplification of <i>rr12</i>                          |
| CP1740REV        | TTTTTCTAGATGCCAGCCTCCTAAGGAACG                 | gene  |
| CP1740REV        | TTTTTCTAGATGCCAGCCTCCTAAGGAACG                 | Amplification of promoter region and <i>rr12</i> gene |
| External primers |  |   |
| C94-68754        | CTAGCCCTGTTTTCTTAGGC                           | Screening of <i>rrp11</i> mutants (RR01)              |
| C87-24906        | CGTGTTGCAAGCGATTCCG                            | Screening of SpaR mutants (RR02)                      |
| C87-10203        | GGATCATTATGAGAGTTACAGC                         | Screening of <i>llrF</i> mutants (RR03)               |
| C85-48527        | CAACCTATTGCCGCTTGCGG                           | Screening of <i>rrp2</i> mutants (RR04)               |
| C85-65285        | GACTATCCGGACTTTTTTCGG                          | Screening of LCABL_10640 mutants (RR05)               |
| C93-82083        | GCAAGCCCATGATGCACTGCC                          | Screening of <i>rrp7</i> mutants (RR06)               |
| C73-25455        | GTGGCCTTGGGTGTGATGG                            | Screening of LCABL_14280                              |

|            |                         |  |
|------------|-------------------------|--|
| C73-15384  | CAACGTATGAAAGAAGTTAATGG | mutants (RR07)<br>Screening of <i>rrp6</i><br>mutants (RR08) |
| C83-40666  | CAGTTAGAATAGAAACTGTCC   | Screening of<br>LCABL_16430<br>mutants (RR09)                |
| C68-7694   | CTTGCAAATATCAAGGAACGTGC | Screening of<br>LACBL_18830<br>mutants (RR10)                |
| C92-145449 | GTCAAAATGAGAAAACACTGTGC | Screening of <i>rrp5</i><br>mutants (RR11)                   |
| C92-59224  | CCTAAAAGCCGGACAAACCC    | Screening of <i>rrp1</i><br>mutants (RR12)                   |
| C89-64634  | GCACGGCAATGGCTTGACC     | Screening of <i>pltR</i><br>mutants (RR13)                   |
| C96-12266  | GCTGACTCACTCTAGTCAGC    | Screening of<br>LCABL_27650<br>mutants (RR14)                |
| C96-50721  | GTCACCTTCTCTAAGGCTGC    | Screening of <i>rrp2</i><br>mutants (RR15)                   |
| C91-15689  | CCTGCATATAAGAACCCC      | Screening of <i>rrp3</i><br>mutants (RR16)                   |

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2 TABLE S4. Maximum specific growth rates and  $\Delta$ O.D 595<sub>nm</sub> of different *L. casei* BL23 mutants

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grown in MRS broth in microplates.

| Strain | Maximum specific growth rate,<br>$\mu_{\max}$ (h <sup>-1</sup> ) | O.D <sub>max</sub> -O.D <sub>min</sub> |
|--------|--|--|
| BL23   | 0.279 ± 0.005  | 2.422 ± 0.06                           |
| TC01   | 0.271 ± 0.006  | 2.344 ± 0.14                           |
| TC02   | 0.291 ± 0.012  | 2.224 ± 0.08                           |
| TC03   | 0.284 ± 0.005  | 2.285 ± 0.11                           |
| TC04   | 0.239 ± 0.007 (P<0.01) <sup>a</sup>                              | 2.390 ± 0.09                           |
| TC05   | 0.268 ± 0.015  | 2.386 ± 0.06                           |
| TC06   | 0.280 ± 0.006  | 2.154 ± 0.12                           |
| TC07   | 0.274 ± 0.010  | 2.316 ± 0.08                           |
| TC08   | 0.275 ± 0.001  | 2.445 ± 0.09                           |
| TC09   | 0.279 ± 0.015  | 2.400 ± 0.03                           |
| TC10   | 0.282 ± 0.006  | 2.382 ± 0.14                           |
| TC11   | 0.213 ± 0.002 (P<0.01)   | 2.318 ± 0.08                           |
| TC12   | 0.285 ± 0.004  | 1.883 ± 0.06 (P<0.01)                  |
| TC13   | 0.284 ± 0.010  | 2.246 ± 0.12                           |
| TC14   | 0.277 ± 0.004  | 2.377 ± 0.13                           |
| TC15   | 0.266 ± 0.016  | 2.269 ± 0.18                           |
| TC16   | 0.276 ± 0.005  | 2.249 ± 0.16                           |
| TC17   | 0.281 ± 0.014  | 2.341 ± 0.18                           |

