# Transcriptomic Response of Enterococcus faecalis V583 to Low Hydrogen Peroxide Levels.

Xue Yan, Aurélie Budin-Verneuil, Nicolas Verneuil, Michael S Gilmore, Sébastien Artigaud, Yanick Auffray, Vianney Pichereau

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# Transcriptomic response of *Enterococcus faecalis* V583 to low hydrogen peroxide levels

Yan Xue [1], Budin-Verneuil Aurelie [1], Verneuil Nicolas [1], Gilmore Michael S. [2], Artigaud Sebastien [3], Auffray Yannick [1], Pichereau Vianney [1, 3, *]

[1] Univ Caen Basse Normandie, Unite Rech Risques Microbiens U2RM, Equipe Stress Virulence, F-14032 Caen, France.
[2] Harvard Univ, Sch Med, Dept Ophthalmol, Boston, MA USA.
[3] Univ Bretagne Occidentale UEB, Lab Sci Environm Marin LEMAR, Inst Univ Europeen Mer, IFREMER,IRD,UBO,CNRS,UMR 6539, F-29280 Plouzane, France.

* Corresponding author : Vianney Pichereau, email address : vianney.pichereau@univ-brest.fr

**Abstract :**

Enterococcus faecalis is a Gram-positive commensal bacterium inhabiting the gastrointestinal tracts of human and other mammals, but is also increasingly recognized as an opportunistic human pathogen. Oxidative stress is one of the major challenges encountered by enterococci, both in their natural environment and during infection. In this paper, we evaluated the transcriptomic response of E. faecalis to oxidative stress, and showed that transcript abundance was reduced for 93 genes and increased for 39 genes during growth in medium containing 1.75 mM H2O2. The presence of hydrogen peroxide affected several metabolic pathways, including a large decrease in ethanolamine utilization and methylglyoxal metabolism, and an increase in transcript abundance for several transport systems. In particular, four operons encoding iron transporters appeared highly induced. By contrast, in our experimental conditions, the expression of most of the genes known to be involved in the enterococcal response to oxidative stress, did not appear significantly altered.

## 1. Introduction

*Enterococcus faecalis* is a Lactic Acid Bacterium naturally inhabiting the gastrointestinal tracts of humans and other mammals, and used as a constituent of some probiotic food supplements [17,18,30]. However, *E. faecalis* also can cause life-threatening infections in humans, especially in the hospital environment [35,46]. Oxidative stress is one of the major challenges encountered by *E. faecalis* during its life cycle [23,34,50]. Nearly two decades of research have revealed that this bacterium possesses genes encoding antioxidant enzymes such as *sodA* (Superoxide dismutase, [41]), *katA* (Catalase, [14]), *gor* (Glutathione reductase), *nox* (NADH oxidase), *trx* (Thioredoxine reductase), *dps* (DNA-binding protein), *msrA* (Peptide methionine-S-sulfoxide reductase) [39], *npr* (NADH peroxidase, [44]), *ohr* (Organic hydroperoxide resistance protein, [43]), *ahpC* (Alkyl hydroperoxide reductase, [57]), *ahpF* (Peroxiredoxine reductase), and several transcriptional regulators involved in the oxidative stress response, as *hypR* [55], *perR*, *fur*, *zur* [56], *soxS* [54].

Global experimental approaches that allow monitoring of gene and protein expression (i.e., transcriptomics and proteomics, respectively) in response to a particular environmental condition, constitute powerful tools to understand the metabolic and regulatory networks involved in stress adaptation on a genome-wide scale. To date, only one proteomic study examined changes in expression of 23 proteins in *E. faecalis* cells exposed to hydrogen peroxide ($H_2O_2$) [12]. In this paper, we report global changes in transcript abundance in *E. faecalis* V583 during growth in the presence of $H_2O_2$.

