

Full Paper

NADH peroxidase plays a crucial role in consuming H_2O_2 in Lactobacillus casei IGM394

Shingo NARAKI^{1*}, Shizunobu IGIMI² and Yasuko SASAKI¹

¹Agricultural Chemistry, Meiji University, 1-1-1 Higashimita, Tama-ku, Kawasaki, Kanagawa 214-8571, Japan ²Agricultural Chemistry, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan

Received October 24, 2019; Accepted December 5, 2019; Published online in J-STAGE December 25, 2019

The facultative anaerobic bacterium Lactobacillus casei IGM394 is used as a host for drug delivery systems, and it exhibits the same growth rate under aerobic and anaerobic conditions. L. casei strains carry several genes that facilitate oxygen and reactive oxygen species (ROS) tolerance in their genomes, but their complete functions have not been uncovered. To clarify the oxygen and ROS tolerance mechanisms of L. casei IGM394, we constructed 23 deficient mutants targeting genes that confer oxidative stress resistance. Significantly decreased growth and high H₂O₂ accumulation were observed in the NADH peroxidase gene-mutated strain (Δnpr) compared with the findings in the wild type. The H₂O₂ degradation capacity of Δnpr revealed that NADH peroxidase is a major H₂O₂degrading enzyme in L. casei IGM394. Interestingly, $\Delta ohrR$, a mutant deficient in the organic hydroperoxide (OhrA) repressor, exhibited higher H₂O₂ resistance than the wild-type strain. Increased Npr expression and H_2O_2 degradation ability were observed in $\Delta ohrR$, further supporting the importance of OhrA to ROS tolerance mechanisms. The other mutants did not exhibit altered growth rates, although some mutants had higher growth in the presence of oxygen. From these results, it is presumed that L. casei IGM394 has multiple oxygen tolerance mechanisms and that the loss of a single gene does not alter the growth rate because of the presence of complementary mechanisms. Contrarily, the H₂O₂ tolerance mechanism is solely dependent on NADH peroxidase in L. casei IGM394.

Key words: NADH peroxidase, oxidative stress, deficient mutants, Lactobacillus casei, H₂O₂

INTRODUCTION

Lactic acid bacteria are facultative anaerobic bacteria that do not require oxygen for growth, and they do not have a respiratory chain and catalase; thus, they rely on anaerobic fermentation to produce energy. There is a wide range of variations in tolerance to oxygen and reactive oxygen species (ROS) stress among lactic acid bacteria, even in the same species. This means that stress tolerance in bacteria depends on the genes present in their genomes. ROS are produced via the conversion of oxygen to the superoxide anion radical, which is further converted to hydrogen peroxide (H_2O_2), and Fe^{2+} in cells induces production of the more toxic hydroxyl radical via the Fenton reaction. ROS damage intracellular proteins and DNA and cause cell death [1]. Many studies have examined the tolerance mechanisms of lactic acid bacteria to oxygen and ROS. Enzymes such as NADH oxidase and pyruvate oxidase, which degrade molecular oxygen [2–7], superoxide dismutase (SOD), which targets superoxide as a substrate [8, 9], and NADH peroxidase, which degrades H_2O_2 [10], are involved in the tolerance mechanisms. Further, lactic

acid bacteria in the Lactobacillus casei group possess multiple types of peroxidase, including NADH peroxidase, glutathione peroxidase, thiol peroxidase and iron-dependent peroxidase. Therefore, several antioxidant enzymes are involved in oxidative stress tolerance in lactic acid bacteria. In addition to enzymes that confer direct resistance to oxygen and ROS, some enzymes contribute to oxidative tolerance. Thioredoxin reductase (TrxB2), which maintains the intracellular redox state balance, has been reported to be involved in oxygen tolerance in Lactococcus lactis, Lactobacillus plantarum WCFS1, and Lactobacillus casei Shirota [11–13], and similar findings have been reported for *Escherichia* coli [14]. Streptococcus mutans carries an iron-binding protein (Dpr) to avoid the Fenton reaction, in addition to antioxidant enzymes such as NADH oxidase [15, 16]. Furthermore, L. casei Shirota expresses the iron-binding protein HprA1, which is involved in H₂O₂ resistance via a different mechanism than Dpr [17]. HprA1 is involved in H₂O₂ resistance, but it does not exhibit H₂O₂-decomposing activity. It has also been reported that the disruption of the NADH peroxidase gene (npx) of L. casei Shirota results in a decreased growth rate under shaking and the

©2020 BMFH Press



This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: https://creativecommons.org/licenses/by-nc-nd/4.0/)

^{*}Corresponding author. Shingo Naraki (E-mail: s naraki@meiji.ac.jp)

loss of H₂O₂-decomposing activity. On the contrary, in recent studies, some lactic acid bacteria in which the electron transfer system is activated by the addition of heme alone or together with menaquinone (vitamin K₂) and oxygen is consumed as the final electron acceptor have been reported [18-20]. Compared with anaerobic fermentation, in which ATP is obtained only from glycolysis, use of the electron transfer system increases the amount of ATP and improves growth rates. Additionally, organic hydroperoxide resistance protein transcriptional regulator (OhrR), which was initially found in the gram-negative bacterium Xanthomonas campestris, is involved in resistance to organic peroxide and H₂O₂ [21]. There are similar reports in the grampositive bacterium Bacillus subtilis [22]. However, OhrR has not been reported in lactic acid bacteria. According to the information on lactobacilli published in KEGG, L. casei and L. plantarum, which are generally considered oxygen-resistant, carry ohrR, but oxygen-sensitive species such as L. acidophilus and L. delbrueckii subsp. bulgaricus do not possess the gene. Thus, the antioxidant factors possessed by lactic acid bacteria vary depending on the genus and species, and the response to oxygen stress differs accordingly.

Comparative genomic analysis of *L. casei* and *L. paracasei* revealed that several genes involved in oxidative stress tolerance are shared between the species [23–31]. However, the functions of these genes remain to be clarified.

L. casei IGM394 has high immunostimulatory capacity, and it is used as a host for drug delivery systems [32, 33]. The bacterium also exhibits an extremely good growth rate under aerobic conditions. Similar to other L. casei group bacteria, this strain has multiple oxidative stress tolerance genes, and thus, it is predicted that it has complex mechanisms of oxygen stress tolerance. However, the details of these mechanisms are unclear. It is important to clarify the functions of genes involved in tolerance to oxidative stress in conducting applied research with this strain as the host.

In this study, we constructed 23 deficient mutants (deficient in a single gene, 14 strains; deficient in multiple genes, 9 strains) targeting antioxidant genes reported in other bacteria via a doublecrossover method. The oxidative stress tolerance mechanisms of these strains were evaluated by examining oxygen resistance in shaking culture as well as based on the consumption and resistance to H_2O_2 generated in metabolic processes. As a result, although no differences were observed in the growth of most of the deficient mutants, the Δnpr strain had a decreased growth rate. We found that NADH peroxidase is an essential enzyme for H_2O_2 degradation in *L. casei* IGM394.

METHODS

Strains, plasmids, media, and growth conditions

The strains and plasmids used in this study are listed in Table 1. L. casei IGM394 was used as the wild type. The L. casei IGM394 was a derivative of L. casei ATCC 393, and the L. casei ATCC 393 was distributed by a European collaborator. The L. casei IGM394 exhibits high transformation efficiency. Escherichia coli DH5 α (Toyobo, Osaka, Japan) was used as the competent cells for DNA transformation. The plasmid pBTE was used as a cloning vector for deficient mutants. Lactic acid bacteria were grown at 37°C in MRS medium (Becton, Dickinson and Company, Sparks, MD, USA) and LAPTg medium (2% glucose, 1% yeast extract, 1% Bacto Proteose Peptone No. 3, 0.1% Bacto Tryptone, 0.1% Tween 80, and 0.01% MgSO₄·7H₂O). *E. coli* was grown at 37°C in LB Miller medium (Becton, Dickinson and Company). Erythromycin was added at a final concentration of 5 μ g/mL for lactic acid bacteria. Ampicillin was added at a final concentration of 100 μ g/mL for *E. coli*. The optical density of the culture was measured at 600 nm (OD₆₀₀) using a UV-1200 UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan). The medium was dispensed into test tubes with loose aluminum caps (static condition) or silicon caps that allowed free air exchange (shaking condition). The cells were cultured with shaking at 180 rpm for the shaking condition. Growth analysis of wild-type and mutant strains was performed in three independent experiments under the static or shaking condition. The data are shown as the mean \pm SE of three independent experiments.

Construction of deficient mutants

pBTE is a derivative of the shuttle and thermosensitive plasmid vector pBT2. The origin of replication for lactic acid bacteria cannot function at 42°C. Recombinant plasmids for deficient mutants were constructed as follows. The upstream and downstream fragments of the target gene were amplified by PCR using L. casei IGM394 genomic DNA as a template, PrimeSTAR Max DNA polymerase (Takara, Shiga, Japan), and the primer pairs listed in Table 2. The fragments were digested at both ends using appropriate restriction enzymes (Table 2). The fragments were cloned into pBTE, which had previously been digested using the same restriction enzymes. Recombinant plasmids were purified using NucleoSpin® Plasmid (Macherey-Nagel, Bethlehem, PA, USA) and transferred into L. casei IGM394 via electroporation. Cells were grown in 10 mL of MRS broth to the stationary phase and harvested via centrifugation, and they were then suspended in 10 mL of MRS broth containing 8% (w/v) glycine and incubated at 37°C for 90 min. The cells were subsequently washed twice with an equal volume of sterile water, followed by washing with an equal volume of 50 mM EDTA solution and washing twice with an equal volume of 0.3 M sucrose solution. They were then suspended in 1 mL of 0.3 M sucrose solution. Electroporation was done with a Gene Pulser (BTX, San Diego, CA, USA) using 100 µL of competent cells and 10 µL of plasmid DNA solution in a 2-mm electroporation cuvette at a capacitance, resistance, and voltage of 25 $\mu F,\,48$ $\Omega,$ and 1.5 kV, respectively. Cells were transferred to 1 mL of MRS broth and then incubated at 37°C for 2 hr. After incubation, cells were plated onto MRS agar containing 5 µg/mL erythromycin and incubated at 37°C for 3 or 4 days under anaerobic conditions using AnaeroPouch[®]-Anaero (MGC, Tokyo, Japan). Erythromycin-resistant colonies were selected, and plasmid introduction was confirmed by PCR with appropriate primers (Table 2). To induce plasmid integration, transformants were incubated at 42°C in MRS broth containing 5 µg/mL erythromycin. After several cycles of subculture, cells were plated onto MRS agar containing 5 µg/mL erythromycin and incubated at 37°C for 3 or 4 days under anaerobic conditions using Anaero Pouch®-Anaero. A colony was selected at random, and plasmid integration was confirmed by PCR with appropriate primers (Table 2). The integrants were incubated at 37°C in MRS broth. After several cycles of subculture, cells were plated onto MRS agar and incubated at 37°C for 3 or 4 days under anaerobic conditions using AnaeroPouch®-Anaero. Colonies were selected at random, and gene disruption was confirmed by PCR with

Strains or plasmid	Phenotype of genotype	Source or reference
Strains		
L. casei		
IGM394	Wild-type	our collection
Δnox	deficient of nox gene	This study
$\Delta nox5$	deficient of nox5 gene	This study
$\Delta poxF$	deficient of poxF gene	This study
$\Delta cidC$	deficient of <i>cidC</i> gene	This study
$\Delta ahpC$	deficient of ahpC gene	This study
$\Delta ohr R$	deficient of ohrR gene	This study
Δsod	deficient of sod gene	This study
Δsuf	deficient of suf gene	This study
Δflp	deficient of <i>flp</i> gene	This study
$\Delta dpsB$	deficient of dpsB gene	This study
$\Delta cydAB$	deficient of cydAB gene	This study
$\Delta gshR1$	deficient of gshR1 gene	This study
$\Delta i pr$	deficient of ipr gene	This study
$\Delta n pr$	deficient of <i>npr</i> gene	This study
$\Delta nox::\Delta npr$	deficient of nox and npr gene	This study
$\Delta nox5::\Delta npr$	deficient of nox5 and npr gene	This study
$\Delta sod::\Delta npr$	deficient of sod and npr gene	This study
$\Delta gshR1::\Delta npr$	deficient of gshR1 and npr gene	This study
$\Delta gshR2::\Delta npr$	deficient of gshR2 and npr gene	This study
$\Delta i pr::\Delta n pr$	deficient of <i>ipr</i> and <i>npr</i> gene	This study
$\Delta gshR1::\Delta gshR2::\Delta npr$	deficient of gshR1, gshR2 and npr gene	This study
$\Delta sod::\Delta gshR1::\Delta gshR2::\Delta npr$	deficient of sod, gshR1, gshR2 and npr gene	This study
$\Delta i pr:: \Delta gshR1:: \Delta gshR2:: \Delta npr$	deficient of ipr, gshR1, gshR2 and npr gene	This study
E. coli		
DH5a	Commercial strain purchased from Toyobo	
Plasmids		
pBTE	<i>E</i> coli-gram positive bacteria shuttle vector carrying pBT2 or region pAMb1	our collection
PDIE	erythromycin resistance gene, multi cloning sites and temperature sensitivity	
pBTE::∆ <i>nox</i>	pBTE carrying deficient fragment of nox	This study
pBTE::∆nox5	pBTE carrying deficient fragment of nox5	This study
pBTE:: <i>\Down</i> F	pBTE carrying deficient fragment of <i>poxF</i>	This study
pBTE::∆ <i>cidC</i>	pBTE carrying deficient fragment of <i>cidC</i>	This study
pBTE::∆ <i>ahpC</i>	pBTE carrying deficient fragment of <i>ahpC</i>	This study
pBTE::ΔohrR	pBTE carrying deficient fragment of <i>ohrR</i>	This study
pBTE::Δsod	pBTE carrying deficient fragment of sod	This study
pBTE::Δ <i>suf</i>	pBTE carrying deficient fragment of suf	This study
pBTE::Δ <i>flp</i>	pBTE carrying deficient fragment of <i>flp</i>	This study
pBTE:: <i>\Delta dpsB</i>	pBTE carrying deficient fragment of <i>dpsB</i>	This study
pBTE::∆ <i>cydAB</i>	pBTE carrying deficient fragment of cydAB	This study
pBTE:: \Delta gshR1	pBTE carrying deficient fragment of gshR1	This study
pBTE::\DeltagshR2	pBTE carrying deficient fragment of gshR2	This study
pBTE::∆ <i>ipr</i>	pBTE carrying deficient fragment of ipr	This study
pBTE::∆ <i>npr</i>	pBTE carrying deficient fragment of <i>npr</i>	This study

Table 1. Bacterial strains and plasmids used in this study

appropriate primers (Table 2).

Quantification of H_2O_2

quantified using the standard curve.

A mixture of the chromogenic reagent DA64 (100 μ M in PIPES buffer [0.1 M, pH 6.8, 0.5% Triton-X 100]) and horseradish peroxidase (100 units/mL) was used to measure H₂O₂ concentrations. Cultures of each strain were harvested via centrifugation (10,000 × g, 3 min). Each supernatant (20 μ L) were added to the mixture, which was incubated at 37°C for 5 min. After incubation, OD₇₂₇ was measured, and H₂O₂ content was

H_2O_2 consumption

Cells precultured at 37°C were inoculated into 10 mL of MRS medium at $OD_{600} = 0.05$. The cells were used after static culture at 37°C for 5 hr. They were then washed twice with PIPES buffer (pH 6.8) and resuspended in 10 mL of H₂O₂ adjusted to 50 μ M, 100 μ M, 300 μ M with PIPES buffer. After incubation at 37°C for 1 hr under a static condition, the cells were harvested via centrifugation (10,000×g, 3 min). Supernatants were used to

Table 2.	Primers	sequence	used i	in this	study
----------	---------	----------	--------	---------	-------