4 <sup>a</sup> Significant difference (P<0.01; one way ANOVA) between the wild-type and the mutant strain.

1 TABLE S5A. Maximal growth rate ( $\mu_{\max}$ ) values and pairwise two way ANOVA under different stress conditions<sup>a</sup>.

| Strain | 0.5% bile              |                    |                   | 0.6 M NaCl             |                  |                  | pH 3.75                |                  |                                   | T 42°C                 |                  |                  |
|--------|------------------------|--------------------|-------------------|------------------------|------------------|------------------|------------------------|------------------|-----------------------------------|------------------------|------------------|------------------|
|        | $\mu_{\max}$           | ANOVA <sup>b</sup> |                   | $\mu_{\max}$           | ANOVA            |                  | $\mu_{\max}$           | ANOVA            |                                   | $\mu_{\max}$           | ANOVA            |                  |
|        |                        | Strain             | Int. <sup>c</sup> |                        | Strain           | Int.             |                        | Strain           | Int.                              |                        | Strain           | Int.             |
| BL23   | 0.17±<10 <sup>-2</sup> |                    |                   | 0.23±0.01              |                  |                  | 0.07±<10 <sup>-2</sup> |                  |                                   | 0.26±0.01              |                  |                  |
| TC01   | NG <sup>d</sup>        | - <sup>e</sup>     | -                 | NG                     | -                | -                | 0.03±<10 <sup>-2</sup> | <0.001           | <0.001                            | 0.24±<10 <sup>-2</sup> | <0.001           | 0.030            |
| TC02   | 0.17±0.01              | 0.778              | 0.010             | 0.23±0.01              | 0.097            | 0.056            | 0.05±<10 <sup>-2</sup> | 0.902            | 0.005                             | 0.25±0.02              | 0.725            | 0.051            |
| TC03   | 0.17±<10 <sup>-2</sup> | 0.848              | 0.070             | 0.20±0.01              | <b>0.002</b>     | <b>&lt;0.001</b> | 0.06±<10 <sup>-2</sup> | 0.179            | 0.003                             | 0.25±0.01              | 0.368            | 0.010            |
| TC04   | 0.13±0.01              | <0.001             | 0.307             | 0.16±0.01              | <b>&lt;0.001</b> | <b>&lt;0.001</b> | 0.05±<10 <sup>-2</sup> | <b>&lt;0.001</b> | <b>&lt;0.001</b> (-) <sup>f</sup> | 0.23±0.01              | <0.001           | 0.033            |
| TC05   | 0.17±<10 <sup>-2</sup> | 0.154              | 0.201             | 0.22±0.02              | 0.037            | 0.560            | 0.05±0.01              | 0.002            | 0.289                             | 0.24±0.01              | 0.006            | 0.444            |