## 2. Materials and methods

### *2.1. Bacterial strains and growth conditions*

In this work, we used *E. faecalis* V583 ery$^S$, an erythromycin sensitive derivative of the clinical isolate *E. faecalis* V583 [45]. Cells were cultivated with shaking in M17 medium supplemented with 0.5% (w/vol) glucose (GM17), supplemented with ±1.75 mM $H_2O_2$

### *2.2. Microarray experiments*

V583 ery$^S$ growth was monitored spectrophotometrically at 600 nm. When the culture reached mid-exponential phase (Fig. 1), cells were harvested and total RNA was extracted, purified, and residual DNA enzymatically removed as described previously [59]. Two independent experiments were performed for each condition. cDNA preparation, fragmentation, labeling and hybridization were performed as described in the Affymetrix manual ("GeneChip Expression Analysis Technical Manual: Procaryotic Target preparation" P/N 702232 Rev 2). Washing and scanning were performed using a GeneChip® Fluidics Station 450. The arrays were read at 570 nm with a resolution of 1.56 µm using an Affymetrix GeneChip Scanner 3000 7G (Affymetrix). Results were analyzed and compared using the GeneChip Operating Software (GCOS) version 1.4. Data were also analysed using R (version 3.0.0; R core team, 2013) and the Bioconductor software (version 2.12; [15]), to perform a t-test with a Benjamini and Hochberg multiple testing correction cut-off of $P < 0.05$ (data not shown). In all, we considered differences in transcript abundance significant if by GCOS they exhibited both a p-value below 0.05, and an absolute expression ratio significantly greater than 2 fold.

### *2.3. Real-Time quantitative PCR*

Real-Time qPCR was performed exactly as described by Giard *et al.* [16]. Specific primers were designed using the Primer3 software available at the web site http://frodo.wi.mit.edu/cgibin/primer3/primer3_www.cgi (Table 1) with the following

parameters: amplicon length (99 to 101 bp), primer length (19 to 21 nucleotides), and primer melting temperature (Tm from 59 to 61 °C). Total RNAs, treated with DNase I (GE Healthcare) and purified using the DNA-free kit (Ambion), were reverse transcribed using the Omniscript enzyme (Qiagen) and random hexamer primers according to the manufacturer's recommendations. RT qPCR was performed using the QuantiTect SYBR Green RT PCR kit (Qiagen). Quantification of 23S rRNA transcript levels was used as an internal control. All experiments were performed twice, and in duplicate with two different RNA samples, using the BioRad iCycler iQ detection system (Bio-Rad Laboratories, Richmond, CA). The value used for the comparison of genes expressions in stressed and unstressed cells was the number of PCR cycles required to reach the threshold cycle ($C_T$). To relate the $C_T$ value to the abundance of an mRNA species, $C_T$ was converted to "*n*-fold difference" by comparing mRNA abundance in the control cells to that obtained with the $H_2O_2$ stressed cells. The *n*-fold difference was calculated by the formula ($n = 2^{-x}$) when the $C_{T\ mutant} < C_{T\ V583}$ and ($n = -2^{x}$) when $C_{T\ mutant} > C_{T\ V583}$, with $x = (C_{T\ mutant} - C_{T\ V583})$.

## 3. Results and discussion

### *3.1. Growth of E. faecalis* V583 ery$^S$ *under oxidative stress.*

*E. faecalis* cells were cultured in the presence of increasing concentrations of $H_2O_2$, from 1.5 mM to 2 mM to identify a condition where $H_2O_2$ had a detectable effect on growth, but where cells were also able to successfully respond to the challenge. Results presented in the Fig. 1 showed that the inhibitory effect of $H_2O_2$ was mainly observable on the duration of the Lag phase, which increased from approx. 30 min to 3 h in the presence of 1.5 mM to 2 mM $H_2O_2$. We aimed at determining the effects of low doses of $H_2O_2$ on the global genes expression profiles of exponentially growing *E. faecalis* cells, which were previously shown to display differences in stress tolerance [12]. Therefore, total RNAs were extracted from

mid-exponential growth phase cells (Fig. 1), and then processed for transcriptome analyses.