Construction of deficient mutuals nov nov_A-forward CAACCIGCAGTITITICETETTGATTAATATGTTTGAAAACAA Nov_A-forward TGGTTGCAATGGATGGAAGCATCGATGCAAACAA Nov_B-forward TGGTTGCAATGGATGGAAGCATCGCTTGAACAACAA Nov_B-forward TGGTTGCCAATGGATGGAAGCATCGCTTGCAACA Nov_B-forward GCAAGACGCTTGTGTGCGGACAGTGCTCGTTTCA Nov_B-forward GCAAGACGCTTGTGTGCGGCGTCGTCGGCGGAAAATTAGAATGCAT Nov_B-forward GCAAGACGCTTGTGGCGCGTCGGTGGGGAAAATTAGAATGCAT Nov_B-forward GCAAGACGCTTGTGGCGGCCGTGGTGGGGAAAATTAGAAAGGAAG Nov_B-forward GCAAGACGCTTGTGGGAGGATTTTGAAGAAAGGAAAGGCAAGT Nov_B-forward GCCAAGACGCTGTGGGGGCGTGGTGGGGAAAATTAGAAAGGAAG Nov_B-forward GCCAGAGCCCAGGGCGCGTGGTGGGGAAAATTAGAAAGGAAG Nov_B-forward GCCAGGACGCGGCGGGGGAAAATGCAAAGGCAAAGG Nov_B-forward GCCAGGACGCGGCGGGGGGAAAATGCAAAGGCAAAGG GCAG-forward GCCAGGAGCGCGGCGGGGGGAAAATGCAAAGGCAAAGG AGACGCAGCGGGGGAAAATGCAAAGGCAAAGGCAAGT AGACGCGGGGGGGGGG	Target gene	Primer name	Primer sequence (5' to 3')	Restriction enzyme site
pace pace, A-reverse CAACCTGC/GAAGTTTTTACATATICTTTGAAAAAT Pal pace, A-reverse TGGAAGGAAGTTTTACATACATCACAAAAAA Hand HI pace, B-reverse TGGAAGCTGGTAACGACGTGCCAACGTGCCCGT Hand HI pace, B-reverse CCATGGATCGTAAAGCATGTGGAAGGTACCTTCTCA Hand HI pace, B-reverse GCCATGGATCGTAAGCGTAGGTGCGGGGGAAAATATAGAATCT Hand HI pace, B-reverse GCCCAAGCTTCTTCATCGGACGTCGCCCTTCTCA Hand HI pace, B-reverse GCCCAAGCTTTGGACGGGGGCACTCTCGCATTGCCCTTCAA Hand HI pace, B-reverse GCCCAAGCTCCTTGGACGGCGCCTTGTAAAAAGGAAGGAA	Construction of d	eficient mutants		
mox A-reverse TGGTAGGAGTOTTIAACCATCGATTGAAACAACAA mox B-roward TTGGTAGGAGTAGTTAAACCATCGATTGCAA mox S-A-forward CCATGGATGCCCCGTGAACGTAGGTAGTTAACCACCATCGTTGCA mox S-A-forward CCAAGGTCCCCGTGAACGTAGGTAGTTGAC mox S-B-forward TGGAAGCCAATGGCGGGGAATATTAGAATTGGATTGGT mox S-B-forward GTGGGATCCCCGGGCAATGGCGGACTTCTGCAA Hud III mox S-A-forward GTGGGATCCACGCCGATGGCGCAATGGCGAA Hud III pox B-forward ATCTTTTGGGAGGATTGTGCAAAAGGAAT Part I pox B-forward ATCTTTTTTTTTGATGGGAGGATTGTCGAA Part I cidC - atoward GCTAGGATCGCAAGGCAATGCGGAA Part I cidC - atoward GCTAGGATCGCAAAGCAAGTGCGAAAATCCAAAAGTCCCCCCAAAAGGAAGTATTGGAAAATCCAAAGTGCGTTTAAAAAAGGAGAATTTGAATGTGGT Hud III cidC - atoward GCTAGGATCGCAATGCGAAAATCCAAAGTCCCCCAAAAGTGCAAGTAGCAAGTGAAGTGAAGTAATGCAAGGTGGTTTAAAAAAGGAGCAATTTGGAAGTGGCTTTAAGTAAAGTGGAGTAAATGTACAAGGTGGCTTTAAGTAAG	nox	nox_A-forward	CAAC <u>CTGCAG</u> TTTTTGCTGTTGATTAATATGTTTGAAAAT	Pst I
nox B-roward TICGTAGCAATGGATGGATAGTAAACACTCCTTCACA nox B-roward CCATGGATCCCAAGGGCCACAGGCCCGT Hud III nox B-A-reverse ACCAATGGATCCAAGGGCACAGTGCCTCCTTCAT Hud III nox B-roverse CCCAATGGATCCCCCAGGATGCGCTCGTCTCTTCA Hun III nox B-roverse CCCAATGGATCCACGCAGGCTCGTCGTCAAC Hun III nox B-roverse CCCAAGGCTCTTCTCAAGAGAATCCCCCCAAAGGAT Ful nox B-roverse CCCAAGGCTTCTCTCAAGAGAATCCCCCAAAGGAT Ful nox B-roverse CCCAAGGCATCTTCCAAGAGAAATCCCAAAGT BanH1 nox B-roverse CCCAAGGCATCTCCAAGGACACCCCCAAAGTGAAGAAATCCAAAGT Ful cidC A-forward CCCACGGCACCAAGTGACGCCCTTATTA BanH1 cidC A-roverse CCACAAGCTCAAGGACATTCCCCAAAGTGACCCCCAAAATCACGTAAAATCCAACGTAATCCCCTTATTAAG Hud III cidC B-roverse CCACAAGCTCCCAACGTGACACAAAATCCACACCTACGTACACCACCCCACACATAGCGCGCAAAATCAACCACCTCCCACACATAGGACACACCACCACACACA		nox_A-reverse	TGTGAAGGAGTGTTTAACTATCCATTCGAATTGCAAACAA	
mox Barverse Tro GAAGCTTGTATCCCCAAGTGCCCT ///mill mox A. Areverse CCAGAGTCCCCCGT0AAGCGTAGTGTTG Baml/I mox J. Barverse GCCAAAGGACCCCGTGAACGGCGTCGTCTGCT Hind III mox J. Barverse GCCAAAGGACCAATGCGGGCATCTGGCT Hind III mox J. Barverse GCCAAAGGACCAATGCGGCACTTCTGGA Hind III park J. Areverse GCCACGCGGGCCGCGCGCGCAAAGGCGCGAA Put I park J. Areverse TGCACTGCGCGGGCGCGCGCGCGAA Put I gark J. Areverse TGCATGCGCGGGCGCGCGCGCGAAAGTCCCCCCCCAAAAGGCGGCC Hind III gark J. Areverse TGCATGGCGCGGGCCGAAAGTCCGCGAGTGCGGAA Put I gark J. Areverse TGCATGGCGGGCGCGAGTGCGGAAATCCCCCCCCCAAAAGGG Hind III gark J. Areverse GCATAGGGCGGTGGAAATCCCCGGGTGGATGCGGCGAAGTCCTGAGGCCGAAAGTCCGGAGGGCCGAAAGTCGGGAAGGACCCCCCCC		nox_B-forward	TTGTTTGCAATTCGAATGGATAGTTAAACACTCCTTCACA	
aox5 nox5_A-loward CCATGEATECCCCGTGATGCTGTTG BanH1 nox5_A-everse AGGAATCCATAGTTCCCCCAGGATGGCGGGGGAAATTAGAATCGT Hind III podF podF_A-forward GTGAAGGAAGGCCAATGGCGGGGGGAAAATTAGAATCGT Hind III podF podF_A-forward GTGGGGGCCAATGGCGGCGCTCCGGAAAGGAAT BanH1 podF podF_B-forward GTGGGGGCCAATGGCGCAATGGCGCAAAAGGAAT BanH1 podF B-forward GCGCAAGGCCTTGGCAATGGCAAAAGTAAAGGAAGTT BanH1 godF B-forward GCGCAAGGCCTTGGCAATGCCAACCCCCCCCCAAAAAGCAAGC		nox_B-reverse	TTC G <u>AAGCTT</u> GTTATCCGCAACGTGCCGT	Hind III
nex5_B-Powerse ACGAATICATAATITTCCCCCAGCATCCCCTTTCA nex5_B-Powerse GCC AAAGCTLCTTGATGCCGGCAAATITAGAATCGT nex5_B-Powerse GCC AAAGCTLCTTGATGCGGCATCGCGTCTATC nex5_B-Powerse ACTITTTGGGAGGGGATTGTTCTAGTGGATAAAAAAGAGAT poxF_A-reverse ACTITTTGGGAGGGGATTGTCAGGGAACTCCTCGGAA poxF_B-reverse TGCATAGGAAGCAACGGAGGGAAAATCAAAAACGAAAAGCAAACGCAAAGCAAACGCAAAGCAAACGCAACGGAAAATCCAAAAACCAAACGCAACGCAGGAAAATCCAAAAACCAAACCTAACGGAACGCAAAAGTCAAAAGCAAACTCCAATAGCGACCTGGACGGCTTTATAA cidC_A-horward GCCATAGGAACCTAGGGCACACAATATCCCGAAAACCCAATAGCGACGCTTGATACGACCA cidC_B-reverse GCCATAAAGGAACCTATCGCCAAAATCCAAATACCCACCTGGACGCCTTTGATACGACCA abpC_A-reverse GCTAAAGGAACCTATCGCCAAAATCCAACACCATTCCCGAACCACCCTTGATACGACCA abpC_B-reverse GCTAGGAACCCACCGTGATATTACCCCCCTTTGATAGCCTA abpC_B-reverse GCTAGGACGCCGCGCACAATCCCGAAATCCCAAATCCCAACCCACCACCACCACCACCCAAATCCGACCACCCTTACAATCACCCGAAATCCCAAATCCCAAATCCCAAATCCCAAATCCCGAAATCCCAAATCCCAAATCCCAAATCCCCAAATCCCCAAATCCCAAATCCCCAAATCCCCAAATCCCCAAATCCCCAAATCCCCAAATCCCCAAATCCCCAAATCCCCCAAAATCCCAAATCCCCCAAAATCCCCCAAATCCCCCAAATCCCCCAAATCCCCCAAATCCCCCAAATCCCCCAAATCCCCCAAATCCCCCAAATCCCCCAAATCCCCCAAATCCCCCAAATCCCCCAAATCCCCCC	nox5	nox5_A-forward	CCAT <u>GGATCC</u> CCCGTGAAGCGTAGTTGTTG	BamH I
nex5_b-reverse GCAAAGGCACGACCATGCGGGGGAAAATTATGAATCCT pxxF pxxF_A-reverse GCCGAAGCCACCATGGCGACCTCTGCAC Hind III pxxF_A-reverse ACTITITGGGAGGGATCTTCTGACCAAAGAGAT pxxI pxxF_A-reverse ACTITITGGGAGGGATCTTCTGAGAAAACCCCCAAAAGGAT pxxI pxxF_B-breverse ICCACTGCAGGGCTTGGCAAAACCCCCCAAAAGGAAAATTATCAAAGAGAT PxxI cidC_A-reverse TGATACAAGCTAATGGAAAATCCCCTCCCAAAAAGGACAAAAGAGAAAATTATCAACGCAAAATCCCCTAAAGAGAAATTATCAACGGTCTTTATAA BauH1 cidC_B-reverse GCAAAGCCCCACCAATTCCCGATAGCTGCTATGCTGTTATCA Hind III ahpC_A-reverse GCATAGCCCCCACAATTCCCGGTTCTGATGCTGCTATCAGAAACT Hind III ahpC_B-reverse GATAGCCACCGCTGAACTCCCCCAAAATTCAGAACCCCTAGAAATC Hind III ahpC_B-reverse GATAGCCACCCCCCCCAAAATTCACAGGATC Hind III ahpC_B-reverse GATAGCCACCCCCCCCCCAAAATTAGAACCCCTCCCAAAATTCAGAATC Hind III ahpC_B-reverse GATGAGCCACCCCCCCCCCAAAATTAGAAGGATC Hind III ada ad_A-reverse CATGATCCAAACCTGAGGACAACCTTCC Hind III sdd second-Areverse GGTGAGGATCCAACCCTGACGCCTAAACCTTC Hind III sdd A-reverse GGTGGAAACTGCCCCAAAACTGGGAGA		nox5_A-reverse	ACGAATTCATAATTTTCCCCCAGCATCTGCCTTCCTTTCA	
mox5 B-revense GCC AAAGCTLCTICATCGGTCCGCTCATC Hand III poxF AppE A-revense ACTITITGGGAGGCCCCCCAAGAAAGCC BamH1 poxF B-revense ACTITITGGGAGGCTCTGGCAAGAATCCACTCCCCAAAAAGCA Fit cidC cidC A-revense TGCATGGAGGCTTGGCAGCGCGCAAAAGCAAAACCCAAAAGCAAAACCCAAAAGCCAAACCCAACCCCCC		nox5_B-forward	TGAAAGGAAGGCAGATGCTGGGGGAAAATTATGAATTCGT	
posF posF A-forward GTTGGCATCCAGCCAATIGCCGATTCTCGGA BamH1 posF A-forward ATCTCTTTTTAGCAGGAAGAATCCCTCCCAAAAGGAT posF posF B-forward ATCTCTTTTTAATCACTAGAAGAATCCCTCCCAAAAAGGT Ps1 cidC A-forward CGTAGGATCCCTAGGAGTGCCGAA BamH1 cidC A-forward CGTAGGATCCCTAGGAGTGCCGAA BamH1 cidC B-forward GGGATAAAGGAGATTTCGTATGGATGATCGTTAACA BamH1 cidC B-forward GGGATAAGGACACATATGCGATAAGCGAGC BamH1 abpC A-reverse GGAAAGGAGCACCATATGCGAAGAGCGC BamH1 abpC A-reverse GAAAGGGAGACAGATCCCCGCACCGTGATTTACGACGAC BamH1 abpC B-reverse GATAAAGGAGAGTACGCCCCGTAATTGCGAGCAGCG BamH1 abpC A-reverse GATAAGGAGAGAGCCCCCCCAAAATAGCGGAGCGTGCTTCTTAAGAGAAT BamH1 abpC A-reverse GATAAGGAGAGAGCACCCCCCCAAAATAGGG BamH1 sidC A-reverse GATAAGGAGAGAGCACCCCCCCAAAATTACCTCCTTTAA BatCCTAATTTTTGGGGGGGGGGGTGCTTCCTCTAA BatCCTAATTTTTTGGGGGGGGGGGGGGGGGGGGGGGGGGGAAAGGAGCACTTGCCCCAAAAGGTG Hind III BatCA <		nox5_B-reverse	GCC A <u>AAGCTT</u> CTTGATCGGCTCGTCTGATC	Hind III
porF.B-Forward ACTITITICGCAGGAGGATICTICIAGTGAAAAAAAGAGAT porF.B-Forward ACCACIGACACCACACAGAGACATCCCCCCCAAAAAGT porF.B-Forward GCCAAGGACACCAGATGCCCACACACACCCCCCCAAAAGT cidC.A-forward GCCTAGGAGCACCACATACGCAAAACICCCATTATCGC cidC.B-reverse GCAAAAGCTIGACACACAATACICCTGAACCCATATCCGACCACATACICCACACACACACCCTGACCCCTATACCACCA cidC.B-reverse GCAAAAGCTIGGACACACAATACICCCGTGACGCCTTATCACACCACAAAGCCGACCCATTCGCACCCATTACCACCACAAAGCCGCGCCCCAAAACCACAAAGCGCGCCCCCAAAACCACAAAGCGCGCCCCCAAAACAAAAAGAGAGGAG	poxF	poxF_A-forward	GTTG <u>GGATCC</u> AGCCAATGGCGACTTCTGGA	BamH I
pors.B. B-ionwardATCTCTTTTTAATCACTAGAAGAATCCCTCCCAAAAAGTParlcidCh-forwardGCTAGGATCCCAGCGGCACGGCTTTTATABamH1cidC. A-forwardGCTAGGATCCCAGCGGCACGGCTTTTTATABamH1cidC. B-forwardGCGACAAAGGAACCAATCGAAAGGAAAATCAAAATCCAAAATCCACGCTHand IIIohgC. A-reverseGCACAAACCTIGGAACACAATCGAATAGCAAAATCAAAATCAAAATCCAACGCTHand IIIohgC. A-reverseGCACAACCTIGGAACCAATATCCGATAGCTCAACGCTHand IIIohgC. A-reverseCACAACCCTIGGAACCAATATCCGATATATCCGACTAATAGCAACGACCAGCAATATACCGCAATTTTTAAGAATCHind IIIohrR. A-forwardCAACCTGACGAGGCGCGTCGTCCCTCTAAParlohrR. A-forwardCTGACTGACAAGCCGCGCCAAAATAAGGParlohrR. B-ForwardTTAGGAGGAAGCAGCCGCCCCAAAAATAAGGParlsod A-reverseGCACACAAGCTTAAGGCTGATGCCTCCTCTAAParlsod A-reverseGCACAGCTCAAGGCGCGCGAAATCACCGAGGACHind IIIsod A-reverseGCACAGCCAAAGCTGAGGCTGGTCCCTCCTAAParlsod A-reverseGGTAAGCTCCTGGTGAACCATCGAGGACCTTGCCACParlsod A-reverseGGTAAGCTCCTGGTGACCATCACGCCCCCAAAAGGTGHind IIIsuf A-forwardCTGAGGCACAGTGGCCCCCCAAAAGGTGHind IIIsuf A-forwardTTGAGGCACAGTGCCCCCCCAAAAGGTGParlsuf A-forwardGCTAGGACCCAACGCCTTGCCCCCCAAASarl H-reversesuf A-forwardGCTGGGGCCCCCGCAGCACCTGCCCCCAAParlflpflp A-reverseGCACCCGCGCGCTGCCCCCAAAAGGTGGCACTGGCCCCCAAParlgdr3HgafA-forwardGCTGGGGCCCCCCGCGCCCCCAABamH1gdr3Hflp A-reverseCGCACCTGGCAGCCTTGCGCAGCGCCCCCCAA		poxF_A-reverse	ACTTTTTGGGAGGGATTCTTCTAGTGATTAAAAAAGAGAT	
posF_B-reverseTGCACTGCAGGGCTTGGCAGTGCCCGAAPs1cidC_A-reverseTGATACAAGCTAGGGGCGGCAGGGCTTTTTATABamH1cidC_A-reverseGCGATAAAGGCACGAATACTGGAAGACTTTTATCGGCHind IIIdidC_B-reverseGGATAAAGCCTGGGACACCAATACTCGGAGGCHind IIIdipC_A-forwardGCTGGGATCCAACACCACATATCCTGGAGGCHind IIIdipC_A-forwardTAGCTGATCAAAGCCGCGGTGGCTGCTTTGATCAACTABamH1dipC_B-reverseCTAAAAGGGAGGATGATGCCCTAAHind IIIohrR_AbornardCAACGTGGAGGAGGAGGATGGTCCCTAGAAATAAGAGGAGGAGAGGAGGAGGAGGAGGAGGAGGA		poxF_B-forward	ATCTCTTTTTTAATCACTAGAAGAATCCCTCCCAAAAAGT	
cid2eid2A-verseGCTAGGATCCCAGCGTGACGGCTTTTATABamH1adpCGCGATAAAGGACAATTTGATTTTGCGATAGCTTGATCA		poxF_B-reverse	TGCA <u>CTGCAG</u> GGCTTGGCAGTGCCGAA	Pst I
eidC -Areverse GATACAGAAGCTAATGGAAAAATCAAAATCTCCTTTATCGA eidC B-reverse GACAAAGGCATATGGATTTGGATTTGGATTGCA eidC B-reverse GACAAAGGCATGGAATTTGGATTTGGATTGGAT alpC A-reverse GACAAAAGGGAGTAATACACAGGGTGGATGGACGACA alpC B-reverse GATAAAGGGAGGAGTGATATGCACGGGGGTTGATCAGGTA alpC B-reverse GATAAAGGGAGGAGTGATATGCACGGGGGTTGATCAGGTA alpC B-reverse GATAAGGTAGAGGAGGGATGGATGCCTCAGATG ohrR A-forward CAAGGTGCAGGGGATGGATGCCCTCAAATGGAG ohrR A-forward CAAGGTGCAGGGATGGATGGCAGGGATG ohrR B-Reverse GCACAAGGCGGCGGAGAGGCGAAGGGATG ohrR B-Reverse GCACAAGGCTGAAATGCAGGCAAATGA ohrR B-Reverse GGACGGAGGATGGATGGCAGAGGGATG sod A-reverse GGACGGAGGCGGAAATGCACGCACCAAAGGT sod A-reverse GGACGGAGGATGAGCATCGGAAATGCACT sod B-reverse GGTAAGGCTGGAGTGGCAGATGGCACAC sod A-reverse GGTAAGGCTGGGAATGCACACTC sod B-reverse GGTAAGGCTGGGAATGCACACTCC sod B-forward CTGGAGGCAGGTGGCTGGGAATGCACT sod A-reverse GGTGAGGCAGGTGGCTGGGAGATGCACT sod B-reverse GGTAAGGCTGGGGCGAAATGCACT sod B-reverse GGTAAGGCTGGGTGCGTGGTGGGCCAAAGGT flp A-forward CTGAGGACGAGTGGCCGAAAAGGT flp A-forward GCAGGGAATGTGCCGTGGAGTGGCCGAAA sof B-reverse TGGAGGACGTGGCCGGAGAGTGGCCGAAAGGT flp A-forward GCTGGGAGACGTGCCGGAGGAGCACT flp A-reverse CGCACGGGGCCAAACGGTGCCGAGGAGC flp B-reverse CGTGCGGGAGCCGAGGGGCGAGGGAGCACT flp B-reverse CGCACGGGGCCGAAGGTGGCCGAGGGAGCAGG flp B-reverse CGCGCGGGGAGCCGAGCGTGCGGCGAAGGC flp B-reverse CGCGCGGGGGGAGACCCACTTGCCCCCCAA dpsB A-forward GCGGGGGGCGAAGCACTTGCCCCCCGGGCAA dpsB A-forward GCGGGGGGGGAGAGAAGCACTGTGGCAGCG fggBA-forward GCGGGTGGAGGGAGAGAAGCCACTGGGCGAAAGA cydAB B-reverse CGCGCGGGGGGAGAGAAGCCCATTGGAGCG gshR-1 A-reverse CAAGGAGGAGCACAGTGGCGCGAAAGA cydAB B-reverse CGCGCGGGGGAGAGGAAGCCACTGGGCGAAAGA fggBA - forward ATGGAGGTGCCGCGCGCGGCGAAAGA fggBA - forward ATGGAGGGAGCGCGAAGGGGGGGAAAGACCCACTGGCGCAA gshR-1 B-reverse CAAGGAGGAGCACATGGTGGAGGCGAAGAGCACGGCGAAAGA fggBAB - forward ATGGCGGGCGAAGAGGAGGGGGCGAAAGACCCCGGCGAAAGA fggBAB - forward ATGGCGGGAGCACAGGTGGCGCGAAGGCAAGGGAAGCACGCGCGAAGGGAGGG	cidC	cidC_A-forward	GCTA <u>GGATCC</u> CAGCGTGACGGCTTTTTATA	BamH I
eide B-reverse GACAAAGGAGATTITGATTITTGATTAGCTTGATCA Hud III ahpC A-reverse GACAAGGCTGGGACACAATATCGTGAGGC Hud III ahpC A-reverse GATAGAGCTGGAGCACCATTGATATTACCTCCATTTTAG ahpC B-reverse GATTAGGCTTAGATGATCACGGATGATTAGCACCATTGATAGATC IIII ohrR ohrR A-forward CAGCTGGATGATGATGACCATGTGATATTACCTCCATTTTAG ohrR ohrR A-forward CAGCTGGAGGAGGAGGCCTGGATGATCAGGATG ohrR A-forward CAGCTGGAGGAGGAGGCCTGGATGATCAGGATG ohrR A-forward CAGCTGGAGGAGGAGGGCTGAGCTGAGGATG ohrR A-forward CAGCTGGAGGAGGAGGCGCCGCAAAATAGG ohrR B-forward GAAGGTTGATGATGATCAGGATG Hind III sod A-forward GAAGGTTGATGATGATCAGGATG IIII ohr III sod A-forward GAAGGTTGATGATGATCAGAAGCACCTGGAAAATGCAGGAAGGA		cidC_A-reverse	TGATACAAGCTAATCGAAAAATCAAAATCTCCTTTATCGC	