| TC06   | NG                     | -                  | -                 | NG                     | -                | -                | 0.03±0.01              | <b>&lt;0.001</b> | <b>&lt;0.001</b>                  | 0.17±0.01              | <b>&lt;0.001</b> | <b>&lt;0.001</b> |
| TC07   | 0.18±0.01              | 0.472              | 0.459             | 0.23±<10 <sup>-2</sup> | 0.077            | 0.743            | 0.04±0.01              | <b>&lt;0.001</b> | <b>0.009</b>                      | 0.26±0.01              | 0.452            | 0.511            |
| TC08   | 0.19±0.01              | 0.153              | 0.009             | 0.23±0.01              | 0.163            | 0.911            | 0.07±<10 <sup>-2</sup> | 0.439            | 0.005                             | 0.25±<10 <sup>-2</sup> | 0.007            | 0.333            |
| TC09   | 0.18±<10 <sup>-2</sup> | 0.557              | 0.556             | 0.22±<10 <sup>-2</sup> | 0.209            | 0.205            | 0.07±<10 <sup>-2</sup> | 0.049            | 0.062                             | 0.26±0.01              | 0.735            | 0.726            |
| TC10   | 0.15±<10 <sup>-2</sup> | <b>&lt;0.001</b>   | <b>&lt;0.001</b>  | 0.22±0.01              | 0.135            | 0.019            | 0.05±<10 <sup>-2</sup> | 0.014            | 0.002                             | 0.24±0.01              | <b>0.003</b>     | <b>&lt;0.001</b> |
| TC11   | 0.13±<10 <sup>-2</sup> | <b>&lt;0.001</b>   | <b>0.001</b> (-)  | 0.17±0.01              | <0.001           | 0.591            | 0.05±<10 <sup>-2</sup> | <b>&lt;0.001</b> | <b>&lt;0.001</b> (-)              | 0.20±0.01              | <0.001           | 0.110            |
| TC12   | 0.08±<10 <sup>-2</sup> | <b>&lt;0.001</b>   | <b>&lt;0.001</b>  | 0.21±0.01              | <b>0.009</b>     | <b>&lt;0.001</b> | NG                     | -                | -                                 | 0.22±0.02              | <b>0.005</b>     | <b>&lt;0.001</b> |
| TC13   | 0.17±0.01              | 0.971              | 0.135             | 0.25±0.01              | 0.031            | 0.260            | 0.04±<10 <sup>-2</sup> | <b>0.001</b>     | <b>0.008</b>                      | 0.24±0.01              | 0.017            | 0.001            |
| TC14   | 0.19±0.01              | 0.108              | 0.037             | 0.22±0.01              | 0.047            | 0.144            | 0.05±<10 <sup>-2</sup> | <b>0.001</b>     | <b>0.006</b>                      | 0.24±0.01              | 0.007            | 0.020            |
| TC15   | 0.16±0.01              | 0.002              | 0.559             | 0.22±0.01              | 0.012            | 0.828            | 0.04±<10 <sup>-2</sup> | 0.025            | 0.487                             | 0.24±0.01              | 0.003            | 0.515            |