*3.2. Transcriptome changes in response to $H_2O_2$ exposure.*

Whole genome expression profiling of *E. faecalis* V583 was performed using affymetrix chips, using total RNAs extracted from both unstressed- and stressed- (1.75 mM $H_2O_2$) cultures at an $OD_{600nm}$ of 0.6 (reached two and four hours after inoculation, respectively, Fig. 1). Comparative analysis showed that 132 genes were significantly differentially expressed in response to 1.75 mM $H_2O_2$, considering a ratio threshold of two fold (Tables 2 and 3). We selected 25 of the mostly deregulated genes, and subjected them to RT-qPCR (Table 4). It is noteworthy that we showed a very strong correlation ($r^2 = 0.86$) between the changes in genes expression observed with the whole genome transcriptomic- and the qPCR- experimental approaches.

Among the 132 genes for which transcript abundance changes significantly, 93 showed reduction and 39 were found to be increased. We classified these 132 genes into 9 groups according to the categories described in the comprehensive microbial resource of The Institute of Genome Research (http://www.tigr.org/tigr-scripts/CMR2/CMRHomePage.spl/). Figure 2 shows the number of differentially regulated genes in each functional class. The main observation is the large dominance of genes encoding proteins for which the function is annotated as unknown (60), and the abundance of genes encoding (i) transport and binding proteins (22 genes increased and 3 reduced), (ii) proteins involved in biosynthesis and metabolism (4 increased and 24 reduced), and (iii) proteins involved in regulation and cell signaling (8). Transcripts for only two genes annotated as involved in stress adaptation and virulence were found to be changed.

*3.3. Classification of genes by their regulation patterns.*

Table 2 and Table 3 identify genes associated with increased and decreased levels of mRNA, respectively, as a result of exposure to 1.75 mM $H_2O_2$. The most highly affected transcripts

are changed by about 10 fold. For example, the *ef3082-ef3085* operon encoding the 4 components of an iron compound ABC transport system, displayed increased transcript abundance from 9 to 11 fold, while the greatest decreases all related to ethanolamine metabolism, and displayed a reduction in transcript abundance reaching almost 12 fold.

To understand these changes in the global context of cellular metabolism, we searched for functional association networks using the STRING program ([13], available at http://string-db.org/). Results presented in Fig. 3 show that of the 132 genes for which significant transcript changes were observed, only 14 were disconnected from any node, and only 22 belonged to networks that included less than 4 partners (thus showing that 73% of the genes affected by $H_2O_2$ stress belonged to larger networks).

Gene ontology enrichment analyses showed that the main biological processes and molecular functions associated with global transcriptional change, again were those related to metal ion transport and ethanolamine metabolism P-values < $10^{-5}$ for each).

*3.3.1. Metabolism*

Ethanolamine metabolism

Both microarray and real time q-PCR (Table 3 and Table 4) showed that $H_2O_2$ decreased the the abundance of transcripts for 18 genes belonging to the *eut* operon involved in ethanolamine metabolism. Ethanolamine (EA) is an abundant compound in the human intestinal tract [24,26] and in processed food [7], and can be used as a source of carbon, nitrogen, and energy. However, growth of the V583 strain, and of RR17 (*ef1633*) a null mutant of *ef1633*, showed that EA provided with CoB12 allowed *E. faecalis* to grow, albeit poorly, only under anaerobic conditions, and the RR17 response regulator was required for this effect. A promoter upstream of RR17 is induced by EA-CoB12 in anaerobiosis [38]. The observation that most of the genes from EF1617 (*eutQ*) to EF1638 (*eutP*) were highly repressed by $H_2O_2$ in this study, could stem from a negative impact of $H_2O_2$ on either EA

and/or CoB12 in the medium, and reflects an important modulation of metabolism in enterococcal cells in response to oxidative stress. It is noteworthy that the Eut system was also shown to be negatively deregulated in (i) a microarray study of the Fsr system [5], and (ii) in *relA* mutants unable to produce (p)ppGpp [59,60] and unpublished data), showing that the regulation of the *Eut* operon is governed by many inputs. This new data adds to the understanding of the role of this system in the physiology of bacteria subjected to changing environments.