eidC B-reverse GACAAAGCTTGGACACAATATCGTGAGGC Hind III ahpC A-reverse CTAAAAATGGAGGTAATATCAACGGTGGCTTTGATCAGGT ahpC A-reverse CTAAAAATGGAGGTAATATCAACGGTGGCTTTGATCAGGTA inpC B-reverse GATTAAGGTTATGTGCGTGATGTCGGTTTGATAGAAATC inpC B-reverse GATAAGCTTGTGTGCGGATGCTCCGTTTTAAGAATC bhrR A-forward CTAGCTGAGGCGGGGGTGCTCCCCTTCATA ohrR A-forward TTAGGAGGAGCAGCGGCGGTGGTCCCCCTCCTAA ohrR B-Reverse CCTACAAGCTGCGGGGTGCTCCCCTCCTAA ohrR B-Reverse GCACAAGCTGCGGGGGTGCTCCCGCAAATC Psr1 sod A-reverse GGTGACGCGAAGCTGCGGAAGTCCGGAAAATC sod B-reverse GGTTAAGGTTGATGCTCGGAGATGCAGGATG sod B-forward GTAGCGGAGCTCTCCTGGGACGTCCCCCAAAAGGTG Hind III suf A-forward GAAGGTGATTCCTCGGAGTGTGTGGGCAGATC sod B-reverse GGTTAAGGTTGGTCAGGATGACGGACCATCAGGTG Hind III suf A-reverse GGTTAAGGTTGGTCAGGTGGATGCCCCAAAAGGTG Hind III suf A-forward GCTAGGAGCACGTGGCGGCGCCCAAAAGGTG Hind III suf B-reverse GGTTAGGCCCAAGTCGGCGCAGATCGCCCAAAGGTG Hind III suf B-reverse GCATCGGCAAACATGCCAAAAATGTTGGC Psr1 fp ffp A-forward GCTAGGGATCAGGTGCGGTGGGTGGCGGCGAGCG fp B-reverse CGCCCGGGGAGCCCCATCGCCCCAA suf B-reverse CGCCGCGGAGCCCGATGCCCCCAACGGTCGCGAGGAGC fp B-reverse CGCCGGGGAGCCCGATGGCCCCAACGGTTGCCACCCTCCTAA fp B-forward GCTAGGGATGCACGGTTGCCACCCTCCCAA fp B-reverse CGCCGCGGAGCACGGTTGCCACCCTCCTAA fp B-reverse CGCGCGGAGCACGGTTGCCACCCTCCTAA fp B-reverse CGCGCGGAGCACGGTTGGCACCCTCCCCAA fp B-reverse CGCGCGGAGCAGCGTTGGGACCCATCGGCA BamH1 fp A-forward CCGGGTGCAGCGGAGCAGAGCACTGTGGACCCCACGG fp B-Reverse CGCGCCGGAGCAGAGCACGGTTGGACCCCACG gbsB B-forward ACAGGAGGAGCAAGTGGAACCCACGGTCGGCAA gbsB A-reverse TGGGAGCCGAGGGGGGAAAAGA ccCGGGTGCAGGGGGGGAAAAGA ccCGGGTGCAGGGGGGGGAAAGAA gbsB A-reverse TGGGGGGCCGAAGGGGGGGGAAAGGA fp C-reverse CACAGGGGGGGGGAAAGGACCCGTGGTGGACCCGCG gbsB B-forward ACAGGAGGAGCAGGTGGGGGGGAAAGGA gbsB A-reverse CGCGGGGGGGGGGGGGGGGGGGGGGGGGGAAAGGA gbsB A-reverse CGCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGAAAGGA gbsB B-forward ATGGGGGGGCTGAAGGGGGGGGGGGGGGGGAAAGGA gbsB B-forward ATGGGGGGGCTGAAGGGGGGGGGGGAAAGGA gbsB B-forward ACAGGGGGGCGGGGGGGGGGGGGGGGGGGAAAGGA gbsB B-forward ACAGGGGGGCGGGGGGGGGGGGGGGGGGGG		cidC_B-forward	GCGATAAAGGAGATTTTGATTTTTCGATTAGCTTGTATCA	
ahpC ahpC A-forward GCTAGGATCCCCTACATTCTCGATATCGGT BamH1 ahpC A-forward TAGCTGATCAAAATGGGGTAATTACACGGTGCTTTGATCAGCTA ahpC B-forward TAGCTGATCAAAGGACGGTGATCACACGGTGGTTGATCACCGTCA ohrR ohrR A-forward GATTAAGGTGGAGGAGGATGATCACACGGTGCTTTATAAGAGATC Hind III ohrR A-Reverse CCTTATTTTTGGGCGGCTGCTTCCTCCTAA Psr 1 ohrR A-Reverse CCTTATTTTTGGGCGGCTGCTTCCTCCTAA Psr 1 sod ohrR A-Reverse CCTAAGTGGAGGAGATGATCAACATAGG Psr 1 sod sod_A-forward CTGACTGCACACTGGAGGAATGCCCAAAAATAGG Psr 1 sod sod_A-forward CTGACTGCACACTCGGAGTTGATCACACTTC Psr 1 sod_A-forward GAAAGGTTGATCCTGGATGATTACCATCCCCAAAATG Psr 1 sod_A-reverse GGTTAGGCTTGATTGCTCGAGGACCAAAATGCCAAAATG Psr 1 suf A-forward GAAGGTTGATTGCTCGGATGATTAAAATGCCAAAATGC BamH1 suf A-forward GAAGGCTGAATCCAAGCACTGCCCCAAAATGGGACACAAGACCCAAAATGTGCAAAATGTTGGAC BamH1 suf B-forward GCTACGGAGACGCAGTGCCCCAACGCCCCCCAAAATGTGGACCAAAATGTGAAATTTTGACAAGAGCAAGAACGACGACGCTGCCAAAGGCCAAGACGATGATGGACGAAGAAAATGTTGGCCAAAGGCCAAGACCATTGCCAAAGCCCAAGACCCATTGCCAAAGCAGAAGAAATGTTGGACAAGAAGAA HamH1 fip B-reverse GCACCTGCGAAGCGTGCAAGCCCCCCCAAAGCGCGGCAAAGAA HamH1 gab B-reverse GCACCTGCGAAGCGTGAACCCCCTCCCCAA		cidC_B-reverse	GACA <u>AAGCTT</u> GGGACACAATATGCTGAGGC	Hind III
ahpC A-reverse CTAAAATGGAGGTAATATCAACGGTGCTTTGATCAGCTA ahpC B-reverse GATT <u>AAGCTT</u> ATGTCCGTGATATTACCTCATTTTAG ahpC B-reverse GATT <u>AAGCTT</u> ATGTCCGTGATCGTCCATTTTAAGAATC Hind III ohrR A-forward CAAGCTGGCAGGCATGATGCCTAG Pr 1 ohrR A-reverse CTTATTTTTGGGCGGCGTCTCTCTCTAA sod A-reverse GCACAAGCTTAGGTGATGAGCAGCGCAGAGAATC Pr 1 sod A-reverse GCACAAGCTGAGGCAGAGAATCCACCTTAC sod A-reverse AGTGACCAAAGCTGAGGAGAATCCACCTTGC sod A-reverse GGTT <u>AAGGTGAAATTCCCGCAAAAATC Pr 1 sod A-reverse GGTTAAGGTGAAATTACCATCCAAGGAGAAAAAAAAAAA</u>	ahpC	ahpC_A-forward	GCTA <u>GGATCC</u> GCTACATTCTCGATATCGGT	BamH I
ahpC_B-forward TAGCTGATCAAAGCACCGTTGATATACCTCCATTTAG bhrR ohrR_A-forward CAAGCTGCAGGAGCGATGATGCCTCGTTTATAAGAATC Hind III ohrR_B-Reverse CCTTATTTTGGGCGGCGTGCTTCCTCCTAA Fsr1 ohrR_B-Forward TTAGGAGGAAGCAGCCGCCCAAAAATAAGG Hind III sod ohrR_B-Reverse GCACAAGCTTGAAGGTTGATGGATCAGGATG Hind III sod sod_A-forward CTGACTGAAGCTTCCTCGAAAATCCAAAATC Psr1 sod_B-forward GAAAGGTTGATCCTCGATGATTGCTCCAAAAGTG Hind III sod_B-forward GAAAGGTTGATCCTCGATGATTGCCCCAAAAGGTG Hind III suf_B-forward GATAGGTTGATCCTCGATGTTGGTCAACT BamH1 suf_B-forward AATAGGAATCCATGGCTCCAAAGGACCAAGGGC BamH1 suf_B-forward AATAGGATCCCAGGCACCATGCCCCAA BamH1 suf_B-forward GCACAGGCACAGTGCCCATGCCCCAA BamH1 flp flp_A-forward GCACCAGGCCCCGATGACCCATGCCCCAA BamH1 flp_B-reverse CCGCTGTCAACAGCCCCATGCCCCAA BamH1 flp_B-forward TTAGGAGGAAGCACTGCCCCCATAGCC Psr1 dpaB dpsB_A-forward GGTCGGGAACGCGTTCCCCCCCTCAA BamH1 flp_B-reverse CCGCTGCAACAGCCCCCATGACCACCACCCCCAA BamH1 dpaB dpsB_A-forward GGTCGGGAAGGAACCAATTGTGCACCACACGCC Psr1 dpaB d		ahpC_A-reverse	CTAAAAATGGAGGTAATATCAACGGTGCTTTGATCAGCTA	
ahpC B-reverseGATTAGCCTTATGTCCGTGATCGTCAGTCGTTATTAAGAATCHind IIIohrRohrR A-ReverseCCATTATTTTGGGGGGGGGTAGTGCGTAGYind IIIohrR B-forwardTTAGGAGGAGGAGGAGTAGGAGTCAGGATGHind IIIsodsod_A-forwardCTGACTGCAGCGCCGCAAAATAGGGHind IIIsodsod_A-forwardCTGACTGCAGCGCCGAAAATGCGCCAGAATGPsr Isod_B-reverseAGTGACCAAACATGAGGAATGCAGCATCAGGTGHind IIIsod_B-reverseGGTTAAGCTTCCTGATGTTGGTCAGTHind IIIsuf_A-reverseGGTGAGCAAAGGTTGATTCCTCGATGTTGGTCAGTBamH1suf_A-reverseGGTGAGGCAAGTGTCCTGGTGAATTACCATCCCCAAAGGTGHind IIIsuf_A-reverseGTGAGGCAAAGTGTCCTGGTGACATGAGCAAAGGTGBamH1suf_A-reverseTTGTAGTCACCAGGACAATGTGCCTAAAATGTTGGCPsr Iflpflp_A-forwardTTTTAGGAGGGCAACGTTGCCGCGATGATGBamH1flp_A-reverseCCACCCTGGCAACGATCACCCAACGCTCAACCPsr Iflp_B-reverseTCGGCTGCAGGCCGATGACCCTCAACPsr IdpsBgBB_A-forwardGGTGCGAGGACGATGTCTCCCCTCTAABamH1dpsBgBB_A-forwardGCGGCTTAGAGGAAAGAACTCAAGCTTGAGAGCGHind IIIcydABdpsB_A-reverseGCGGCTTGCAAGGCCGAAGGACACGTGTGTGCAGCAGGGCGAAAGAFsr IcydABgBR-1_A-forwardGCTCGGAGGAGGAAGGACACCCAGCTGTGTGCAGCGGCAATFsr IgshR1gshR-1_A-forwardCCTCGGAGCCCGCGCGCGCGCGCGCGCGCGCGCGCGCGAATFsr IgshR1gshR-1_A-forwardCCTCGGAGCCCGCGCGCGCGCGCGCGCGCGCGCGGCGCG		ahpC_B-forward	TAGCTGATCAAAGCACCGTTGATATTACCTCCATTTTAG	
ohrR ohrR A-forward CAAGCTCCAGGAGCATGATGCCTAG Pst1 ohrR A-Reverse CCTATTTTTGGGGGGGCGCTAGATGCCTCAA ohrR B-Reverse CCACAAGCTTAGGAGCAGCGCCCAAAAATAGG ohrR B-Reverse GCACAAGCTTAAGGTTGATGGGAACAAGCAGCGCCCAAAAATC Pst1 sod A-forward CTGACTGCAGGCGGCAAAATCGGAGAAACC Pst1 sod A-forward CGACAGCAACATCGAGGAGAACACCTTG Sod A-forward GAAGGTTGATTCCTCGATGTTGGTCACT sod B-forward GATAGCTGGTAAATCCCATCCCAAAAGGTG Hind III suf A-forward AATAGGATCCTTGTGGTCCAAACACTGAGGAC BamH1 suf A-forward AATAGGATCCTGGGCAAACATGCTCAAAA BamH1 suf B-forward TTTTAAGTCACCAGGACACAGCCTCAAA BamH1 fip fh p.A-forward GGTCGGGTCCAAGCGTTGCCGAGGTGACCTCAA BamH1 fip. heverse GGCTGGCAGGCCCGAGGCCCTCAAC BamH1 dpsB B-forward GGTCGGTCGAGGCCCGAGGACCTCAACC BamH1 dpsB B-forward GGCCGGTAAGAGGTGACAAGCTTGTGGAAGGCGAGAGGC BamH1 dpsB B-forward GGCCGGCGCAGGAGAAGCATCAGGTTGTGGAAGGCGA BamH1 dpsB B-forward GGCGGTCGAGGCCCTAAGCTTACGGTACAGGT Pst1 gpsB B-forward GGCCGGAGGAGAAGCATTGTTGAAAGCTCAGGT Pst1 gpsB J-reverse GGCGGTGGCAACGGTGCCCTAATC BamH1 gpshR.1 pshorward CCCGGGTGGCCCGAGGAGAAGCA		ahpC_B-reverse	GATT <u>AAGCTT</u> ATGTCCGTGATCGTCCGTTTATTAAGAATC	Hind III
ohrR_A-ReverseCCTTATTTTGGGCGGCTGCTTCCTCAAohrR_B-ReverseGCACAAGGCTGACGCCCCAAAAATAAGGohrR_B-ReverseGCACAAGGTGACGGCGCCCAAAAATAAGGsodsod_A-forwardCTGACTGCAGCGCGCGAAATTGGCCAAAATCsod_A-reverseAGTGACCAAACGAGGAGAATCAAGCGAGGATCATTCCsod_B-ReverseGGTGACCAACGTGGTGGTCAATTGCCCCAAAAGGTGsod_B-reverseGGTGAGCATGTGGTGGTCAAGGATCAGGGACsuf_A-forwardAATAGGATCCTTGGTGTCCCCCAAAAGGTGsuf_B-reverseGGTGGCAGATGTCCTGGTTGACTTAAAAsuf_B-reverseGCACTGCGCAGCACTGCCCAAGCACCAGGACsuf_B-reverseGCACTGGCAGAATCATGCTGCAAAATGTTGGCflpflp_A-forwardGTTAGGAGGCAACGCTTGCCAACGCATTGCflpB-reverseCACCCTCGGCAAGCACCAGCCCTTAGflpB-reverseCCGCCTGCAGACCCTTGCCAACGCCCTCCAAflp_B-reverseCCGCGTGCAGGCCCGATGCCTTACGGTAGGCTGflp_B-reverseCGCGCTGCAGGAGCCCTCAACflp_B-reverseCGCGCTGCAGGAGCCCTATCGGTTACGGCAflp_B-reverseCGCGCTGCAGGAGAGAAGAACTCAAGCTGTGTGAAAGCGCGdpsB_B-forwardGCGGCTGCAGGAGAGAAGAACTCAAGCTGTTGACAGCGdpsB_B-reverseCGCGCTGCAGGAGAGAAGAACTCAAGGTGGAGAAGAcydAB_A-reverseTCTTTGCCCGCCGCTTGATCACCTTATCgshR1p-forwardATAGAGGGGACCATGGTTGACACCTGTgshR1A-reverseTCTTGGCGCCCCGCCGgshR1B-reverseTCTTGGCGCGCCGCGgshR1A-reverseTCTGGAGCCTTCACTATCATCATTGTCgshR1B-forwardATAGGAGGAGCCAATGGTTGATCCCTGgshR1B-reverseGCGCGCGCGGCGAAAGGCTAATGGTCCTTGTgshR1B-reverseGCGCCTCCACCTCA	ohrR	ohrR_A-forward	CAAG <u>CTGCAG</u> GAGCGATGATGCCTAG	Pst I
ohrR_B-forwardTTAGGAGGAAGCAGCCGCCCAAAAATAAGGsodohrR_B-ReverseGCACAAGCTTAAGGTTAGGATCAGGATGHind IIIsodsod_A-forwardCTGACTIGCAGCGGGAAATTGCGCAAAATCPst Isod_B-forwardGAAAGGTTGGATCCTGGAGGATCAACCTTTCsod_B-forwardAGTGAACAAACATCGAGGAATCAACCTTTCsod_B-reverseGGTTAAGCTTGGTGACTCAGGACCBamH1suf_A-forwardAATAGGATCCTTGGTGGTCCAAGCATCAGGACBamH1suf_A-forwardTTTGAAGCAAGAGTGCTGGTGGTTCAATAAAsuf_B-reverseGCATCGCCAAGAAGTGCCTGGTGGGTTAAATAAASuf_B-reverseGCATCGCCAAGAACGTGCCTAAAAsuf_B-forwardTTTTAAGTCAACCAGGACACTTTGGCTCCAABamH1flpflp_A-reverseCATCACTCGGCCAAGACGACCGCTGCCCAAABamH1flp_B-reverseCATCACTCGGCGCAAGCACGACCCCTCTAABamH1flp_B-reverseCGCGCGGGACCCAACGACCGTGCCCAAAACBamH1dpsBdpsB_A-reverseCGGCTGGGAACGCGTAACGGCCGAACGGCTGATGGCBamH1dpsBdpsB_A-reverseGGGTCGGAACGCCTAAGGACTCAAACCCTCTACCCCBamH1dpsBdpsB_A-reverseGCGGCGGCGAGAAGCAATCAGCCTGTGTGAACGCGCBamH1cydABeydAB_A-forwardGCGCGCAGGAGAAGCAATCAGCCTGTGTGAACGGCGSal1cydABeydAB_A-forwardCCTCGGGACGCACCACTCACTCTATeydAB_A-forwardATAGAGGGAGTCAAAGCGCGCGCAAAGAcydABB-reverseTAGAGGGGATCAAAGCGGCGGCGAAAAGAeydAB_B-reverseCAACGCGCCCCTGAACCCTCTCATgshR1gshR-1_A-reverseATAGAGGGAGTCAAAGGCGGCGAAAGASal1gshR2gshR-2_A-forwardATCAAGGAGGAGCCTAAGGCGCCGCAATSal1gshR2gshR-2_A-revers		ohrR_A-Reverse	CCTTATTTTTGGGCGGCTGCTTCCTCCTAA	
ohrR_B-ReverseGCACAAGCTTAAGGTTGATGGATCAGGATCHind IIIsodsod_A-forwardGCACAAGCTGCAAATTGCGCAAAATCPst Isod_A-reverseAGTGACCAAACATCGAGGAATCAACCTTTCsod_A-reverseGGTAAGGTCGTGTGTGATCACCTTCsod_B-reverseGGTTAAGCTTGGTCAAGCATCCCCCAAAAGGTGHind IIIsuf_A-forwardAATAGGATCCTTGGTCCAAGCATCAGGACBamH1suf_A-reverseTTGGAGGCAAGTGTCTGGTGTCAAGCATCAGGACBamH1suf_B-reverseGCATCGCAACAGGACCCTTGCCCAASaf B-reverseflpflp_A-forwardGCTAGGATCCACGAGCACCTGCCCAABamH1flp_B-reverseCATCACCGGCAACCGTGCCCAAGCACCGTGCCCAABamH1flp_B-reverseCCATCACCGGCACCGTTGCCCAGTGATGBamH1flp_B-frewerseTTAGGAGGTGGCAACGGTTGCCGAGTGAGGBamH1dpsBdpsB_A-forwardGGTCGGGATCCAATGCCTTAAGGTTAGGCACBamH1dpsBdpsB_A-forwardGGCGGTAAGAGGAAGAACTCAAGCTGTGTGACGAGAGGdpsB_B-forwardcydABgesdA-reverseCCTGTGTCCCCAATCACTACCATTCBamH1cydABeydAB_A-reverseCCTGTTTCCCCCTAACATCACTTCBamH1cydABgshR-1A-forwardCCAGGCTGAACACCTGGCGAAAGCAFst IgshR1gshR-1B-forwardATAGGGGCGTAAAGCGGCGGAAACGGCGAAAGGASal IgshR1gshR-1B-forwardCAATGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC		ohrR_B-forward	TTAGGAGGAAGCAGCCGCCCAAAAATAAGG	
sod sod_A-forward CTGACTGCAGCGCTGAAATTGCGCAAAATC Pst1 sod_A-reverse AGTGACCAAACATCGAGGAATCAACCTTTC sod_A-reverse GGTGAACCATCACATCGCATGTTTGGTCACT sod_B-reverse GGTTAAGCTTGGTGACCTCCCCAAAGGTG Hind III suf_A-forward AATAGGATCCTTGGTGGTCCAAGGATCAAGGTG BamH1 suf_A-reverse TGGAGGCAAGTGTCCTGGTGGCACTAAAA BamH1 suf_B-reverse GCATCTTGCCAGGACACTTGCCCAAA BamH1 flp Mp_A-reverse CCATCGTCGCAGAACCATTGCCCCAAA BamH1 flp A-forward GCTAGGATCCCCAAGCACATTGG Pst1 flp_A-reverse CATCACTCGGCAACGACGTTGCCCACAA BamH1 flp_B-forward TTAGGAGGTGGCAACGGTTGCCAGGACACGTCCTAAA BamH1 dpsB_A-reverse CGCGCTGCAGGACCAATGGCTCCAAGAC Pst1 dpsB_A-reverse CGCGCTGCAGGACCAATGGTTGACGACA BamH1 dpsB_B-reverse CGCGCTGCAGGAAAGACTCAAAGCTTGATGACAGAGGG HamH1 cydAB eydAB_A-reverse CGCGCTGCAGGAAAGACCTAAGCTTGTGGAACAGGG Pst1 cydAB eydAB_A-reverse CCTCGCGAGACACACGTGCAAAGAGA EydAB cydAB eydAB_A-reverse TGAACGGCCTGAACACGTGCAAAAGA EydAB cydAB_B-reverse TAGACGGACCAATGAGGCCGCAAAAGA EydAB gshR1 gshR-1_A-reverse TAGACGGCGCGCAAACGGCGCGCAAAAGAA		ohrR_B-Reverse	GCAC <u>AAGCTT</u> AAGGTTGATGGATCAGGATG	Hind III
sod_A-reverse AGTGACCAAACATGAGAGAATCAACCTTC sod_B-forward GAAGGTGATCCCTCGATGTTGGCACCT sod_B-reverse GGTTAAGCTTGTGATGACTCCCCAAAAGGTG Hind III suf_a-reverse TTGGAGCAAGTGCCTGGTGACTTAAAAA suf_A-reverse GCATCTGCAAGGATCAGGACCACTTGCCCCAA suf_B-forward GCTAGGAACGTGCCGGGCACCGTCCCAA flp_h-a-reverse GCATCTGCCAAGGACCCATTTG BanH1 flp_A-reverse GCATCTGCGCAACGATCAGGACCCATTTG BanH1 flp_A-reverse GCATCTGCGCAACGATCAGGACCCATTTG BanH1 flp_A-reverse GCATCTGCGCAACGGTGCCGAGTGACT flp_B-reverse TCGGCTGCCAAGGACCGTTGCCCACCTCCTAA flp_B-reverse TCGGCTGCCAGGCCCGATGACCCACTGC dpsB_A-freverse CGCTCTGTCAACCAGGACCCATTGGCACCGC dpsB_A-freverse CGCTCTGTCAACAAGCTTGAGTACGGCA BanH1 dpsB_A-reverse CGCTCTGTCAACAAGCCTGAGGCAGTGGCAAGGCG dpsB_B-forward GCGGTGCAGGAGCAATCGTGTGACAGGCGG dpsB_B-forward CCCGGTGCAGGCCCGATGACCCATTC cydAB_A-forward CCCGGGTGCCGAGGCCATCATTG BAAGTCAGGCG dpsB_B-forward CCCGGGTGCACGGCCGATGAGTCTTCCCCTCTAACGGCA dpsB_B-forward CCCGGGTGCACGGCCGCGCGCAAAGGCAGTCGTTGACAGGCG cydAB_B-reverse TCTTTTCGCCGCCCGCTTTGATCACGCCG gshR-1_A-forward CAAGGAGGACGCTGCGTGAACGAGCAT gshR-1_B-forward ATAGAGGTGCAACGGTGCGAAAGAA cydAB_B-reverse TCTTTTCGCCGCCGCTTGATCCCCGG gshR-1_A-forward CCACGGAGACCATGGTTGTAGCGGCGAAATGA gshR-1_B-forward ATAGAGGTGCAAAGCGTGCGAAAGA fgrA-reverse TAGACTGCAACCGTGCATCACTG gshR-1_B-forward ATGGAGGCGCAACCTTGATCCCGG gshR-1_B-forward ATGGAGGCGCAATTGTGTGCCCGGCGAAT gshR-1_B-forward ATGCGCGCCAATTAGGTGTCACCTCCTTGT gshR-1_B-forward ATGCGCGCACCATGACCGTGCAATGATGATC gshR-2_B-forward ATGCGCGCACCATGAGGCGCAAT fipr_A-reverse GCCGG <u>GCGAACGAATGATGATC</u> gshR-2_B-forward ACAGGGGCGAACGATAAGGTGCTAATGATCC gshR-2_B-forward ACAGGGCGAACGATAAGGTGCTAATGATCC fipr_A-reverse GCCGG <u>GCGAACGAATTAAGTGCCTCCTTGT</u> fipr_A-reverse GCCGG <u>CCAACGAATTAAGTGCCTCCCTCAA</u> fipr_A-reverse GCCG <u>GCCGAACGAATTAAGTGCCTCCCTCAA</u> fipr_A-reverse GCCG <u>CCAACGACTTACGGCCCCCAAA</u> fipr_A-reverse GCCC <u>AAGGAACGAACGATAAGGTTGCCCCTCAAA</u> fipr_A-reverse ATTAGGAGGACAATTTACCTTTAAAGGCCCCCAAAGGACAATTGATCCCTCAACGACCTCAACGACCTTCAACGACCTCCAACGACTTACGACCAACGACTTACCTCCTCAACGACTTACGACCT	sod	sod_A-forward	CTGA <u>CTGCAG</u> CGCTGAAATTGCGCAAAATC	Pst I