|      |           |       |       |           |       |       |                        |                  |              |           |       |       |
|------|-----------|-------|-------|-----------|-------|-------|------------------------|------------------|--------------|-----------|-------|-------|
| TC16 | 0.17±0.01 | 0.162 | 0.624 | 0.21±0.02 | 0.015 | 0.046 | 0.05±<10 <sup>-2</sup> | <b>&lt;0.001</b> | <b>0.004</b> | 0.24±0.01 | 0.043 | 0.166 |
| TC17 | 0.16±0.01 | 0.252 | 0.123 | 0.20±0.02 | 0.015 | 0.008 | 0.06±<10 <sup>-2</sup> | 0.254            | 0.063        | 0.24±0.01 | 0.092 | 0.042 |

1 <sup>a</sup> Contribution of treatment is omitted since it was always significant; significant differences are indicated in bold characters.

2 <sup>b</sup> P values

3 <sup>c</sup> Interaction.

4 <sup>d</sup> No growth.

5 <sup>e</sup> Analysis not performed.

6 <sup>f</sup> Negative interaction.

1

2 TABLE S5 B. Increment in optical density ( $\Delta$  O.D.) values and pairwise two way ANOVA at different stress conditions<sup>a</sup>.

| Strain | 0.5% bile     |        |       | 0.6 M NaCl    |                  |                      | pH 3.75       |                  |                  | T 42°C        |                  |                  |
|--------|---------------|--------|-------|---------------|------------------|----------------------|---------------|------------------|------------------|---------------|------------------|------------------|
|        | $\Delta$ O.D. | ANOVA  |       | $\Delta$ O.D. | ANOVA            |                      | $\Delta$ O.D. | ANOVA            |                  | $\Delta$ O.D. | ANOVA            |                  |
|        |               | Strain | Int.  |               | Strain           | Int.                 |               | Strain           | Int.             |               | Strain           | Int.             |
| BL23   | 1.15±0.14     |        |       | 1.83±0.04     |                  |                      | 0.56±0.07     |                  |                  | 2.19±0.10     |                  |                  |
| TC01   | NG            | -      | -     | NG            | -                | -                    | 0.19±0.06     | <b>&lt;0.001</b> | <b>&lt;0.005</b> | 2.07±0.15     | 0.112            | 0.741            |
| TC02   | 1.16±0.10     | 0.061  | 0.046 | 1.86±0.05     | 0.010            | 0.003                | 0.40±0.02     | <0.001           | 0.569            | 1.97±0.08     | <0.001           | 0.848            |
| TC03   | 1.13±0.03     | 0.114  | 0.240 | 1.75±0.05     | 0.003            | 0.419                | 0.35±0.02     | <0.001           | 0.337            | 2.11±0.17     | 0.072            | 0.591            |
| TC04   | 0.85±0.07     | 0.004  | 0.014 | 1.81±0.02     | 0.404            | 0.873                | 0.38±0.04     | 0.006            | 0.043            | 2.18±0.07     | 0.653            | 0.751            |
| TC05   | 1.21±0.09     | 0.781  | 0.317 | 1.88±0.07     | 0.911            | 0.174                | 0.37±0.04     | 0.001            | 0.015            | 2.15±0.15     | 0.420            | 0.962            |
| TC06   | NG            | -      | -     | NG            | -                | -                    | 0.14±0.01     | <b>&lt;0.001</b> | <b>0.009</b>     | 0.46±0.03     | <b>&lt;0.001</b> | <b>&lt;0.001</b> |
| TC07   | 1.11±0.07     | 0.126  | 0.495 | 1.84±0.01     | 0.128            | 0.086                | 0.40±0.02     | 0.001            | 0.037            | 2.02±0.11     | 0.008            | 0.489            |
| TC08   | 1.11±0.05     | 0.820  | 0.484 | 1.88±0.08     | 0.304            | 0.725                | 0.41±0.02     | 0.086            | 0.025            | 2.10±0.12     | 0.409            | 0.192            |
| TC09   | 1.23±0.10     | 0.632  | 0.375 | 1.91±0.03     | 0.278            | 0.056                | 0.40±0.02     | 0.007            | 0.027            | 2.12±0.10     | 0.287            | 0.579            |
| TC10   | 0.98±0.03     | 0.070  | 0.245 | 1.82±0.03     | 0.516            | 0.685                | 0.36±0.04     | 0.022            | 0.111            | 2.14±0.14     | 0.425            | 0.927            |
| TC11   | 1.09±0.11     | 0.135  | 0.730 | 1.73±0.11     | 0.023            | 0.966                | 0.32±0.02     | <0.001           | 0.071            | 2.11±0.12     | 0.082            | 0.805            |
| TC12   | 0.52±0.03     | <0.001 | 0.297 | 1.81±0.03     | <b>&lt;0.001</b> | <b>&lt;0.001 (-)</b> | NG            | -                | -                | 1.02±0.05     | <b>&lt;0.001</b> | <b>&lt;0.001</b> |
| TC13   | 1.15±0.05     | 0.132  | 0.138 | 1.89±0.03     | 0.114            | -0.008               | 0.34±0.01     | <0.001           | 0.622            | 2.16±0.08     | 0.054            | 0.156            |
| TC14   | 1.13±0.02     | 0.549  | 0.847 | 1.79±0.06     | 0.316            | 0.930                | 0.45±0.04     | 0.097            | 0.477            | 2.14±0.09     | 0.359            | 0.991            |