Methylglyoxal metabolism

The production of MG (methylglyoxal) in bacterial cells is maintained in balance with the capacity for detoxification and protection against this toxic electrophile, which can react with the nucleophilic centres of macromolecules such as DNA, RNA and proteins [4,11]. Table 3 and Table 4 show that the abundance of transcripts for 3 genes encoding glyoxalase family proteins (among the 9 genes identified in the genome with related function) is significantly reduced during the growth in the presence of $H_2O_2$. It should be noted that all other glyoxalase genes, and the *ef0939* gene encoding the methyl glyoxal synthase, were also negatively impacted, but by differences < 2 fold (Table 5). This indicates an important role for methylglyoxal metabolism under this stress. In enteric bacteria, three methylglyoxal detoxification routes have been identified, *i.e.* the glutathione-dependent glyoxalase I-II system [19], (ii) glutathione-independent glyoxalase III [31], and (iii) methylglyoxal reductases and dehydrogenases [19,32]. The 5 glyoxalase genes for which mRNA abundance decreased in the presence of $H_2O_2$ share sequence identity with genes of the glyoxalase I-II system. Although methylglyoxal is considered as an extremely toxic electrophile, potentially leading to the death of bacterial cell, several studies suggest that this compound is important for bacterial homeostasis. Indeed, methylglyoxal is thought to be important in bacteria for growth regulation, D-lactate production [8], the uncoupling of anabolism and catabolism [49],

191 the prevention of accumulation of phosphorylated intermediates [6,21], virulence [3],
192 programmed cell death [22,37], and oxidative stress [58]. Therefore, the reduction in the
193 abundance of transcripts encoding cellular glyoxalases suggests that tuning of methylglyoxal
194 intracellular content in $H_2O_2$ stressed cells is important in *E. faecalis* oxidative stress
195 adaptation.

196 *3.3.2. Transport*

197 Iron uptake

198 Table 2 and Table 4 show a notable increase in mRNA encoded by 10 genes belonging to 4
199 putative operons, with functions related to iron transport. Iron is an essential element for most
200 microbes, since many enzymes use iron as a cofactor [1]. During pathogenic processes, iron
201 acquisition systems are often found to be highly expressed to compensate for low iron
202 availability within the host [47]. Interestingly, the greatest increase in mRNA encoding an
203 iron transporter in our study (ef3082-3085), was also shown to be affected by growth in blood
204 [53]. Conversely, the expression of the *mntH* gene (ef1057), that was recently shown to be
205 strongly repressed in response to iron excess in *E. faecalis* OG1-RF [29], was not modified in
206 our experimental conditions. There is also an intimate relationship between iron metabolism
207 and oxidative stress. First, through the Fenton/Haber-Weiss reaction, iron can promote the
208 formation of hydroxyl radicals, which indiscriminately damage all cellular components [9,51].
209 Second, iron acts as a co-factor for several enzymes, including some involved in oxidative
210 stress defence, such as KatA or Dps.

211 The induction by $H_2O_2$ of genes with functions in iron uptake was also observed in *Bacillus*
212 *subtilis*, and could reflect an adaptive response to iron limitation [33]. Taken together, our
213 results suggest that $H_2O_2$-stressed cells encounter an intracellular iron limitation that could
214 result from iron recruitment by certain oxidative defence proteins (*e.g.* catalase), necessitating
215 increased transport capacity to meet the competing intracellular iron needs.