sod_B-forward GAAAGGTTGATTCCTCGATGTTGGTCACT sod_B-reverse GGTT <u>AAGCTTGGTCAATAACATCCCCCAAAAAGGTG Hind</u> III suf_A-forward ATA <u>GGATCCTTGTGGTCCAACCATCAGGAC BamH</u> 1 suf_A-reverse TTGGAGGCAAGTGTCCTGGTTGACTTAAAA suf_B-forward TTTTAAGTCAACCAGGACACTTGCCTCCAAC flp_A-forward GCTA <u>GGATCCCAAGCACAGCACCATTTGG</u> BamH1 flp_A-forward GCTA <u>GGATCCCAAGCACCGGTGCCCACCATTTG</u> BamH1 flp_B-forward GCT <u>AGGATCCCAAGCACCGGTGCCCACCTCTAA</u> flp_B-reverse CATCACTCGGCAACCGTTGCCCCACCTCTAA flp_B-reverse TCGC <u>GTGCAGGCCAACGGTGCCCACCCG</u> gbs_B_A-forward GGT <u>GGATCCAATGCTAACAGCTACGCCA</u> BamH1 dpsB_A-forward GGT <u>GGATCCAATGCTTACGGCAACGGCTGCAGCC</u> dpsB_B-reverse CGCCTGTCAACAAGCTTGGTGACGCCA gbs_B-reverse GCGC <u>GTGCAGGACCCAATGCCTTAACGGCA</u> BamH1 cydAB_A-forward GGT <u>GGATCCCAATGCCTTACGGCA</u> BamH1 cydAB_A-forward GCGCTTAAGAGGAAGAACTCAAGCTTGTTGACAGAGCG dpsB_B-reverse GCGC <u>GTGCAGGAGAAGCAATTGTTGAAAGTCAGT</u> Pst 1 cydAB_A-forward CCT <u>GGATCCCCTACCATCACCTCTT</u> cydAB_A-forward ATAGAGGGAGAAGAACTCAAGCTTGTTGACAGCAGCC gshR1 eydAB_A-forward ATAGAGGGGATCAAAGCGGGGGGGAAAAGA cydAB_B-forward ATAGAGGTGATCCAACCTTGCTCTT cydAB_B-forward ATAGAGGTGATCCAACGCTGCCTATC gshR1_B-forward ATAGAGGGGATCCAATGCTTGTTGACCGCCGCG gshR1 gshR-1_A-reverse TAGAC <u>TTGCAGCGCCTCGTTGCCCCCTGT</u> gshR1_B-forward ATAGAGGGCGTCCGGTTCGGTCCCCTGT gshR2_B-forward ATGCGGCTCGCTCGGTCCCCTGTG gshR2_B-forward ATGCGGCTCGCTCGTTCGTCCTGT gshR2_A-reverse CACAGGAGACGAAGAAGGCGTAATGGTCTCCTCTTGT gshR2_B-reverse GCGG <u>GGTCGACCCCTTCGTCCTTGT</u> gshR2_A-reverse CACAGGAGACGAAGAAGGCGTAATTGATAC ps_B-reverse CCGC <u>AGGCCGCCCCCCCCCCCCCCCCCCCCCCCCCCCC</u>		sod_A-reverse	AGTGACCAAACATCGAGGAATCAACCTTTC	
suf and bereverse GGTTAAGCTTGGTAAATTACCATCCCCAAAGGTG Hind III suf A-forward AATAGGATCCTTGTGGTCCAAGCATCAGGAC BanHI suf A-reverse TTGGAGGCAAGTGTCCTGGTTGACTTAAAA suf B-reverse GCATCTGCAGAATCATGCCCAAGCACTTGCCCAA suf B-reverse GCATCGCAGGATCATGCTGACTAAAATGTTGGC Pst I fip. A-reverse CATCACTCGGCAAGCACGTGCCCAAGCACCAGTACCAGACCAGTG fip.B-forward TTAGGAGGTGCCAAGCCCATTTG BanHI fip.B-forward TTAGGAGGTGCCAAGCACGGTGCCGAGTGATG fip.B-forward GGTCGGAGCCCAGTGCCGAGTGACTAACGAGAGCG dpsB_A-reverse CGCGCTGCCAGTGCCCAACCAGTCCCTCAAC BanHI dpsB_A-reverse CGCGCTGCCAACGGTTGCCGAGTGCCAAGGCG dpsB_A-reverse GGCGCTGCCAAGGAGAAGAAGCACTCAAGGCTGTGACGAGGG dpsB_B-reverse GGCGCTGCCAAGGAGAAGAAGAACTCAAGGCTGTGACAGAGG dpsB_B-reverse GGCGCTGCCAAGGCAGAGAAGAAGAACTCAAGGTTGTGACAGAGG dpsB_B-reverse GCGGGCTGCAAGGAGAAGAAGAACTCAAGGCTGTGTGACAGAGG dpsB_B-reverse GCGGGCTGCAAGGAGAAGAAGAACTCAAGGCTGTGTGACAGGGG dpsB_B-reverse TCTTTCGCCGCCCCTATCATCC BanHI cydAB_A-reverse TCTTTCGCCGCCGCCTTGATCAGGT Pst I gshR1_A-forward CAAGGGGGTGATCAAAGGGGGCGAAAAGA cydAB_B-reverse TAGACTGCAGGCTGAACACGTGCATACTG Pst I gshR1_B-forward CAATGCGGCTAAGGGTGATACCGGGCAAT gshR2 gshR1_B-forward CAAGGGGTCCAATGGTGTACGCGCCAAT gshR2 gshR2_A-reverse GTGTGAAGCGCTCGATATCCGG BanHI gshR2_A-reverse GTGGAGCCCCAATCAGGTGTATCAGGGCAAT gshR2 B-forward AACGGAGTCCAATGAGGGTCATATCATCC gshR2 B-forward AACGGAGTCCCAATGAGGTGTATACGGGCCAAT gshR2 B-forward AACGGAGTCCCAATGAGGTGTATACGGCGCCAAT gshR2 B-forward AACAGGAGTCCCAATGAGGTCATAGCGCCAAT gshR2 B-forward AACAGGAGTCCCAATAAGGTGCTAAGGGGCAAT gshR2 B-forward AACAGGAGTCCCAATAAGGTGCTGCCC BanHI gshR2 B-forward AACAGGAGTCCCAATGAGGTCCAAGG gshR2 B-forward AACAGGAGTCCCAATAAGGTGCTGCC Pst I gshR2 B-forward AACAGGAGTCCCAATGAGGTCAAAGCGCGCGCGAATGCATCCCCAAA fip_B-forward AACAGGAGCCAATGCGTCCCAAA fip_B-forward ACAAGGGAGCCAATGCCTCCAAA fip_B-forward ACAAGGAGCAATGCCTCCAACG fif fip_B-forward ACAAGGAGCAATTCCTTCGGTGCCG BanHI fip_B-forward ACAAGGAGCAATTACGGTCCCCAAA fif fip_B-forward ACAAGGAGTCCAATCCCTCAAA fip_B-forward ACAAGGAGTCCAATCCCTCAAA fip_B-forward ACAAGGAGCAATTACGGTCCACAGCTCCAAA fip_B-forward ACAAG		sod_B-forward	GAAAGGTTGATTCCTCGATGTTTGGTCACT	
suf suf_A-reverse TTGGAGGCAAGTGTGCTGGTGACATCAGGAC BamH1 suf_B-reverse GCATCTGCCAGGAAGTGTGACTTAAAA suf_B-reverse GCATCTGCACAGGACCTTGGCTCCAA suf_B-reverse GCATCGCAAGCACAGACCATGGCTAAAATGTTGGC Pst I flp flp_A-forward GCTAGGATCCCAAGCACAGACCATTTTG BamH1 flp_B-reverse CATCACTCGGCAACCGTTGCCAACCATTTTG BamH1 dpsB flp_B-forward TTAGGAGGTGGCAACGGTTGCCCACTCTCAA Pst I dpsB dpsB_A-forward GCGGCTGCAAGGCCATGCCTACGGTAAGGCACCTCAAC Pst I dpsB dpsB_A-forward GCGGTGCAAGGAGAAGCAATTGTTGTGAACAGAGGCG dpsB_Areverse cydAB eydAB_A-reverse GCGGCTGCAAGGAGAAGCAATTGTTGTGAACAGAGGCG dpsB_Areverse cydAB eydAB_A-reverse GCGGCTGCAAGGCCGCATCACTATCATTC BamH1 cydAB eydAB_A-reverse TGCTTTTCGCCGCCGCGTTGATCACTCTCTT Pst I cydAB B-forward ATAGAGTGATCAAAGGGGGCGCGAAAAGA Pst I cydAB B-forward ATAGAGGAGATCAATGGTTGATCACCTCTGT Pst I gshR1 gshR-1_A-reverse TGTAGACGCTTCGGTACACGGCGCGAAAAGA Sal I gshR1 gshR-1_A-reverse ATGCAGGCGCACACGTTGGGCGCGAAAGGAAGAATTGATAC Pst I gshR2 gshR-1_B-reverse GTTGAACACAGGAGGAGAAGGAAGGAAGGAAGGAAGGAATTGATAC gshR-1 </td <td></td> <td>sod_B-reverse</td> <td>GGTT<u>AAGCTT</u>GGTAAATTACCATCCCCAAAAGGTG</td> <td>Hind III</td>		sod_B-reverse	GGTT <u>AAGCTT</u> GGTAAATTACCATCCCCAAAAGGTG	Hind III
suf_A-reverse TIGGAGGCAAGTGTCCTGGTTGACTTAAAAA suf_B-forward TTTTAAGTCAACCAGGACACTTGCCTCAAA auf_B-reverse GCATC <u>TGCAGA</u> AATCATGCTGAAAATGTTGGC <i>Pst</i> 1 fip fip fip fip fip fip A-forward GCTA <u>GGATCCC</u> AAGCACAGACCCATTTTG BamH 1 fip_B-forward TTAGGAGGTGGCAACCGTTGCCACCTCCTAA fip_B-reverse TCGG <u>CTGCCAGGCCCGATGACCCCTAAC Pst</u> 1 dpsB dpsB_A-forward GGTC <u>GGATCCCAATGCCTTACGGTTACGGCA</u> BamH 1 dpsB_b-reverse CGCTCTGTCAACAAGCTTGACTCATCCCTTAACGC dpsB_b-forward GCGGTTAAGAGGAGAAGAACTCAAGCTTGTTGACAGAGGGG dpsB_b-reverse GCGG <u>CTGCCGGAGGACCCCATTCCCCTTACGGCTGCAAGAGGAGAAGAACTCAAGCTGTGTGACAGAGAGG</u> cydAB_A-reverse TCTTTTCGCCCGCGCTTCACCATTCTC BamH 1 eydAB_A-reverse TCTTTTCGCCCGCGCCTTCGGCAAGAGAAGAACT cydAB_b-forward CCTC <u>GGATCCCCCTACTCACTATCATTC BamH 1 eydAB_A-reverse TAGACTGCAGCCCGATGACCCCCTACTCACTTCT gshR-1_A-reverse TAGACTGCAGCGCGGCGGCGCAATAGG gshR-1_A-reverse ACAAGGAGGATCAATGGTTGTAGCGGCAAT gshR-1_A-reverse ACAAGGAGGATCAATGGTTGTAGCGGCAAT gshR-1_A-reverse CCCGGCTTCGGTATCCCGG Hind III gshR-2_A-reverse CACAGGAGCGAGCAGCAGCATTCCTCTGTG gshR-2_A-reverse CCCGGCTACTCACTTCTCTGTGT gshR-2_A-reverse CCCGGCTCCCATTAAGGTCATATCATCCC BamH 1 ipr_A-forward CCGACTGCAACGCGCGCAATAGC pshR-2_A-reverse CCCGGGTCATCACGCCTCACTTCATCCC BamH 1 ipr_A-forward CCGACTGCACACTTCGTTCGTGT gshR-2_B-reverse GCGGGTCGAACACGTTCCTCTTGTGT ipr_A-reverse GCGCGCTAATTAAGGTCATACGTCTCTGTGT ipr_A-reverse GCCGGCTAACGGTAAAGGGCCTAATGCCCCAATTAAGGTCATACCGCTTCGTCTGTGT ipr_A-reverse GCCGCCAATTAAGGTCATACCGTTCCCCAAA ipr_B-reverse GCCGCCAATTAACGCTTCCTCCTCGTGAT ipr_B-reverse GCCCGCATTAGGATAAGTTCCTTCACGCCCCAATTAAGGACACTTATCGGTACGCCTCCAAA ipr_B-reverse GCCCGCTAATTCCGCCCCCAAA ipr_B-reverse GCCGCCTAATTCAGGCTCCCTCAAG ipr_B-reverse GCCCGCTAATTGGTTACGCCTCCAAA ipr_B-reverse GCCCGCATTCACGGCCTAAGCCTTCAGG BamH 1 ipr_A-reverse GCCCGCATTAGGATAAGTTCCTTCAACG ipr_B-reverse GCCCGCATTCGCTCCTAAT ipr_B-reverse GCCCGCAATTCCCTCAAA ipr_B-reverse GCCCGCATTCCCTCAATTCCTCAACGCCTCCAAA ipr_B-reverse GCCCGCAATTCCCTCAATTCCTCCAAATTCCTTCAAAGCAACTTTATTCGTACGCCTCCAAATTCCCTCAATT ipr_B-reverse GATACTGCAATTGCTTCTCAAGGTACAGCTTACCCCTCAATT ipr_B</u>	suf	suf_A-forward	AATA <u>GGATCC</u> TTGTGGTCCAAGCATCAGGAC	BamH I
suf_B-forward TITIAAGTCAACCAGGAACCTIGCCTCCAA suf_B-reverse GCATCTGCAAGAATCATGCTAAAATGTTGGC Psr1 flp A-forward GCTAAGGATCCCAAGCACAGACCCATTTG BamH1 flp_B-reverse CATCACTCGGCAACCGTTGCCCACTCCTAA Imp Broward TTAGGAGGTGCCAACCGTTGCCCACTCCTAA dpsB dpsB_A-forward GGTCGGATCCCAATGCCTTACGGTACGCAC BamH1 dpsB dpsB_A-forward GGTCGGATCCAATGCCTTACGGTACGGCA BamH1 dpsB A-forward GGTCGGATGCAATGCTTAAGAGTCAAGGCAGAGGCG BamH1 dpsB A-reverse CGCGCTGCAGGAGAAGAAGTTGTTGACAGGAGGGGGGGGG		suf_A-reverse	TTGGAGGCAAGTGTCCTGGTTGACTTAAAA	
		suf_B-forward	TTITTAAGTCAACCAGGACACTTGCCTCCAA	D 1
<i>ipp</i> np_A-forwardGCIAGGATCCCAAGCACAGACCCTTTIGBamH Ifp_A-reverseCATCACTCGGCAACCGTTGCCAACCGTTGCCAACFitfp_B-reverseTTAGGAGGTGGCAACCGTTGCCGAGTGATGFit <i>dpsB_dpsB_A-forward</i> GGTC <u>GGATCCAATGCCTTACGGCTACGGCA</u> BamH I <i>dpsB_b-crverse</i> CGCTCTGTCAACAAGCTTGAGGTCTTCTCCTCTAACCGCJamH I <i>dpsB_b-crverse</i> GCGGTTAAGAGAGAAGAACTCAAGCTTGTTGAACAGAGCGJamH I <i>cydAB_b-forward</i> GCGG <u>TCGCAGG</u> GAGAAGAACTCAAGCTGATGTTGACAGAGCGJamH I <i>cydAB_b-forward</i> CCTC <u>GGATCCCCTACTCACTATCCTTTGCACAGAGCGCGCGAAAAGAAPst I<i>cydAB_b-forward</i>CCTC<u>GGATCCCCTGACTACACCTCTAT</u><i>samH IcydAB_b-forward</i>ATAGAGGGGATCAAAGGGCGGCGAAAAGAAPst I<i>gshR1gshR-1_A-forward</i>CAAT<u>GTGCAGCCTGGAACACGTGCGGCGAAAAGAA</u><i>sal IgshR2gshR-1_A-reverse</i>ACAAGGAGGATCAATGGTTGTAGCGGCCAAT<i>sal IgshR2gshR-1_A-reverse</i>ACAAGGAGGATCAATGGTCGTCCTTGT<i>gshR IgshR2gshR-2_A-forward</i>AACC<u>AGGACCCAATTAAGGCCTAATGATCCCCGGGGCGAAATGAGGGCTAATTGATCCCCGGshc2_A-reverse</u><i>Sal IgshR2_A-forward</i>AACC<u>AGGACCCCAATTAAGGCCTAATGATCCCCGGGCCGCGGCGGCGGCGGCGGCGGCGGCGCCGCCG</u></u>	a	suf_B-reverse	GCAT <u>CTGCAG</u> AATCATGCTAAAATGTTGGC	Pst I
	flp	flp_A-forward	GCTA <u>GGATCC</u> CAAGCACAGACCCATTTTG	BamH I
		np_A-reverse		
$ \begin{array}{c} \mbox{lp} B-reverse & \mbox{lc} GGTCGATGCCCGATGACCCGATGACCCGAATGCCCCAATGCGCTAACGGCA & BamH1 \\ \mbox{dpsB} A-forward & \mbox{GC}GGTTCAACAAGGCTGAGGTCTTCTCCTCTTAACGGC & \\ \mbox{dpsB} B-forward & \mbox{GC}GGTTAAGAGGAGAAGAACTCAAGGCTGGTGACAGAGCG & \\ \mbox{dpsB} B-reverse & \mbox{GC}GGCTGCAGGAGAAGAACTCAAGGCTAGGT & Pst 1 \\ \mbox{eydAB} A-forward & \mbox{CCC}GGGGCGCAAAAGCAATTGTTGAAAGTCAGT & \\ \mbox{eydAB} B-forward & \mbox{CCC}GCGCGCTTGGATCACTCTCATT & \\ \mbox{eydAB} B-forward & \mbox{CCC}GCGGCTGACACGGGCGGCGAAAAGA & \\ \mbox{eydAB} B-reverse & TAGACTGCAGCGCGGCGGCGAAAAGA & \\ \mbox{eydAB} B-reverse & TAGACTGCAGCCTGAACACGTGGATACTG & \\ \mbox{eydAB} B-reverse & TAGACTGCAGCCTGGATACCCGG & \\ \mbox{gshR-1} B-reverse & ACAAGGAGGATCAATGGTGTAGCGGCAAT & \\ \mbox{gshR-1} B-reverse & ACAAGGAGGATCCAATGGTGTAGCGGCAAT & \\ \mbox{gshR-2} & \mbox{gshR-2} B-forward & \mbox{ACAGGAGCCCAATGAGCCCTCCTTGT & \\ \mbox{gshR-2} & \mbox{gshR-2} & \mbox{GCGGGTGGACGCAAGGAAGGCGTAATTGATAC & \\ \mbox{gshR-2} & \mbox{gshR-2} & \mbox{GCGGGTGGACGCATCAGGCCTTCCTTGTGT & \\ \mbox{gshR-2} & \mbox{gshR-2} & \mbox{GCGGGTGGACGCATCACGGCGTAATTGATAC & \\ \mbox{gshR-2} & \mbox{gshR-2} & \mbox{GCGGGTGGACGCATCACGGCTTCCTTGTGT & \\ \mbox{gshR-2} & \mbox{gshR-2} & \mbox{GCGGGTGAACGAAGGAAGGCGTAATTGATAC & \\ \mbox{gshR-2} & \mbox{forward} & \mbox{GCGGGTGGACGCATCACGGCTCCAAA & \\ \mbox{gshR-2} & \mbox{forward} & \mbox{GCGGGTGAACGATTACGGCTCCCAAA & \\ \mbox{gshR-2} & \mbox{forward} & \mbox{GCGGCGTAACGGATCACGGTTGAT & \\ \mbox{gshR-2} & \mbox{forward} & \mbox{GCGGGTGAACGATTACGGCCTCCAAA & \\ \mbox{gshR-2} & \mbox{forward} & \mbox{ACAGGAGCGTAACGGTTGATGAGC} & \\ \mbox{fign } & \mbox{fign } & \mbox{fign } & \\\ \mbox{fign } & \mbox{fign } & \mbox{fign } & \\\ fign $		np_B-forward		D / I
dpsBdpsB_A-reverseCGTCUGALCLAATGCCTTACGCCABamH 1dpsB_A-reverseCGCTGTCAACAAGCTTGAGTTCTTCTCCTCTTAACCGCdpsB_B-forwardGCGGTTAAGAGGAGAAGAACTCAAGCTTGAGAGCAGGcydABeydAB_A-forwardCCTC <u>GGATCC</u> CCTACTCACTATCATTCBamH 1cydABeydAB_A-forwardCCTC <u>GGATCC</u> CCTACTCACTATCATTCBamH 1cydABeydAB_A-reverseTCTTTTCGCCGCCGCTTGATCACCTCTATFst IcydAB_B-reverseTCTTTTCGCCGCCGCGCTTGATCACCTCTATeydAB_B-reverseTAGACTGCAGCCTGAACACGTGCATACTGgshR1gshR-1_A-forwardCAATGCCAGCGCTGAACACGTGCATACTGPst IgshR1gshR-1_A-reverseACAAGGAGGATCAATGGTTGTAGCGGCAAATgshR-1gshR1gshR-1_A-reverseGTTGAAGCTTTGATCGGCGCGCGHind IIIgshR2gshR-1_B-reverseGTTGAAGCTTTGATCGGCGCGCGCGBamH 1gshR2gshR-2_A-forwardAACAGGAGTCCCAATTAAGGTCATATCATCCCBamH 1gshR2gshR-2_A-reverseGCAGGAGCAAGGAAGGCAGGCGCGCGHind IIIgshR2gshR-2_B-reverseGCAGGGCGCACACGCGCTCAAGGSal 1ipripr_A-forwardGTATCAATTACGCCTCCTTGTGTCTGTGTFst Iipripr_A-forwardGCGCCAAGCGTATCACGGCCTCAAGSal 1ipripr_A-forwardCCGACTGCAGCTTATCATCGCTCCAAAFst Iipr_B-reverseGGCCAAGCTTATGAGGACCATGCCTCCAAAipr_B-reverseipr_B-forwardCCAAGGCACTTATCGTCGGAGCATCCCCAAAHind IIInpr_A-forwardCCAAGGCTCATGGAGCATCCCTCGAAABamH 1npr_A-forwardCCAAGGCTCATGGAGCATACGCTCCCAAAAipr IIInpr_B-reverseGGCCAAGCTTA	de a D	пр_B-reverse		Pst I