|      |           |       |       |           |       |        |           |       |       |           |       |       |
|------|-----------|-------|-------|-----------|-------|--------|-----------|-------|-------|-----------|-------|-------|
| TC15 | 1.16±0.08 | 0.261 | 0.220 | 1.89±0.02 | 0.328 | -0.044 | 0.49±0.09 | 0.042 | 0.413 | 2.15±0.09 | 0.119 | 0.348 |
| TC16 | 1.19±0.06 | 0.266 | 0.105 | 1.92±0.03 | 0.322 | 0.012  | 0.48±0.02 | 0.018 | 0.341 | 2.09±0.09 | 0.027 | 0.528 |
| TC17 | 1.15±0.03 | 0.489 | 0.485 | 1.72±0.05 | 0.072 | 0.773  | 0.58±0.04 | 0.605 | 0.356 | 2.01±0.11 | 0.054 | 0.434 |

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1 <sup>a</sup> Abbreviations and symbols as in Table 3 A.

TABLE S6. MIC values of *L. casei* BL23 and TCS-defective derivative mutants.

| Strain  | Bacitracin <sup>a</sup> | Vancomycin <sup>b</sup> | Gramicidin <sup>a</sup> | Nisin <sup>a</sup> |
|---------|-------------------------|-------------------------|-------------------------|--------------------|
| BL23    | 3                       | 0.97 <sup>c</sup>       | >250                    | 1                  |
| TC01    | 1                       | 0.19 <sup>c</sup>       | >250                    | 0.25               |
| TC02    | 3                       | >1.2                    | >250                    | 1                  |
| TC03    | 3                       | >1.2                    | >250                    | 1                  |
| TC04    | 4                       | >1.2                    | >250                    | 1.5                |
| TC05    | 3                       | >1.2                    | >250                    | 1                  |
| TC06    | 3                       | 0.45 <sup>c</sup>       | >250                    | 0.25               |
| TC07    | 3                       | >1.2                    | >250                    | 1                  |
| TC08    | 3                       | >1.2                    | >250                    | 1                  |
| TC09    | 2                       | >1.2                    | >250                    | 0.25               |
| TC10    | 2                       | >1.2                    | >250                    | 0.25               |
| TC11    | 3                       | >1.2                    | >250                    | 0.5                |
| TC12    | 3                       | 0.17 <sup>c</sup>       | >250                    | 0.25               |
| TC13    | 3                       | >1.2                    | >250                    | 1                  |
| TC14    | 3                       | >1.2                    | >250                    | 1                  |
| TC15    | 4                       | >1.2                    | >250                    | 1                  |
| TC16    | 4                       | >1.2                    | >250                    | 1                  |
| TC17    | 4                       | >1.2                    | >250                    | 1                  |
| ΔRR01   | 1.5                     | 0.29 <sup>c</sup>       | >250                    | 0.25               |
| ΔRR01-c | 3                       | 0.74 <sup>c</sup>       | >250                    | 1                  |
| ΔRR06   | 3                       | 0.43 <sup>c</sup>       | >250                    | 0.5                |
| ΔRR06-c | 3                       | 0.97 <sup>c</sup>       | >250                    | 1                  |

$\Delta$ RR12                      3                      0.45<sup>c</sup>                      >250                      0.125

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1    <sup>a</sup>  $\mu\text{g ml}^{-1}$

2    <sup>b</sup>  $\text{mg ml}^{-1}$

3    <sup>c</sup> IC<sub>50</sub> values are shown. MIC values higher than 1.2  $\text{mg ml}^{-1}$ .