Stress related transporters

We observed in Table 2 and Table 3 that hydrogen peroxide resulted in increased abundance of transcripts of *kdpA, kdpB, kdpC, kdpD,* encoding the high affinity ABC potassium transport system Kdp, and *ef0575-ef0576* (cationic ABC transporter). At the same time, $H_2O_2$ exposure resulted in reduced levels of *cadA* (cadmium-translocating P-type ATPase), *ef0986* (cation transporter) and *nhaC* ($Na^+/H^+$ antiporter) mRNA. Select changes were verified by RT-qPCR (Table 4). All bacterial *kdp* operons investigated so far are repressed during growth in media of high external $K^+$ concentration ($[K^+]e$) [2]. Increased abundance of mRNA encoding the *kdp* operon under our experimental condition, suggests that $H_2O_2$ led to an intracellular $K^+$ limitation. Thus, the Kdp-ATPase is an efficient $K^+$ scavenging system that isexpressed when other $K^+$ transporters cannot support cellular requirement for $K^+$, which not only plays a vital role in bacterial osmotic adaptation [10,40] but is also important for pH regulation, gene expression and activation of cellular enzymes [42]. The most notable deficiency observed in the *nhaC* deletion strain of *Bacillus firmus* was its poor growth at pH 7.5 and $Na^+$ concentrations up to 25 mM [20], suggesting its contribution in osmotic stress. The cadmium cation is toxic to most microorganisms, probably by binding to essential respiratory proteins [52] and through oxidative damage by production of reactive oxygen species [48]. One of the best-characterized bacterial cadmium resistance mechanisms is determined by the cadmium-transporting ATPase encoded by *cadA* found in *Staphylococcus aureus* [36] and *Listeria monocytogenes* [27,28]. Various stresses (NaCl, heat, ethanol, acidity and alkalinity) induced weak or strong $H_2O_2$ cross-protection in *E. faecalis* ATCC19433 [12]. Combined results suggest that the pre-treatment of $H_2O_2$ could increase the bacterial osmotolerance and resistance to antibiotic, and decrease the bacterial resistance to cadmium.

*3.3.3. Genes involved in the adaptation to oxidative stress*

Expression of other genes clearly involved in the adaptation to oxidative stress did not

241 significantly change in GM17 medium supplemented with 1.75 mM $H_2O_2$ (Table 6). These
242 genes may already be expressed at a high level in laboratory growth. Interestingly, although
243 not reaching the criteria used for significance in this study, the abundance of transcripts
244 encoding *msrB* (Peptide methionine-S- sulfoxide reductase [25]) was reduced 1.65 fold by
245 $H_2O_2$. The repression of *msrB* in 2.5 mM $H_2O_2$ adaptation for 30 minutes and the resistance of
246 Δ*msrB* double crossing over mutant to the lethal challenge of 7 mM $H_2O_2$ for 6 hours
247 observed in *E. faecalis*, suggests that *msrB* contributes negatively to adaptation to oxidative
248 stress [61]. It therefore was not surprising that when *E. faecalis* is facing the oxidative stress
249 of $H_2O_2$, a reduction in transcripts for *msrB* was observed.

250 *3.4. Concluding remarks*

251 Global changes in the abundance of mRNA for all genes in exponentially growing *E. faecalis*
252 cells, cultured in the presence of a low dose (1.75 mM) of $H_2O_2$, was measured. At this time
253 (4 h), *E. faecalis* had almost completely adapted as evinced by restoration of its growth rate
254 (Fig. 1). Analysis of changes in transcript abundance in response to this stress revealed that (i)
255 the expression of most of the well-known oxidative stress genes of *E. faecalis* was not
256 significantly altered, possibly because they already were highly expressed in rapid growth in
257 laboratory conditions, or because they may be regulated post-transcriptionally to minimize the
258 time delay associated with response to a potentially lethal condition. Among the responses we
259 did observe, we found that under $H_2O_2$ stress, *E. faecalis* restricted the metabolism of
260 ethanolamine, and limited the metabolism of methylglyoxal, but increased its use of transport
261 systems to satisfy the specific demand for essential metal ions, such as iron. Concomitantly,
262 we observed decreased expression of some genes known to contribute to adaptation to other
263 stresses (*e.g. nhaC*, *cadA*). Future work will aim at determining whether and how (i)
264 metabolic changes in ethanolamine and methylglyoxal metabolism, and iron transport,
265 impact the adaptation of *E. faecalis* to these specific conditions; and (ii) how changes in

expression of genes clearly involved in the adaptation to other stresses affect cross adaptation. It will also be of importance to determine the role of the 60 genes encoding hypothetical proteins that were shown to be deregulated in these conditions.