dpsB_A-reverseCGCGTGTAAGAGGAGAAGAACTCAAGCTTGTTGAAGGGCGdpsB_b-forwardGCGGTTAAGAGGAGAAGCAATTGTTGAAAGCTGAGAGGCGcydABeydAB_A-forwardCCTC <u>GGATCCCCTACTCACTATCATTC</u> gydAB_A-reverseTCTTTTCGCCGCGCGCTTTGATCACTCTCATcydAB_B-forwardATAGAGGTGATCAAAGCGGCGGCGAAAAGAcydAB_B-reverseTAGACTGCAGCCTGAACACGTGCATACTGgshR-1gshR-1_A-forwardCAATGTCGACGCTTCGGTATCCCGGgshR-1gshR-1_A-forwardCAATGTCGACGCTTCGGTATCCCGGgshR-1B-forwardATTGCCGCTACAACCATTGATCCCGGgshR-1B-reverseACAAGGAGGATCAATGGTTGTAGCGGCAATgshR-1B-forwardATTGCCGCTACAACCATTGATCCCGGgshR-1B-forwardATTGCCGCTACAACCATTGATCCCGGGgshR-2A-forwardAACAGGATCCCAATTAAGGTCATATCATCCCgshR2gshR-2A-forwardgshR2GATCAATTACGCCTCCTTCGTCTTGTGgshR2gshR-2A-forwardgshR2GCGGGTCGACGCATCACGGCCTAAGgshR2B-forwardGTATCAATTACGCCTTCCTTCGTCTGTGgshR2B-forwardCCGACTGCAGCGTAACGGCCTAAGgshR2B-forwardCCGACTGCAGCGTAACGGCCTAAGgshR2B-forwardCCGACGCGCATCACGGCCTAAGgshR2B-forwardCCGACTGCAGCGTAACGGTACACGTTGCCgshR2B-forwardCCGACGCGCACCTTATGCGTCCCCCCAAAiprA-forwardCCGACGCGCGACCACGGACACGCTTCGCipripr_B-forwardCCGACGCGCGACACGACTTATCCCTCCAAAiprnpr_A-forwardCAATGGAGGACCATTATCCTTCCAAAiprB-forwardCATGGAACCTTTATGCTTAACGCCTCCCTAATipr <td>apsв</td> <td>dpsB_A-forward</td> <td></td> <td>BamH 1</td>	apsв	dpsB_A-forward		BamH 1
dpsB_B-lorwardGCGGTTAAGAGGAGAAGCAACTCAAGCTAGTPst IcydABdpsB_B-reverseGCGGCTGCAGGAGAAGCAACTCAAGCTAGTPst IcydAB_A-reverseTCTTTTCGCCGCCCCACTCACTACCACTCATcydAB_B-reverseTCTTTTCGCCGCGCCGCTTTGATCACCTCATcydAB_B-forwardATAGAGGTGATCAAAGCGGCGGCGAAAAGAcydAB_B-reverseTAGACTGCAGCCTGAACACGTGCATACTGPst IgshR1gshR-1_A-forwardCAATGTCGACGCTTCGGTATCCCGGSal IgshR-1gshR-1_A-reverseACAAGGAGGATCAATGGTTGACGCGCAATgshR1gshR-1_B-forwardATTGCCGCTACAACCATTGATCCTCGTgshR-1_B-reverseGTTGAAGCCCCAACCATTGATCCTCCTGTgshR2gshR-2_A-forwardAACAGGAGCTTTGATCGGCGGCGCGHind IIIgshR2gshR-2_B-forwardACAAGGAAGCAATCACGGCCTAATGATCACCCCBamH IgshR2gshR-2_B-reverseCACAGGAGCGAACGAAGGAAGGCGTAATTGATACpst Igrbripr_A-forwardGTATCAATTACGCCTTCCTTCGTCTCTGTGgshR-1ipripr_A-forwardCCGACTGCAGCGATAAGGTACGCTCAAGFst Iipripr_A-forwardCCGACTGCAGCGTAACGGTCAAGCTTCGATpst Iipripr_B-forwardATCAAGCAACTTTATCGTTACGCCTCCAAABamH Iipr_B-forwardCAATGGAGCGTAACGAGAGTTGCCTTCGATipr_B-reverseHind IIInprnpr_A-forwardCAATGGAGCAACTTTATCGTTACGCCTCCAAABamH Iipr_B-forwardATCAAGCAACTTTATCGTTACGCCTCCAAABamH Inpr_B-forwardCAATGGAGGACAACGAGAGCTCCTCCAAABamH Inpr_B-forwardCAATGGAGGACCACTTTATCGTTCAAGCCTCCCAAApst Inpr_B-forwardGTCTTTTTAAAGGAGAAGTTCCCTCCAAATTAAGACApst I		dpsB_A-reverse		
cydABcydAB_A-forwardCCTCGGATCCCCTACTCAATGATGAAGGAAGGAAGGAAGG		dpsB_B-forward		Det I
cydABcydAB_A-reverseTCTTTCGCCGCCGCTTGCACACCTCTATcydAB_A-reverseTCTTTTCGCCGCCGCCGCTTGATCACCTCTATcydAB_B-forwardATAGAGGTGATCAAAGCGGCGGAAAAGAcydAB_B-reverseTAGACTGCAGCCTGAACACGTGCATACTGgshR1gshR-1_A-forwardCAATGTCGAGCGTTCGGTATCCCGGgshR-1_B-forwardACAAGGAGGATCAATGGTTGTAGCGGCCAATgshR-1_B-reverseGTTGAAGCTTTGATCGGCGCGCGCGgshR-1_B-reverseGTTGAAGCTTTGATCGGCGGCGCGCgshR-2_A-forwardAACAGGAGTCCCAATTAAGGTCATATCATCCCgshR-2_B-forwardGTATCAATTACGCCTTCCTTCGTCTGTGgshR-2_B-forwardGTATCAATTACGCCTTCCTTCGTCTGTGgshR-2_B-forwardGTATCAATTACGCCTTCCTTCGTCTGTGgshR-2_B-forwardCCGACTGCAGCGCATCACGGCCTAAGjpripr_A-forwardCCGACTGCAGCGTATATCAGTTGCipr_A-reverseGTGAGGCGTAACGATAACGTTGCTipr_B-forwardATCAAGCAACTTTATCGTTACGCTCCAAAipr_B-reverseGGCCAGCCTACAGCGACAAGACGCTTCAGipr_B-reverseGGCCAAGCTTAGGAGGAACAGCTTCCCCnpr_A-reverseAATTGGAGGAGAACAACTTTATCGTTACGCCTCCAAAipr_B-reverseGGCCAAGCTTAGGAGGAAAAGTTGCCTTCAGnpr_A-reverseAATTGGAGGAGAATTCCCTTCAGmpr_A-reverseAATTGGAGGAGATTTACTTTTAAAAAGACAnpr_B-reverseGATACTGCAGAATTTGGCCGGGACAAGTGnpr_B-reverseGATACTGCAGATTTGCCCCTCAATTnpr_B-reverseGATACTGCAGATTTGCCCCCCAATT	and AP	aud A P A forward	CCTCCCATCCCCTACTCACTATCATTC	PSt 1 Pam H I
cydAB_ArleverseICTITICOCCCCCCAATTCOATCACCTCTATcydAB_B-forwardATAGAGGTGATCAAAGGGGCGCGAAAAGAcydAB_B-reverseTAGACTGCAGCCTGAACACGTGCATACTGgshR1gshR-1_A-forwardCAATGCCGCACGCGCTCCGGTATCCCGGgshR-1_A-reverseACAAGGAGGATCAATGGTTGTAGCGCGCAATgshR-1_B-forwardATTGCCGCTACAACCATTGATCCTCTTGTgshR-1_B-reverseGTTGAAGCTTTGATCGGCGCGCCGgshR-2gshR-2_A-forwardAACAGGATCCCAATTAAGGTCATATCATCCCgshR-2gshR-2_A-reverseCACAGAGCGAAGGAAGGAAGGCGTAATTGATACgshR-2gshR-2_B-forwardGTATCAATTACGCCTTCCTTCGTCTGTGgshR-2gshR-2_B-forwardGTATCAATTACGCCTTCCTTCGTCTGTGgshR-2gshR-2_B-reverseGCGGGTCGACGCATCACGGCCTAAGjpripr_A-forwardCCGACTGCAGCGTATTAATCACGTTGCpripr_A-forwardCCGACTGCAGCGTACACGATACGATACGCTTCCAAAnprnpr_A-forwardCAATGGATCCATCGGAGCATATCCCTCCAAAnpr_B-reverseGGCCAAGCTTATGAGGTACAGCTTCGCnpr_B-forwardTGTCTTTTTAAAAAGTAAATTCCTCCTAATTnpr_B-reverseGATACTGCAGATTTGGCCGGGACAAGTGps-B-reverseGATACTGCAGATTTGGCCGGGACAAGTGpr_B-reverseGATACTGCAGATTTGGCCGGGACAAGTGpr_B-reverseGATACTGCAGATTTGGCCGGGACAAGTGpr_B-reverseGATACTGCAGATTTGGCCGGGACAAGTGpr_B-reverseGATACTGCAGATTTGGCCGGGACAAGTG	суаль	cydAB_A-Iorward		<i>Δαμι</i> Π Ι
cydAB_B-reverseTAGACGTGCAGCCTGAACACGGGCATACTGPst IgshR1gshR-1_A-forwardCAATGTCGACGCTTCGGATCCCGGSal IgshR1gshR-1_A-reverseACAAGGAGGATCAATGGTTGTAGCGGCAATSal IgshR-1_B-forwardATTGCCGCTACAACCATTGATCCTCCTTGTgshR-1gshR-1_B-forwardATTGCCGCTACAACCATTGATCCTCCTTGTBamH IgshR2gshR-2_A-forwardAACAGGAGCGAAGGAGGAGGAAGGCGTAATTGATACBamH IgshR2gshR-2_B-reverseCACAGAGACGAAGGAGAGGCGTAATTGATACBamH IgshR2gshR-2_B-reverseCCGAGTGCACGCATCACGGCCTAAGSal Iipripr_A-forwardCCGAGTGGACGCATCACGGCCTAAGSal Iipripr_A-forwardCCGACTGCAGCGTATTAATCACGTTGCPst Iipr_B-reverseGGCCAAGCTTATGAGGTACAGCTTCGCHind IIInprnpr_A-forwardCCAATGGATCCATCGGAGCATATCCCTCCAAAHind IIInprnpr_A-reverseTTTGGAGGCGTAACGAGCTTCGCHind IIInprnpr_A-reverseGGCCAAGCTTATGAGGTACAGCTTCGCHind IIInprnpr_A-forwardCAATGGATCCATCGGAGCATATCCCTCCAAAHind IIInprnpr_A-reverseATTAGGAGGAATTTACTTTTAAAAGACAHind IIInpr_B-reverseGGCCAAGGTCATCGGAGCATATCCCTCCAAAHind IIInpr_B-reverseGATACTGCAGGAAGTTAGCTCCTCCTAATTNor ATTAGGAGGAATTTACTTTTAAAAAGACAnpr_B-reverseGATACTGCAGAGTTGGCCGGGACAAGTGPst I		cydAB_R forward		
gshR1gshR-1_A-forwardCAATGTCGACGCTTCGGTATCCCGGSal IgshR1gshR-1_A-reverseACAAGGAGGATCAATGGTTGTAGCGGCAATSal IgshR-1_B-forwardATTGCCGCTACAACCATTGATCCTCCTTGTgshR-1_B-forwardATTGCCGCTACAACCATTGATCCTCCTTGTgshR2gshR-2_A-forwardAACAGGATCCCAATTAAGGTCATATCATCCCBamH IgshR2gshR-2_B-forwardGTATCAATTACGCCTTCCTTCGTCTCGTGSal IgshR2gshR-2_B-forwardGTATCAATTACGCCTTCCTTCGTCTCGTGSal Iipripr_A-forwardGCGGGTCGACGCATCACGGCCTAAGSal Iipripr_A-forwardCCGACTGCAGCGTATTAATCACGTTGCPst Iipr_B-forwardATCAAGCAACTTTATCGTTACGCCTCCAATInd IIInprnpr_A-forwardCAATGGATCCATCGGAGCATAACGCTTCGCHind IIInprnpr_A-forwardCAATGGATCCATCGGAGCATAACGCTTCGCHind IIInprnpr_A-forwardCAATGGATCCATCGGAGCATATCCCTTCAGBamH Inprnpr_A-forwardCAATGGATCCATCGGAGCATATCCCTTCAGBamH Inpr_B-reverseGGCCAAGCTTATGAGGAGCATATCCCTTCAGBamH Inpr_B-reverseAATTAGGAGGAATTTACTTTTAAAAAGACAYest I		cydAB_B reverse	TAGACTGCAGCCTGAACACGTGCATACTG	Pet I
gshR1gshR1_A-reverseACAAGGAGGATCAATGGTTGTAGCCGGCAATsal 1gshR-1_A-reverseACTGCCGCTACAACCATTGATCCTCCTTGTgshR-1_B-forwardATTGCCGCTACAACCATTGATCCTCCTTGTgshR-1_B-reverseGTTGAAGCTTTGATCGGCGCGCCGHind IIIgshR2gshR-2_A-forwardAACAGGATCCCAATTAAGGTCATATCATCCCBamH IgshR-2_B-forwardGTATCAATTACGCCTTCCTTCGTCTCTGTGgshR-2_B-forwardGTATCAATTACGCCTTCCTTCGTCTCTGTGgshR-2_B-reverseGCGGGTCGACGCATCACGGCCTAAGSal Iipripr_A-forwardCCGACTGCAGCGTATTAATCACGTTGCPst Iipr_B-forwardATCAAGCAACTTTATCGTTACGCCTCCAAAipr_B-reverseGGCCAAGCATCATCGGAGCATATCCCTTCAGnprnpr_A-forwardCAATGGATCCATCGGAGCATATCCCTTCAGBamH Inpr_A-reverseGGCCAAGCTTATGAGGTACAGCTTCGCHind IIInpr_B-forwardCAATGGATCCATCGGAGCATATCCCTTCAGBamH Inpr_B-forwardCAATGGAGGAATTTACTTTTAAAAAGACATGTCTTTTTAAAAAGTAAATTCCTCCTAATTnpr_B-forwardTGTCTTTTTAAAAGTAAATTCCTCCTAATTpst I	ashR1	gshR_1_A_forward	CAATGTCGACGCTTCGGTATCCCGG	Sal I
gshR 1_A fervationATTGCCGCTACAACCATTGATCCTCCTTGTgshR-1_B-forwardATTGCCGCTACAACCATTGATCCTCCTTGTgshR-1_B-reverseGTTGAAGCTTTGATCGGCGCGCCGHind IIIgshR2gshR-2_A-forwardAACAGGATCCCAATTAAGGTCATATCATCCCBamH IgshR-2_B-forwardGTATCAATTACGCCTTCCTTCGTCTCTGTGgshR-2_B-forwardGTATCAATTACGCCTTCCTTCGTCTCTGTGipripr_A-forwardCCGACTGCAGCGCATCACGGCCTAAGSal Iipripr_A-forwardCCGACTGCAGCGTATTAATCACGTTGCPst Iipr_B-reverseGTCCAAGCAACCATCACGGCCTCCAAAipr_B-reverseGGCCAAGCTTATGAGGTACAGCTTCGCnprnpr_A-forwardCAATGGATCCATCGGAGCATATCCCTTCAGBamH Inpr_A-reverseAATTAGGAGGAATTTACTTTTAAAAAGACAipr_B-forwardTGTCTTTTTAAAAAGTAAATTCCTCCTAATTnpr_B-forwardTGTCTTTTTAAAAGTAAATTCCTCCTCAATTnpr_B-forwardTGTCTTTTTAAAAGTAAATTCCTCCTCAATTnpr_B-reverseGATACTGCAGAGATTTGGCCGGGACAAGTGPst I	gsniti	gshR-1_A-reverse	ACAAGGAGGATCAATGGTTGTAGCGGCAAT	Sull
gshR 1_D forwardINFOCCOUNT FUNCTIONgshR-1_B-reverseGTTGAAGCTTTGATCGGCGCGCCGHind IIIgshR-2gshR-2_A-forwardAACAGGATCCCAATTAAGGTCATATCATCCCBamH IgshR-2_B-forwardGTATCAATTACGCCTTCCTTCGTCTCTGTGgshR-2_B-forwardGTATCAATTACGCCTTCCTTCGTCTCTGTGipripr_A-forwardCCGACTGCAGCGCATCACGGCCTAAGSal Iipripr_A-forwardCCGACTGCAGCGTAACGATAAAGTTGCTTGATPst Iipr_B-forwardATCAAGCAACTTTATCGTTACGCCTCCAAAipr_B-reverseGGCCAAGCATCATCGGAGCATACGATAAAGTTGCTTGATnprnpr_A-forwardCAATGGATCCATCGGAGCATATCCCTTCAGBamH Inpr_A-reverseATTAAGGAGGAATTTACTTTTAAAAAGACAipr_B-forwardFGTCTTTTTAAAAGTAAATTCCTCCTAATTnpr_B-forwardTGTCTTTTTAAAAGTAAATTCCTCCTAATTpst I		gshR_1_R-forward		
gshR1_D-reverseGTTO_AGGATCCCAATTAAGGTCATATCATCCCBamH IgshR2gshR-2_A-reverseCACAGAGACGAAGGAAGGCGTAATTGATCATCCCBamH IgshR-2_B-reverseCACAGAGACGAAGGAAGGCGTAATTGATACgshR-2_B-reversegshR-2_B-reverseGCGGGTCGACGCATCACGGCCTAAGSal Iipripr_A-forwardCCGACTGCAGCGTATTAATCACGTTGCPst Iipr_B-reverseTTTGGAGGCGTAACGATAAAGTTGCTTGATInnd IIInprnpr_A-reverseGCCCAAGCTTATGAGGTACAGGCTCCAAAInnd IIInprnpr_A-forwardCCGACTGCAGCGTATTAATCACGTTCGCHind IIInprnpr_A-forwardCAATGGATCCATCGGAGCATATCCCTTCAGBamH Inpr_B-reverseAATTAGGAGGAAATTTACTTTTAAAAAGACAInngr_B-reverseAATTAGGAGGAATTTACTTTTAAAAAGACAnpr_B-forwardTGTCTTTTTAAAAGTAAATTCCTCCTCAATTPst I		gshR_1_B_reverse	GTTGAAGCTTTGATCGGCGCGCG	Hind III
gsindgsind 2_A reverseCACAGAGACGAAGGAAGGAAGGCGTAATTGATACgshR-2_A-reverseCACAGAGACGAAGGAAGGCGTAATTGATACgshR-2_B-forwardGTATCAATTACGCCTTCCTTCGTCTCTGTGgshR-2_B-reverseGCGGG <u>TCGAC</u> GCATCACGGCCTAAGgshR-2_B-reverseGCGCACTGCAGCGTATTAATCACGTTGCipr_A-forwardCCGACTGCAGCGTAACGATAAAGTTGCTTGATipr_B-forwardATCAAGCAACTTTATCGTTACGCCTCCAAAipr_B-reverseGGCCAAGCTTATGAGGTACAGCTTCGCnprnpr_A-forwardcAATGGATCCATCGGAGCATATCCCTTCAGBamH 1npr_A-reverseAATTAGGAGGAAATTACTTTTAAAAAGACAnpr_B-forwardTGTCTTTTTAAAAGTAAATTCCTCCTAATTnpr_B-reverseGATACTGCAGATTGGCCGGGACAAGTGPst I	oshR?	gshR-2 A-forward	A A CAGGATCCCA ATTA A GGTCATATCATCCC	RamH I
gshR 2_B-forwardGTATCAATTACGCCTTCCTTCGTCTCTGTGgshR-2_B-forwardGTATCAATTACGCCTTCCTTCGTCTCTGTGgshR-2_B-reverseGCGGG <u>TCGAC</u> GCATCACGGCCTAAGipripr_A-forwardCCGACTGCAGCGTATCACGGTGCPst Iipr_B-forwardATCAAGCAACTTTATCGTTACGCCTCCAAAipr_B-reverseGGCCAAGCTTATGAGGTACAGCTTCGCipr_B-reverseGGCCAAGCTTATGAGGTACAGCTTCGCnprnpr_A-forwardCAATGGATCCATCGGAGCATATCCCTTCAGmpr_B-forwardCAATGGAGGAATTTACTTTTAAAAAGACAnpr_B-forwardTGTCTTTTTAAAAGTAAATTCCTCCTCAATTnpr_B-reverseGATACTGCAGATTGGCCGGGACAAGTGPst I	551112	gshR-2_A-reverse		Dumiti
gshR 2_B forwardGCGGGTCGACGCATCACGGCCTAAGSal Iipripr_A-forwardCCGACTGCAGCGTATCACGGCCTAAGSal Iipr_A-reverseTTTGGAGGCGTAACGATAATCACGTTGCPst Iipr_B-forwardATCAAGCAACTTTATCGTTACGCCTCCAAAIntel IIInprnpr_A-forwardCAATGGATCCATCGGAGCATATCCCTTCAGBamH Inpr_A-reverseATTAGGAGGAGAATTTACTTTTAAAAAGACAIntel IIInpr_B-forwardCAATGGATCCATCGGAGCATATCCCTTCAGBamH Inpr_B-forwardGTCTTTTTTAAAAAGTAAATTCCTCCTAATTIntel IIInpr_B-forwardGATACTGCAGATTGGCCGGGACAAGTGPst I		gshR-2 B-forward	GTATCA ATTACGCCTTCCTTCGTCTCTGTG	
<i>ipr</i> ipr_A-forward CCGA <u>CTGCAG</u> CGTATCAATCACGTTGC <i>Pst I</i> <i>ipr_A-reverse</i> TTTGGAGGCGTAACGATAAAGTTGCTTGAT <i>ipr_B-forward</i> ATCAAGCAACTTTATCGTTACGCCTCCAAA <i>ipr_B-reverse</i> GGCC <u>AAGCTT</u> ATGAGGTACAGCTTCGC <i>Hind III</i> <i>npr_A-forward</i> CAATGGATCCATCGGAGCATATCCCTTCAG <i>BamH 1</i> <i>npr_A-reverse</i> AATTAGGAGGAATTTACTTTTAAAAAGACA <i>npr_B-forward</i> TGTCTTTTTAAAAAGTAAATTCCTCCTAATT <i>npr_B-reverse</i> GATACTGCAGATTGGCCGGGACAAGTG <i>Pst I</i>		gshR-2_B-reverse	GCGGGTCGACGCATCACGGCCTAAG	Sal I
ipr_A-reverse TTTGGAGGCGTAACGATAAAGTTGCTTGAT Fort ipr_B-forward ATCAAGCAACTTTATCGTTACGCCTCCAAA Hind III npr npr_A-forward CAATGGAGGCGTACCGGAGCATATCCCTTCAG BamH I npr_B-forward CAATGGAGGAATTTACTTTTAAAAAGACA Fort Inor_A-reverse npr_B-forward CAATGGAGGAATTTACTTTTAAAAAGACA Fort Inor_A-reverse npr_B-forward TGTCTTTTTAAAAAGTAAATTCCTCCTAATT Pst I	ipr	ipr A-forward	CCGACTGCAGCGTATTAATCACGTTGC	Pst I
ipr_B-forward ATCAAGCAACTTTATCGTTACGCCTCCAAA ipr_B-reverse GGCC <u>AAGCTT</u> ATGAGGTACAGCTTCGC npr npr_A-forward cAATGGAGGACATCTCATCGGAGCATATCCCTTCAG BamH I npr_B-forward rgTCTTTTTAAAAAGTAAATTCCTCCTAATT npr_B-forward GATACTGCAGCATTTGGCCGGGACAAGTG Pst I	T.	ipr A-reverse	TTTGGAGGCGTAACGATAAAGTTGCTTGAT	
ipr_B-reverse GGCCAAGCTTATGAGGTACAGCTTCGC Hind III npr npr_A-forward CAATGGATCCATCGGAGCATATCCCTTCAG BamH I npr_B-forward AATTAGGAGGAATTTACTTTTAAAAAGACA TGTCTTTTTAAAAAGTAAATTCCTCCTCAATT npr_B-forward GATACTGCAGGATTTGGCCGGGACAAGTG Pst I		ipr B-forward	ATCAAGCAACTTTATCGTTACGCCTCCAAA	
npr npr_A-forward CAATGGATCCATCGGAGCATATCCCTTCAG BamH1 npr_A-reverse AATTAGGAGGAATTTACTTTTAAAAAGACA TGTCTTTTTAAAAAGTAAATTCCTCCTAATT npr_B-forward TGTCTTTTTAAAAAGTAAATTCCTCCTCAATT Pst I		ipr B-reverse	GGCCAAGCTTATGAGGTACAGCTTCGC	Hind III
npr_A-reverse AATTAGGAGGAATTTACTTTTAAAAAGACA npr_B-forward TGTCTTTTTAAAAGTAAATTCCTCCTAATT npr_B-reverse GATACTGCAGATTTGGCCGGGACAAGTG Pst I	npr	npr A-forward	CAATGGATCCATCGGAGCATATCCCTTCAG	BamH I
npr_B-forwardTGTCTTTTTAAAAGTAAATTCCTCCTAATTnpr_B-reverseGATACTGCAGATTTGGCCGGGACAAGTGPst I	T	npr A-reverse	AATTAGGAGGAATTTACTTTTAAAAAGACA	
npr_B-reverse GATACTGCAGATTTGGCCGGGACAAGTG Pst I		npr_B-forward	TGTCTTTTTAAAAGTAAATTCCTCCTAATT	
		npr_B-reverse	GATACTGCAGATTTGGCCGGGACAAGTG	Pst I