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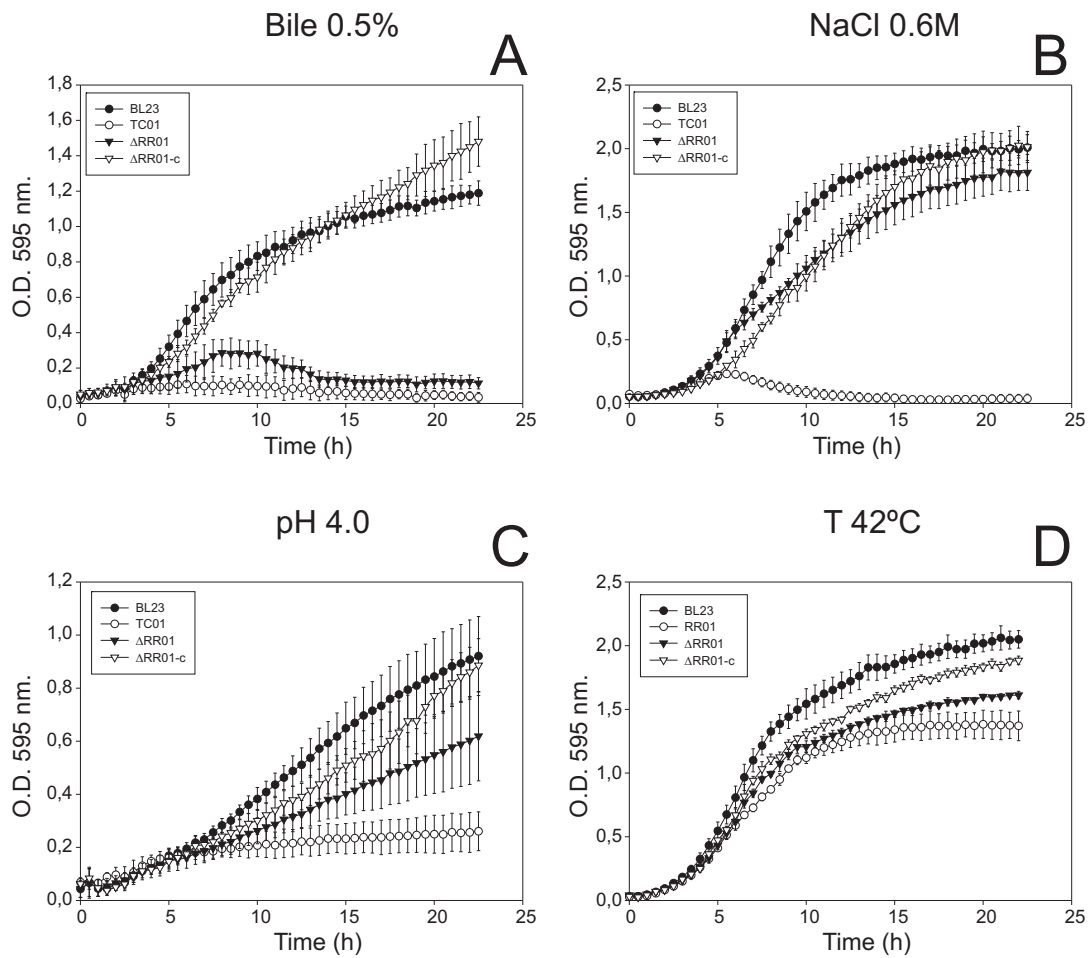
6