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## Legends to figures

Fig. 1. Growth of V583 ery$^S$ with shaking in GM17 medium or supplemented with 1.5mM, 1.75mM and 2mM $H_2O_2$. The arrows indicate the time at which RNA was extracted.

Fig. 2. Functional classification of genes with statistically significant increases (■) and decreases (□) in mRNA level upon 4 h exposure to hydrogen peroxide (total of 213 genes). The total number of genes associated with increased/decreased mRNA abundance as the result of $H_2O_2$ exposure in each functional class is shown.

Fig. 3. Network analysis of genes shown to be associated with significant transcript changes (built using STRING v9.1 [13]).

447 Table 1. Oligonucleotide primers used for real-time quantitative PCR in this study.

| | |
|---|---|
| 0188L: TCGCAGCTTGTGGTAATACG | 0188R: ACTCGCAATAATCCGTTTGG |
| 0191L: GGAACACCGCAAGAAGTGAT | 0191R: GCCGCTGTGTCTGACTATGA |
| 0475L: TCGAATTGCGCTGACAGATA | 0475R: TAGCGCGTTCTCCAACTTTT |
| 0567L: CGCTATGCACTGAATGTGCT | 0567R: ACCTCCCGGTAAAAAGTGCT |
| 0570L: GCTGGTGTTGGGAAAACCTA | 0570R: GGACGATCATGAGGTTCGAT |
| 0575L: AGGCTTTGGAAAAGGTCGAT | 0575R: AAATCCGCATTTTGAGCAAG |
| 0630L: AGTCAACCACGTGGGGATTA | 0630R: TTCCACCAATTTCATCACGA |
| 0666L: TCTTGAGCAAGCGGGTATCT | 0666R: AGCGCCATTTGCGTATTATC |
| 1120L: TTAAAGTGATGGGCGTTTCC | 1120R: TGCGAAGGATAAGCAGAAGC |
| 1617L: ACCCGAAATCGACGTAACAG | 1617R: GGCGCGGACTTTCTTCTAA |
| 1620L: TGAAGGTAAGCCGGTATTGG | 1620R: GTCCACGTTTTCCTGAATCG |
| 1621L: CAACCTGGACAGTTCGTTTGT | 1621R: TGTGATTCGCTACGTTCAGG |
| 1624L: AAATTGGCACCGATTATTGC | 1624R: ATTGTATGACCCGCACCTTC |
| 1627L: CACGTCACTGAAACGACAGAA | 1627R: CAGGATCTACGGCATTTTTCA |
| 1629L: ATCCATCGCTGGTTCAGAAA | 1629R: AGCTGCTTGACCACTGTGAA |
| 1633L: TTAGGCGCACTGGGTTATTT | 1633R: TAACAGCTGCGTTTGTTTGC |
| 1635L: CAGGGATGGCGTTTAATGTT | 1635R: TTGCATTCATTCGTCCATGT |
| 1638L: ATAAAACACAGGCGGTGGAA | 1638R: GCCGCTGTGACGTTTAATG |
| 1669L: GCCCAAGTCGTTTTGGTAGA | 1669R: TGATTTACGATCCGCTAGGC |
| 3085L: AACCGATAGTGCAAGGTTGG | 3085R: CCGCTTAGCGCAAAAATAAA |

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449

Table 2. Genes of *Enterococcus faecalis* V583 ery$^S$ associated with increased transcript abundance as the result of exposure to $H_2O_2$.