Target gene	Primer name	Primer sequence (5' to 3')	Restriction enzyme site
Confirmation of d	leficient mutants		
nox	279962_F	GTAGCATCGGCAATTGTCATGTAGTGTCAC	
	282934 R	CTGTTTTGAGTCATACCGTGCAACCCG	
nox5	177892_F	CTGCGGTTCGATGGTGCTAAGGTCACCTTC	
	180877_R	GTTTTGACGCATTCATCGAATCGAGTCGCG	
poxF	2268013_F	GTCTGACTAATATGCAGTGGCGCAAAGTGAG	
	2270930_R	CGAGGCAGCCAAAGCTTTCGTTAAGAAGCAC	
cidC	498129_F	CGTTGCTTCGATCATGGTCTGGCAGAATTC	
	501457_R	GGCCAGTGGCATTCCTGATTACACCGAG	
ahpC	2611951_F	GAATAACCATAGAAAGAAGGGAGGCAGTTG	
	2614120_R	AATTATTACCAGCCGGACCCGAGCACAAAG	
ohrR	1040892_F	CAATTTTAGATCCGGATACCATGGCGATTTC	
	1043371_R	CTCCATTGCACACAAATTGCACACAAATTC	
sod	2004800_F	CAATCGCATGCTCGGAAATGAGTTTCAAAC	
	2007409_R	GGAAATAGGTATGCGATATTCATTTACGAC	
flp	69484_F	CTTATGGAGGAGGTTTCGATCCTATAGAAC	
	71774_R	GCAGTATACCAACGTTCCAACCGCTATC	
dpsB	68755_F	GAAAAGGTGATGTTTGTCGGTGACGGGATC	
	70973_R	GTATTTAAAAAACATCACTCGGCAACCTCACCAAG	
cydAB	14573_F	GAAGCTTAGAGTGACGGCTAATGAAC	
	18661_R	CCGCAAAATGGACGGGTATTATCCATC	
gshR1	2311599_F	CAATGGGTTGCGGTTCGCATTCCTGAC	
	2314729_R	CTGTCGGAACGTTACTCGTCATGCTTG	
gshR2	2748254_F	CAGTGACCAAAGATTTTGACCATCATAAAC	
	2751189_R	GTTGATCCAACGAGCGGCGTCATC	
ipr	706255_F	GGGTAATAAACCAGCAATGACCACAAGACG	
	708795_R	CTAGAATTCAATCGAAATAATATTCGGATTGTCGG	
npr	464266_F	CCAATTTTTTTCTGCAAAGTCCTTTTGAGAG	
	467233_R	CGTTTTACAAGCATGGGAAAATACGGC	
qRT-PCR			
NADH peroxida	ase	ACGGCAATCCACAAGTTTGC	
		TTGTTGTTGAACGGCGAGTG	
Elongation factor Tu		AACCGCGAACAAGTTGAACG	
-		ACGGCCACCTTCTTCTTTTG	
Glyceraldehyde	3-phosphate dehydrogenase	AACACGATTCCTCACAGCAC	
		ACAACAGAAACACGCTGTGC	