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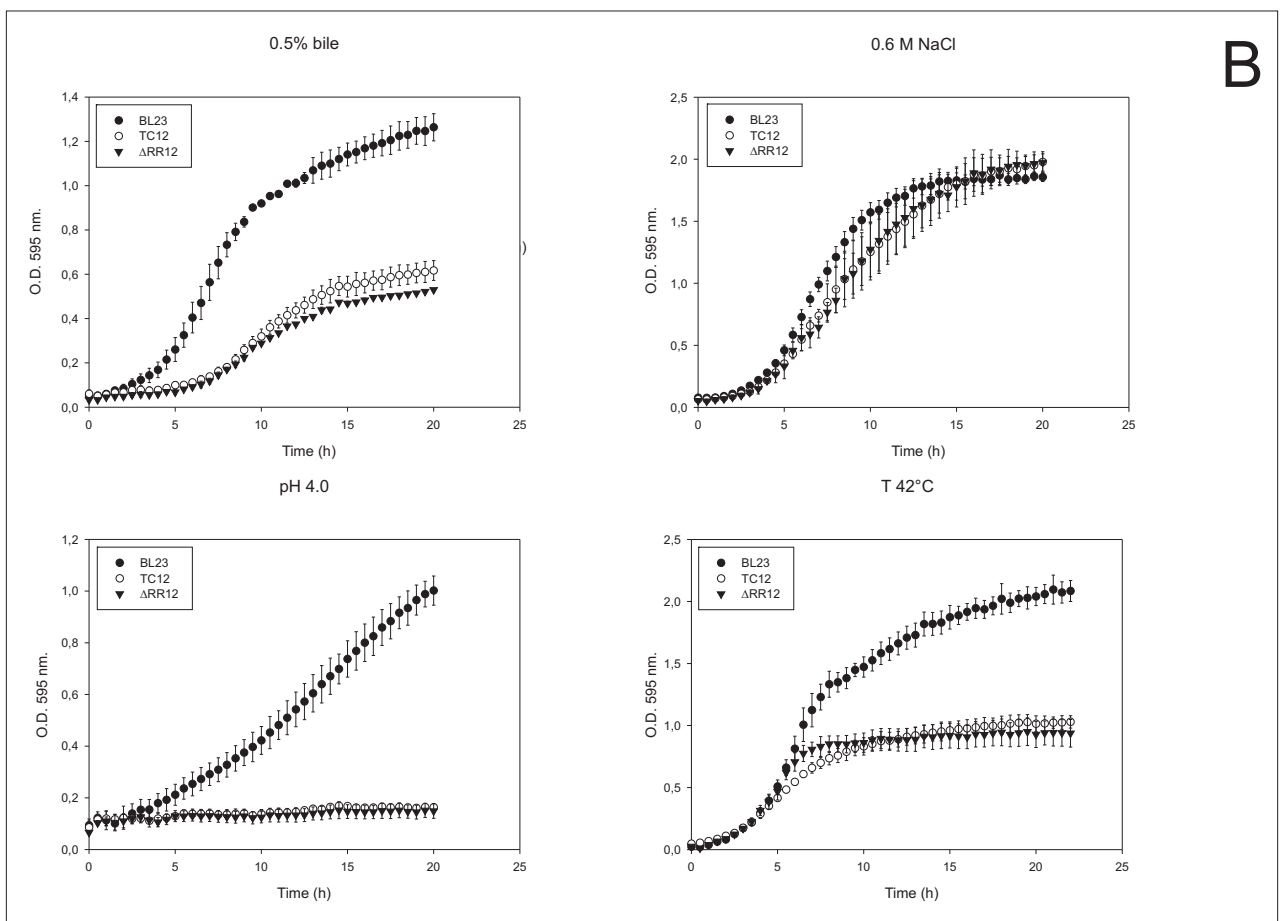
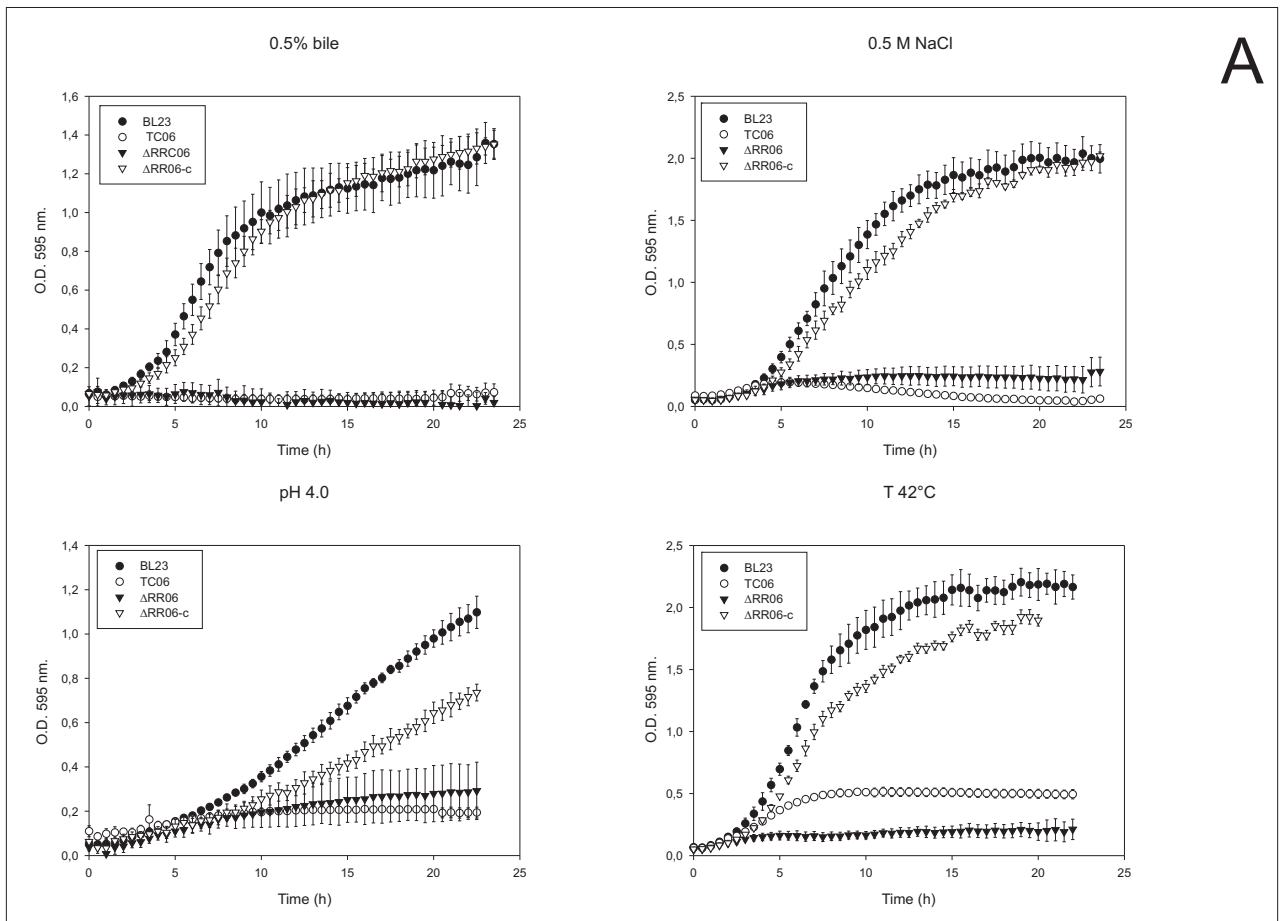
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**Fig. S1.** Growth of BL23 and TC01-defective mutants under different conditions (TC01, insertional mutant; RR01, deletion mutant; RR01-c, deletion mutant complemented with plasmid pT1-RR01). Error bars indicate SD (at least three replicates).





**Fig. S2.** Growth of *L. casei* BL23 and TC06-defective mutants (A) or TC12-defective mutants (B) under different conditions. Error bars indicate SD (at least three replicates).