Table 2. continued.

measure H₂O₂ concentrations.

Repressive effect of H_2O_2 on bacterial growth

Cells precultured overnight at 37°C were inoculated into fresh MRS medium at OD₆₆₀ = 0.05. These bacterial cultures were aliquoted (180 μ L/well) into a 96-well plate, and 20 μ L of H₂O₂ solution was added to a final concentration of 0.5, 1.0, or 2.0 mM; the plate was then incubated at 37°C for 24 hr. The OD₆₀₀ was measured using a multiplate reader, and the value at each concentration was calculated on the basis of the value at 0 mM for each strain. Significance was indicated by p<0.05 (for each wild-type concentration).

RNA isolation

 H_2O_2 treatment was performed as follows. Samples precultured at 37°C were inoculated into 20 mL of MRS medium at OD_{600} = 0.05. The cultures were grown for 5 hr at 37°C under static conditions and divided into two 10 mL aliquots. The cells were harvested via centrifugation (10,000 × g, 3 min) and washed twice with PIPES buffer (pH 6.8). One aliquot was resuspended in 10 mL of PIPES buffer and incubated at 37°C for 1 hr. The other aliquot was resuspended in H₂O₂ adjusted to 0.5 mM with PIPES buffer and incubated at 37°C for 1 hr. The 10 mL cultures were added to 20 mL of RNAprotect Bacteria Reagent (Qiagen). The mixtures were kept at room temperature for 5 min. The cells were then harvested via centrifugation for 10 min at 5000×g, suspended in 500 µL of TE buffer (50 mM Tris–HCl, pH 8.0) containing 5 mg/mL lysozyme and 20 µL/mL mutanolysin, and incubated at 37°C for 30 min. Total RNA was purified using a Direct-zol[™] RNA MiniPrep kit (Zymo Research) according to the manufacturer's protocol. DNA was digested using DNase I in the purification step. RNA was isolated from three independent cultures.

Quantitative real-time PCR assays

cDNA was synthesized using a PrimeScript RT reagent kit (Takara) according to the manufacturer's protocol. In total, 0.1 mg of total RNA was used as a template. Quantitative realtime PCR assays were performed using a CFX96 Real-Time PCR Detection System (Bio-Rad) with THUNDERBIRD SYBR qPCR Mix (Toyobo). The primers were designed to amplify products of approximately 80 bp in length (Table 2). The reaction mixture contained 25 μ L of THUNDERBIRD SYBR qPCR Mix, 1 μ L of 15 μ M forward primer, 1 μ L of 15 μ M reverse primer, 1 μ L of 50× ROX reference dye, 20 μ L of dH₂O, and 2.5 μ L of diluted cDNA templates. All reactions were run in duplicate for each of the three independent RNA samples. The gene expression values were normalized using the elongation factor Tu and glyceraldehyde 3-phosphate dehydrogenase as an internal standard. Standard curves for both the internal standard and target genes were generated by amplifying 10-fold serial dilutions of cDNA. The gene expression data from quantitative real-time PCR were analyzed using Student's t-test.

RESULTS

Construction of mutants deficient in genes involved in oxygen and ROS tolerance in L. casei IGM394

To elucidate the mechanisms of oxygen and ROS tolerance in *L. casei* IGM394, we constructed gene-deficient mutants targeting enzymes or factors that are expected to be involved in oxygen tolerance (Table 3). The target gene was completely deleted via the double-crossover method using a thermosensitive suicide vector. Fourteen out of 16 targeted genes were successfully disrupted in mutants. However, disruptants were not obtained for trxB2 and the chaperone protein gene groEL.

Growth of deficient mutants under static and shaking conditions

We evaluated the growth rates of 23 deficient mutants under static and shaking conditions (Fig. 1A, 1B). The growth rate of Δnox , which is an NADH oxidase (nox, H₂O-forming) genedeficient mutant, was decreased under both culture conditions. Compared with the findings for the wild type, the OD decreased from 2.2 to 1.5 under the static condition and from 2.2 to 1.6 under the shaking condition after 24 hr of culture. In the Δnpr mutant, the growth rate was decreased only under shaking culture. Under the static condition, the OD of Δnpr was 2.0, which was similar to that of the wild type (2.2). However, under the shaking condition, the OD of Δnpr was 1.5, whereas that for the wild type was 2.2. Conversely, the ODs of four strains, namely the NADH oxidase (nox5, H₂O₂-forming) gene-deficient mutant $\Delta nox5$, pyruvate oxidase gene-deficient mutants $\Delta poxF$ and $\Delta cidC$, and DNA-binding protein gene-deficient mutant $\Delta dpsB$, were slightly increased (approximately 0.1–0.2) under the shaking condition. The other eight deficient mutants did not exhibit different growth rates versus the wild type under either of the culture conditions. Decreased growth under shaking was observed only for Δnpr . Therefore, we constructed mutants deficient in multiple genes using Δnpr as a host and evaluated the effect on viability. We constructed six double-deficient mutants, one triple-deficient mutant, and two quadruple-deficient mutants. The target genes of the six double-deficient mutants were nox, nox5, sod, glutathione reductase (gshR1 or gshR2), and iron-dependent peroxidase (ipr). The triple-deficient mutant featured mutations of gshR1 and gshR2. Finally, the quadruple-deficient mutants featured mutations of *sod* or *ipr* using the triple-deficient mutant $\Delta gshR1::\Delta gshR2::\Delta npr$ as a host. However, these deficient mutants exhibited the same growth rate as Δnpr (Fig. 1B). These results indicated that the Npr gene is important for the growth of L. casei IGM394 under the shaking condition. Measuring the amount of H2O2 accumulated

 Table 3. Targeting enzymes or factors that are expected to be involved in oxidative stress tolerance

	Gene name
NADH peroxidase	npr
Organic hydroperoxide resistance protein transcriptional regulator	ohrR
NADH oxidase (H ₂ O - forming)	nox
NADH oxidase (H_2O_2 - forming)	nox5
Pyruvate oxidase	poxF
Pyruvate oxidase	cidC
Alkyl hydroperoxide reductase subunit C	ahpC
Superoxide dismutase	sod
Fe-S cluster assembly protein	suf
Probable transcriptional regulator	flp
DNA binding protein	dpsB
Cytochrome bd ubiquinol subunit I, II	cydAB
Glutathione reductase	gshR1
Iron-dependent peroxidase	ipr
Thioredoxin reductase	trxB2
Chaperon protein	groEL

in LAPTg medium after 24 hr, H_2O_2 was detected only in Δnpr and nine multiple-deficient mutants (data not shown). In addition, the evaluation by bacterial turbidity included dead cells, and there was a possibility that the results might be inaccurate. Therefore, we measured colony-forming units, and the results were in line with the measured OD values. Thus, only the results for turbidity are presented.

Growth of the wild-type and Δnpr strains under the shaking condition and H_2O_2 concentrations in the culture

 H_2O_2 concentrations in the MRS culture medium of wild-type and Δnpr cultures were measured over time under the shaking condition (Fig. 2). Although H_2O_2 was not detected in the wildtype culture, following overnight culture of Δnpr , approximately 1,000 μ M H_2O_2 had accumulated. In addition, no accumulation of H_2O_2 was observed in either strain under the static condition (data not shown). These results suggest that *L. casei* IGM394 converts oxygen in its growth process under shaking and that the generated H_2O_2 is degraded by NADH peroxidase.

Growth suppression by H_2O_2

The effect of H₂O₂ on the growth of each mutant was evaluated under various H₂O₂ concentrations (0-2.0 mM) in MRS medium (Fig. 3). The suppressive effect of H_2O_2 on growth was concentration dependent. In particular, 0.5 mM H₂O₂ had little effect on growth, whereas 1.0 mM H₂O₂ reduced proliferation. Bacterial growth was completely inhibited by 2.0 mM H₂O₂. The influence of 0.5 and 1.0 mM H_2O_2 on the growth rates of nine mutants was similar to that observed in the wild-type strain. However, the growth rate of Δnpr was reduced by 0.5 mM H₂O₂, and the rate was significantly lower than that of the wild type in the presence of 1.0 mM H₂O₂. The growth of $\Delta ohrR$ was not suppressed by 1.0 mM H_2O_2 . In addition, $\Delta ohrR$ also proliferated in the presence of 2.0 mM H₂O₂, and H₂O₂ resistance was improved by the deletion of *ohrR*. From these findings, it was presumed that ohrR is one of the tolerance mechanisms in L. casei IGM394.



■ static 24 h □ aerobic 24 h

Fig. 1. (A) Growth rates of wild type and deficient mutants under static and shaking conditions. Strains precultured overnight at 37° C were inoculated into fresh LAPTg medium at a final OD₆₀₀ of 0.05. After 24 hr, we measured the OD₆₀₀ using a spectrophotometer. The black bar shows the static condition, and the gray bar shows the shaking condition. (LAPTg medium has no ability to consume H₂O₂.) The data are shown as the mean ± SE of three independent experiments. asterisk (*) Student's t-test; p<0.05. (B) Growth rates of Δ npr and multiple deficient mutants under static and shaking conditions. Strains precultured overnight at 37° C were inoculated into fresh LAPTg medium at a final OD₆₀₀ of 0.05. After 24 hours, we measured the OD₆₀₀ using a spectrophotometer. The black bar shows the static condition, and the gray bar shows the shaking condition. (LAPTg medium has no ability to consume H₂O₂.) The data are shown as the mean ± SE of three independent experiments. At a final OD₆₀₀ of 0.05. After 24 hours, we measured the OD₆₀₀ using a spectrophotometer. The black bar shows the static condition, and the gray bar shows the shaking condition. (LAPTg medium has no ability to consume H₂O₂.) The data are shown as the mean ± SE of three independent experiments. asterisk (*) Student's t-test; p<0.05.

H_2O_2 consumption in PIPES buffer

 H_2O_2 accumulation in the medium was estimated for 14 deficient mutants under the shaking condition, and H_2O_2 was only detected in the Δnpr culture. This illustrated that only Δnpr could not consume H_2O_2 . H_2O_2 accumulation in the medium under the shaking condition was confirmed for Δnpr , and Δnpr could not consume H_2O_2 generated during the growth process

(Fig. 4). Therefore, the ability to consume added H_2O_2 in PIPES buffer was measured. In this experiment, the wild-type, Δnpr , and $\Delta ohrR$ strains were used after 5 hr of logarithmic growth. After the cells were exposed to 0, 50, 100, or 300 μ M H_2O_2 for 1 hr under the static condition, H_2O_2 concentrations in PIPES buffer were measured. On average, the H_2O_2 concentration was decreased by 84% compared with the initial concentration in the



Fig. 2. Growth and accumulated H_2O_2 concentration of wild type and Δnpr under the shaking condition.

Strains precultured overnight at 37°C were inoculated into fresh MRS medium at a final OD_{600} of 0.05. We measured the OD_{600} using a spectrophotometer every 3 hr. Subsequently, 1 mL of the culture was collected and centrifuged (10,000 g, 3 min), and 20 μ L of the supernatant was used for measuring the H₂O₂ concentration. After measuring the wavelength at 727 nm, chromogenic reagent DA64 was used to quantify H₂O₂ based on the standard curve. The black square represents the OD value of wild type, and the black triangle represents that of Δnpr . The gray bar shows the concentration of H₂O₂ in the Δnpr culture. The data are shown as the mean ± SE of three independent experiments.





presence of wild-type cells. The wild-type strain decreased the supplemented H_2O_2 concentration from 50 to 0 μ M, from 100 to 25 μ M, and from 300 to 204 μ M. The Δnpr strain could not consume H_2O_2 efficiently. The H_2O_2 concentration for the Δnpr strain after 1 hr of incubation was similar to or slightly higher

than the control level. In $\Delta ohrR$ culture buffer, H₂O₂ could not be detected after adding 50 or 100 μ M H₂O₂. The $\Delta ohrR$ strain completely consumed 100 μ M H₂O₂ and decreased the H₂O₂ concentration from 300 to 174 μ M. This indicated that the H₂O₂ consumption ability of the $\Delta ohrR$ strain was greater than that of



Fig. 4. H_2O_2 concentration of wild type and Δnpr in PIPES buffer.

Strains precultured overnight at 37°C were inoculated into fresh MRS medium at a final OD_{600} of 0.05. The cells were used after static culture at 37°C for 5 hr. They were washed twice with PIPES buffer (pH 6.8) and resuspended in 10 mL H₂O₂ adjusted to 0 to 300 μ M with PIPES buffer. After incubation at 37°C for 2 hr, the cells were harvested by centrifugation (10,000 g, 3 min). Twenty microliters of the supernatant was used for measuring the H₂O₂ concentration. After measuring the wavelength at 727 nm, the chromogenic reagent DA64 was used to quantify H₂O₂ based on the standard curve. The data are shown as the mean ± SE of three independent experiments.

the wild-type strain.

Gene expression analysis of NADH peroxidase via quantitative real-time PCR

The aforementioned results revealed that H_2O_2 consumption was mainly performed by Npr and that deletion of *ohrR* eliminated the growth-suppressing effects of H_2O_2 in *L. casei* IGM394. It was presumed that OhrR was involved in H_2O_2 consumption; therefore, we examined the expression level of *npr* in the wildtype and $\Delta ohrR$ strains via quantitative real-time PCR. In addition, we observed that the expression level changed depending on the presence or absence of H_2O_2 . In this experiment, the treatment conditions were exposure to H_2O_2 adjusted to 0.5 mM with PIPES buffer for 1 hr at 37°C. In the wild-type strain, the expression level of *npr* was constant regardless of the presence of H_2O_2 . However, the expression level of *npr* in the $\Delta ohrR$ strain was 2.5fold higher in the absence of H_2O_2 and 3-fold higher than that of the wild-type strain in the presence of H_2O_2 .

DISCUSSION

Aerobic organisms have various tolerance mechanisms against oxygen and ROS. Lactic acid bacteria, which are facultative anaerobes, do not require oxygen to grow, but they can grow in the presence of oxygen. Several factors have been reported to be involved in oxidative stress tolerance, but the mechanisms differ by species and strain. The similar growth of *L. casei* IGM394 (wild type) under static and shaking conditions observed in this study indicated that this strain has multiple mechanisms to respond to oxidative stress.

SOD, which converts highly toxic superoxide substrates into H_2O_2 , is important in the mechanism of oxidative tolerance. Serata *et al.* reported that *sod* of *L. casei* Shirota was transcribed but that its protein was inactive, and they reported that superoxide was eliminated via the intracellular accumulation of Mn^{2+} [34]. The possibility that *L. casei* IGM394 has the same Mn^{2+} accumulation

mechanism as *L. casei* Shirota could explain why *sod* disruption did not affect the growth rate of the former bacterium (Fig. 1A).

Higuchi *et al.* reported that AhpC degrades H_2O_2 into water and that H_2O_2 is produced by Nox as a byproduct of oxygen consumption in *S. mutans* [3]. *L. casei* IGM394 expresses Nox5, which produces H_2O_2 in a manner similar to that observed in S. mutans. However, H_2O_2 was not detected in the culture medium of $\Delta ahpC$ under shaking. It is predicted that *L. casei* IGM394 carries a number of enzymes for degrading H_2O_2 , such as NADH peroxidase, and that these enzymes complement the function of AhpC to degrade H_2O_2 under shaking.

There are reports that Fnr-like protein (Flp) is a potential sensor protein and regulator, although the genes it regulates in Lc. lactis and L. casei remain to be clarified [35, 36]. When Flp is oxidized, an intramolecular disulfide bond is formed, thereby conferring the ability to bind to the promoter region. Although double deletion of flpA and flpB leads to hypersensitivity to H_2O_2 in Lc. lactis ssp. cremoris MG1363 [37], Aflp of L. casei IGM394 exhibited the same growth rate as the wild type under static and shaking conditions (Fig. 1A) or in the presence of $2 \text{ mM H}_2\text{O}_2$. It is unclear whether the different responses of the two strains are due to different functions of Flp. The DNA-binding protein Dps is an H₂O₂ resistance factor in *E. coli* that has been identified as a nonspecific DNA-binding protein accumulated in stationary cells [38]. It has been reported that Dps forms a ferritin-like complex, binds to DNA to form an extremely stable complex, and protects DNA against H_2O_2 [39, 40]. The suf cluster may participate in Fe-S cluster assembly or repair. Under oxidative stress, OxyR (regulator protein) activates the expression of the suf cluster in E. coli [41]. Cytochrome bd oxidase (CydAB) is the terminal electron acceptor that finally reduces oxygen to water. In all lactic acid bacteria, cydAB is clustered [42, 43]. GshR is one of the enzymes constituting the glutathione-ascorbic acid cycle, a metabolic pathway that detoxifies H₂O₂ generated in the process of metabolism. Yamamoto et al. reported that GshR may be important in protecting S. mutans against oxidative stress

53

[44, 45]. Despite reports of their involvement in oxidative stress resistance, deletion mutants of these genes ($\Delta dpsB$, Δsuf , $\Delta cydAB$, $\Delta gshR$) had similar growth rates as the wild-type strain under static and shaking conditions. It is presumed that the mechanisms of oxygen consumption or tolerance were complemented by other mechanisms in L. casei IGM394.It should be noted, however, that we predicted the presence of genes that are essential for growth under oxidative stress conditions but are not complemented by other mechanisms. In addition, we tried to disrupt trxB2, which plays a significant role in cellular redox processes, including protein repair and defense against oxidative stress, but these efforts were unsuccessful. Serata et al. succeeded in constructing a trxB2-deficient mutant in L. casei Shirota, and the ability of this strain to grow under aerobic conditions was significantly reduced. This suggested that TrxB2 is an important enzyme for oxygen tolerance in L. casei Shirota [13]. Our different findings may be due to the fact that we did not perform experimentation under an anaerobic condition.

Our finding that only Δnox exhibited decreased growth versus the wild type indicated that Nox may have different functions than other oxygen-consuming enzymes. One reason for this is that Nox converts oxygen to water without producing H₂O₂. Other oxygenconsuming enzymes, such as NADH oxidase (Nox5, H₂O₂forming) and pyruvate oxidase (PoxF, CidC), convert oxygen to H₂O₂. It is considered that the oxygen consumption function depends on the H₂O₂-generating enzyme following the deletion of Nox, and that the influence of H₂O₂ or ROS produced by the Fenton reaction explains the decreased growth rate of Δnox . One other reason is that Nox works to maintain the redox potential in cells. Futhermore, the increased growth rates observed for $\Delta nox5$, $\Delta poxF$, and $\Delta cidC$ suggested that the decreased production of H₂O₂ by these proteins eased the stress on cells.

 Δnpr , in which the NADH peroxidase gene was disrupted, only displayed decreased growth under the shaking condition relative to the wild type. As NADH peroxidase is an H₂O₂-degrading enzyme, it was considered that Δnpr could not degrade the H₂O₂ generated as a byproduct of oxygen consumption under the shaking condition. A high concentration of H_2O_2 was detected in the Δnpr culture under the shaking condition. The H₂O₂ concentration increased with the incubation time and reached about 500 μ M after 15 hr and about 950 µM after 24 hr. However, in cultures of the wild-type and all other deficient mutant strains, H₂O₂ was not detected under either condition (Fig. 2). This revealed that the loss of H2O2 degradation could not be compensated for by other genes in L. casei IGM394. Other mutants featuring deficiencies of multiple genes displayed no changes in phenotype under shaking. From this finding, it was suggested that the oxidative stress tolerance mechanisms of L. casei IGM394 are multiple and diverse, and thus, no effect on growth was observed because missing functions could be complemented by other genes. However, the data indicated that H₂O₂ consumption is critical for the oxidative stress tolerance mechanism in this strain because decreased growth under the shaking condition was only observed for Δnpr . As lactic acid bacteria cannot synthesize heme, there is no catalase-based H₂O₂ degradation system. Previous studies reported that L. plantarum carries manganese catalase, which uses manganese as a cofactor [46], and that *L. sakei* synthesizes heme catalase when heme is added to the medium [47]. Genomic data revealed that L. casei IGM394 possesses four peroxidases: NADH peroxidase, iron-dependent peroxidase, glutathione

peroxidase, and thiol peroxidase; however, the bacterium does not carry manganese catalase. Our findings revealed that peroxidases other than NADH peroxidase cannot efficiently degrade H_2O_2 .

Interestingly, $\Delta ohrR$, which is a deficient mutant of the transcriptional regulator gene (ohrR), showed strong resistance to H_2O_2 (Fig. 3). The $\triangle ohrR$ could grow under 0.5 mM and 1.0 mM H₂O₂ supplemented conditions as well as 0 mM supplemented conditions. Furthermore, $\Delta ohrR$ could grow under even 2.0 mM supplemented conditions, that is, conditions in which wild type could not grow. OhrR is a transcriptional repressor of organic hydroperoxide resistance protein (OhrA). As the ohrR disruption resulted in the constitutive expression of the OhrA protein, it induced strong resistance to H_2O_2 in $\Delta ohrR$. The OhrR gene was first identified in Xanthomonas campestris [20] and subsequently reported in many gram-negative bacteria. In gram-positive bacteria, the Ohr family was reported to be involved in resistance to organic peroxide and H₂O₂ in *Bacillus subtilis* [21], and OhrA overexpression induced H₂O₂ tolerance. In B. subtilis, OhrR repressed ohrA expression by binding to the inverted repeat (IR) sequence (TACAATT-N-AATTGTA) presented upstream of ohrA. However, there is no detailed report on the Ohr family in lactic acid bacteria, and a similar IR sequence upstream of ohrA was not detected in L. casei IGM394. Our results suggested that deletion of ohrR induced greater H2O2 resistance, and these effects appear to be related to the constitutive expression of ohrA. Meanwhile, deletion of ohrR induced higher expression of NADH peroxidase (Fig. 5). In L. casei IGM394, ohrA expression might be regulated by the recognition of different IR sequences or a different mechanism from that observed for *ohrR* in *Bacillus*. The association between the constitutive expression of ohrA and the higher expression of NADH peroxidase is unclear at present. However, it was suggested that the OhrR protein participates in the mechanisms combating oxygen and ROS in lactic acid bacteria. Analyses of ohrA expression control and function will be required in the future.

The findings of decreased growth under the shaking condition



Fig. 5. NADH peroxidase expression level with and without H_2O_2 in the wild type and $\Delta ohrR$.

Target gene: NADH peroxidase gene (npr).

Housekeeping gene: elongation factor Tu gene and glyceraldehyde 3-phosphate dehydrogenase gene.

The relative expression levels were calculated using 2 housekeeping genes. Black bars represent untreated; gray bars represent H_2O_2 treated. The data are shown as the mean \pm SE of three independent experiments.

and the loss of H_2O_2 consumption following disruption of *npr* were similar to those reported for *L. casei* Shirota [16]. Although *npx* expression was increased by approximately 10-fold in *L. casei* Shirota in response to H_2O_2 exposure according to quantitative real-time PCR, the expression level of *npr* in *L. casei* IGM394 was constant under the shaking condition. Serata *et al.* reported that *L. casei* Shirota exhibited the ability to consume H_2O_2 only after 1 hr of pretreatment with 0.5 mM H_2O_2 added to the culture medium. However, *L. casei* IGM394 could consume H_2O_2 without this pretreatment. This difference in the regulation of H_2O_2 consumption remains to be clarified.

According to our study, *L. casei* IGM394 has multiple oxygen consumption mechanisms, and disruption of a single gene is not sufficient to eliminate the ability to consume oxygen or alter growth. It is presumed that NADH oxidase efficiently converts oxygen to water in the wild-type strain, and thus, multiple H_2O_2 consumption mechanisms may not be necessary. NADH peroxidase plays a key role in H_2O_2 consumption, and other genes could not compensate for its function. Thus, it was concluded that the NADH peroxidase has a critical role in the oxidative stress response mechanisms in *L. casei* IGM394.

REFERENCES

- Imlay JA. 2008. Cellular defenses against superoxide and hydrogen peroxide. Annu Rev Biochem 77: 755–776. [Medline] [CrossRef]
- Higuchi M, Shimada M, Yamamoto Y, Hayashi T, Koga T, Kamio Y. 1993. Identification of two distinct NADH oxidases corresponding to H2O2-forming oxidase and H2Oforming oxidase induced in *Streptococcus mutans*. J Gen Microbiol 139: 2343–2351. [Medline] [CrossRef]
- Higuchi M, Yamamoto Y, Poole LB, Shimada M, Sato Y, Takahashi N, Kamio Y. 1999. Functions of two types of NADH oxidases in energy metabolism and oxidative stress of *Streptococcus mutans*. J Bacteriol 181: 5940–5947. [Medline] [CrossRef]
- Lorquet F, Goffin P, Muscariello L, Baudry JB, Ladero V, Sacco M, Kleerebezem M, Hols P. 2004. Characterization and functional analysis of the *poxB* gene, which encodes pyruvate oxidase in *Lactobacillus plantarum*. J Bacteriol 186: 3749–3759. [Medline] [CrossRef]
- Sedewitz B, Schleifer KH, Götz F. 1984. Physiological role of pyruvate oxidase in the aerobic metabolism of *Lactobacillus plantarum*. J Bacteriol 160: 462–465. [Medline] [CrossRef]
- Sasaki Y, Horiuchi H, Kawashima H, Mukai T, Yamamoto Y. 2014. NADH Oxidase of Streptococcus thermophilus 1131 is required for the effective yogurt fermentation with Lactobacillus delbrueckii subsp. bulgaricus 2038. Biosci Microbiota Food Health 33: 31–40. [Medline] [CrossRef]
- Patton TG, Rice KC, Foster MK, Bayles KW. 2005. The *Staphylococcus aureus cidC* gene encodes a pyruvate oxidase that affects acetate metabolism and cell death in stationary phase. Mol Microbiol 56: 1664–1674. [Medline] [CrossRef]
- Chang SK, Hassan HM. 1997. Characterization of superoxide dismutase in Streptococcus thermophilus. Appl Environ Microbiol 63: 3732–3735. [Medline] [CrossRef]
- Sanders JW, Leenhouts KJ, Haandrikman AJ, Venema G, Kok J. 1995. Stress response in *Lactococcus lactis*: cloning, expression analysis, and mutation of the lactococcal superoxide dismutase gene. J Bacteriol 177: 5254–5260. [Medline] [CrossRef]
- Miyoshi A, Rochat T, Gratadoux JJ, Le Loir Y, Oliveira SC, Langella P, Azevedo V. 2003. Oxidative stress in *Lactococcus lactis*. Genet Mol Res 2: 348–359. [Medline]
- Vido K, Diemer H, Van Dorsselaer A, Leize E, Juillard V, Gruss A, Gaudu P. 2005. Roles of thioredoxin reductase during the aerobic life of *Lactococcus lactis*. J Bacteriol 187: 601–610. [Medline] [CrossRef]
- Serrano LM, Molenaar D, Wels M, Teusink B, Bron PA, de Vos WM, Smid EJ. 2007. Thioredoxin reductase is a key factor in the oxidative stress response of *Lactobacillus plantarum* WCFS1. Microb Cell Fact 6: 29. [Medline] [CrossRef]
- Serata M, Iino T, Yasuda E, Sako T. 2012. Roles of thioredoxin and thioredoxin reductase in the resistance to oxidative stress in *Lactobacillus casei*. Microbiology 158: 953–962. [Medline] [CrossRef]
- Holmgren A. 1985. Thioredoxin. Annu Rev Biochem 54: 237–271. [Medline] [CrossRef]
- Yamamoto Y, Higuchi M, Poole LB, Kamio Y. 2000. Role of the dpr product in oxygen tolerance in *Streptococcus mutans*. J Bacteriol 182: 3740–3747. [Medline] [CrossRef]
- Yamamoto Y, Higuchi M, Poole LB, Kamio Y. 2000. Identification of a new gene responsible for the oxygen tolerance in aerobic life of *Streptococcus mutans*. Biosci

Biotechnol Biochem 64: 1106-1109. [Medline] [CrossRef]

- Serata M, Kiwaki M, Iino T. 2016. Functional analysis of a novel hydrogen peroxide resistance gene in *Lactobacillus casei* strain Shirota. Microbiology 162: 1885–1894. [Medline] [CrossRef]
- Brooijmans RJ, de Vos WM, Hugenholtz J. 2009. Lactobacillus plantarum WCFS1 electron transport chains. Appl Environ Microbiol 75: 3580–3585. [Medline] [CrossRef]
- Sijpesteijn AK. 1970. Induction of cytochrome formation and stimulation of oxidative dissimilation by hemin in *Streptococcus lactis* and *Leuconostoc mesenteroides*. Antonie van Leeuwenhoek 36: 335–348. [Medline] [CrossRef]
- Winstedt L, Frankenberg L, Hederstedt L, von Wachenfeldt C. 2000. *Enterococcus faecalis* V583 contains a cytochrome *bd*-type respiratory oxidase. J Bacteriol 182: 3863–3866. [Medline] [CrossRef]
- Mongkolsuk S, Praituan W, Loprasert S, Fuangthong M, Chamnongpol S. 1998. Identification and characterization of a new organic hydroperoxide resistance (ohr) gene with a novel pattern of oxidative stress regulation from *Xanthomonas campestris* pv. phaseoli. J Bacteriol 180: 2636–2643. [Medline] [CrossRef]
- Fuangthong M, Atichartpongkul S, Mongkolsuk S, Helmann JD. 2001. OhrR is a repressor of *ohrA*, a key organic hydroperoxide resistance determinant in *Bacillus subtilis*. J Bacteriol 183: 4134–4141. [Medline] [CrossRef]
- 23. Makarova K, Slesarev A, Wolf Y, Sorokin A, Mirkin B, Koonin E, Pavlov A, Pavlova N, Karamychev V, Polouchine N, Shakhova V, Grigoriev I, Lou Y, Rohksar D, Lucas S, Huang K, Goodstein DM, Hawkins T, Plengvidhya V, Welker D, Hughes J, Goh Y, Benson A, Baldwin K, Lee JH, Díaz-Muñiz I, Dosti B, Smeianov V, Wechter W, Barabote R, Lorca G, Altermann E, Barrangou R, Ganesan B, Xie Y, Rawsthorne H, Tamir D, Parker C, Breidt F, Broadbent J, Hutkins R, O'Sullivan D, Steele J, Unlu G, Saier M, Klaenhammer T, Richardson P, Kozyavkin S, Weimer B, Mills D. 2006. Comparative genomics of the lactic acid bacteria. Proc Natl Acad Sci USA 103: 15611–15616. [Medline] [CrossRef]
- Cai H, Thompson R, Budinich MF, Broadbent JR, Steele JL. 2009. Genome sequence and comparative genome analysis of *Lactobacillus casei*: insights into their nicheassociated evolution. Genome Biol Evol 1: 239–257. [Medline] [CrossRef]
- Mazé A, Boël G, Zúñiga M, Bourand A, Loux V, Yebra MJ, Monedero V, Correia K, Jacques N, Beaufils S, Poncet S, Joyet P, Milohanic E, Casarégola S, Auffray Y, Pérez-Martínez G, Gibrat JF, Zagorec M, Francke C, Hartke A, Deutscher J. 2010. Complete genome sequence of the probiotic *Lactobacillus casei* strain BL23. J Bacteriol 192: 2647–2648. [Medline] [CrossRef]
- Zhang W, Yu D, Sun Z, Wu R, Chen X, Chen W, Meng H, Hu S, Zhang H. 2010. Complete genome sequence of *Lactobacillus casei* Zhang, a new probiotic strain isolated from traditional homemade koumiss in Inner Mongolia, China. J Bacteriol 192: 5268–5269. [Medline] [CrossRef]
- Ai L, Chen C, Zhou F, Wang L, Zhang H, Chen W, Guo B. 2011. Complete genome sequence of the probiotic strain *Lactobacillus casei* BD-II. J Bacteriol 193: 3160–3161. [Medline] [CrossRef]
- Chen C, Ai L, Zhou F, Wang L, Zhang H, Chen W, Guo B. 2011. Complete genome sequence of the probiotic bacterium *Lactobacillus casei* LC2W. J Bacteriol 193: 3419–3420. [Medline] [CrossRef]
- Hochwind K, Weinmaier T, Schmid M, van Hemert S, Hartmann A, Rattei T, Rothballer M. 2012. Draft genome sequence of *Lactobacillus casei* W56. J Bacteriol 194: 6638. [Medline] [CrossRef]
- Koryszewska-Baginska A, Aleksandrzak-Piekarczyk T, Bardowski J. 2013. Complete genome sequence of the probiotic strain *Lactobacillus casei* (formerly *Lactobacillus paracasei*) LOCK919. Genome Announc 1: e00758–e13. [Medline] [CrossRef]
- Wang S, Zhu H, He F, Luo Y, Kang Z, Lu C, Feng L, Lu X, Xue Y, Wang H. 2014. Whole genome sequence of the probiotic strain *Lactobacillus paracasei* N1115, isolated from traditional Chinese fermented milk. Genome Announc 2: e00059–e14. [Medline]
- Kajikawa A, Igimi S. 2011. Development of recombinant vaccines in lactobacilli for elimination of *Salmonella*. Biosci Microflora 30: 93–98. [Medline] [CrossRef]
- Komatsu A, Igimi S, Kawana K. 2018. Optimization of human papillomavirus (HPV) type 16 E7-expressing lactobacillus-based vaccine for induction of mucosal E7-specific IFNγ-producing cells. Vaccine 36: 3423–3426. [Medline] [CrossRef]
- Serata M, Yasuda E, Sako T. 2018. Effect of superoxide dismutase and manganese on superoxide tolerance in *Lactobacillus casei* strain Shirota and analysis of multiple manganese transporters. Biosci Microbiota Food Health 37: 31–38. [Medline] [CrossRef]
- Scott C, Guest JR, Green J. 2000. Characterization of the Lactococcus lactis transcription factor FlpA and demonstration of an *in vitro* switch. Mol Microbiol 35: 1383–1393. [Medline] [CrossRef]
- Gostick DO, Green J, Irvine AS, Gasson MJ, Guest JR. 1998. A novel regulatory switch mediated by the FNR-like protein of *Lactobacillus casei*. Microbiology 144: 705–717. [Medline] [CrossRef]
- Scott C, Rawsthorne H, Upadhyay M, Shearman CA, Gasson MJ, Guest JR, Green J. 2000. Zinc uptake, oxidative stress and the FNR-like proteins of *Lactococcus lactis*. FEMS Microbiol Lett 192: 85–89. [Medline] [CrossRef]
- Almirón M, Link AJ, Furlong D, Kolter R. 1992. A novel DNA-binding protein with regulatory and protective roles in starved *Escherichia coli*. Genes Dev 6 12B:

2646–2654. [Medline] [CrossRef]

- Grant RA, Filman DJ, Finkel SE, Kolter R, Hogle JM. 1998. The crystal structure of Dps, a ferritin homolog that binds and protects DNA. Nat Struct Biol 5: 294–303. [Medline] [CrossRef]
- Wolf SG, Frenkiel D, Arad T, Finkel SE, Kolter R, Minsky A. 1999. DNA protection by stress-induced biocrystallization. Nature 400: 83–85. [Medline] [CrossRef]
- Zheng M, Wang X, Templeton LJ, Smulski DR, LaRossa RA, Storz G. 2001. DNA microarray-mediated transcriptional profiling of the *Escherichia coli* response to hydrogen peroxide. J Bacteriol 183: 4562–4570. [Medline] [CrossRef]
- Giuffrè A, Borisov VB, Arese M, Sarti P, Forte E. 2014. Cytochrome bd oxidase and bacterial tolerance to oxidative and nitrosative stress. Biochim Biophys Acta 1837: 1178–1187. [Medline] [CrossRef]
- Cesslein B, Derrē-Bobillot A, Fernandez A, Lamberet G, Lechardeur D, Yamamoto Y, Pedersen MB, Garrigues C, Gaudu P. 2010. Respiration, a strategy to avoid oxidative

stress in *Lactococcus lactis*, is regulated by the heme status. Lactic Acid Bacteria 21: 10–15. [CrossRef]

- Sherrill C, Fahey RC. 1998. Import and metabolism of glutathione by *Streptococcus mutans*. J Bacteriol 180: 1454–1459. [Medline] [CrossRef]
- Yamamoto Y, Kamio Y, Higuchi M. 1999. Cloning, nucleotide sequence, and disruption of *Streptococcus mutans* glutathione reductase gene (*gor*). Biosci Biotechnol Biochem 63: 1056–1062. [Medline] [CrossRef]
- Barynin VV, Whittaker MM, Antonyuk SV, Lamzin VS, Harrison PM, Artymiuk PJ, Whittaker JW. 2001. Crystal structure of manganese catalase from *Lactobacillus plantarum*. Structure 9: 725–738. [Medline] [CrossRef]
- Hertel C, Schmidt G, Fischer M, Oellers K, Hammes WP. 1998. Oxygen-dependent regulation of the expression of the catalase gene katA of *Lactobacillus sakei* LTH677. Appl Environ Microbiol 64: 1359–1365. [Medline] [CrossRef]