| Locus | Descriptions (Gene symbol) | M* | SD |
|---|---|---|---|
| Biosynthesis and metabolism | | | |
| EF0693 | 1-phosphofructokinase (*fruK-1*) | 1.1 | 0.3 |
| EF1222 | adenine deaminase (*ade*) | 1.4 | 0.2 |
| EF1511 | mandelate racemase/muconate lactonizing enzyme family protein | 1.1 | 0.4 |
| EF2999 | allantoinase, putative | 1.1 | 0.1 |
| Transport and binding protein | | | |
| EF0188 | iron compound ABC transporter, substrate-binding protein | 2.0 | 0.6 |
| EF0191 | ferrichrome ABC transporter, ATP-binding protein | 1.1 | 0.3 |
| EF0192 | ferrichrome ABC transporter, permease protein | 1.0 | 0.1 |
| EF0193 | ferrichrome ABC transporter, permease protein (*fhuG*) | 1.0 | 0.3 |
| EF0475 | ferrous iron transport protein A (*feoA*) | 1.3 | 0.0 |
| EF0476 | ferrous iron transport protein B (*feoB*) | 1.6 | 0.1 |
| EF0567 | potassium-transporting ATPase, subunit A (*kdpA*) | 1.2 | 0.4 |
| EF0568 | potassium-transporting ATPase, subunit B (*kdpB*) | 1.2 | 0.4 |
| EF0569 | potassium-transporting ATPase, subunit C (*kdpC*) | 1.2 | 0.1 |
| EF0575 | cationic ABC transporter, ATP-binding protein | 1.0 | 0.2 |
| EF0576 | cation ABC transporter, permease protein | 1.1 | 0.1 |
| EF0694 | PTS system, fructose-specific family, IIBC components | 1.0 | 0.6 |
| EF1053 | ABC transporter, ATP-binding protein | 1.0 | 0.5 |
| EF1117 | amino acid ABC transporter, permease protein | 2.2 | 0.4 |
| EF1118 | amino acid ABC transporter, permease protein | 2.3 | 0.2 |
| EF1119 | amino acid ABC transporter, amino acid-binding protein | 2.0 | 0.1 |
| EF1120 | amino acid ABC transporter, ATP-binding protein | 1.8 | 0.1 |
| EF1220 | spermidine/putrescine ABC transporter, ATP-binding protein | 1.0 | 0.2 |
| EF3082 | iron compound ABC transporter, substrate-binding protein | 3.1 | 0.8 |
| EF3083 | iron compound ABC transporter, ATP-binding protein | 3.2 | 0.6 |
| EF3084 | iron compound ABC transporter, permease protein | 3.2 | 0.7 |
| EF3085 | iron compound ABC transporter, permease protein | 3.3 | 0.6 |
| Regulation and signal | | | |
| EF0570 | sensor histidine kinase (*kdpD*) | 1.0 | 0.1 |
| EF0578 | helix-turn-helix protein, iron-dependent repressor family | 1.3 | 0.1 |
| Cell envelope and cell division | | | |
| EF0887 | glycosyl transferase, group 2 family protein | 1.5 | 0.1 |
| EF3314 | cell wall surface anchor family protein | 1.5 | 0.1 |
| Stress and Virulence | | | |
| EF2068 | multidrug resistance protein, putative | 1.2 | 0.1 |
| Unknown | | | |
| EF0477 | hypothetical protein | 1.7 | 0.8 |
| EF0886 | hypothetical protein | 1.4 | 0.2 |
| EF0888 | conserved hypothetical protein | 1.0 | 0.1 |
| EF0889 | conserved hypothetical protein | 1.2 | 0.4 |
| EF1223 | chlorohydrolase family protein | 1.2 | 0.6 |
| EF1512 | conserved hypothetical protein | 1.1 | 0.3 |
| EF1808 | agaS protein | 1.1 | 0.4 |
| EF2067 | conserved hypothetical protein TIGR00481 | 1.1 | 0.0 |

*M is the mean Log2(fold ratio); SD is the standard deviation.

454 Table 3. Genes of *Enterococcus faecalis* V583 ery$^S$ associated with transcript reductions as
455 the result of exposure to $H_2O_2$.

| Locus | Descriptions (Gene symbol) | M* | SD |
|---|---|---|---|
| Biosynthesis and metabolism | | | |
| EF0098 | L-serine dehydratase, iron-sulfur-dependent, beta subunit (*sdhB-1*) | -2.4 | 0.2 |
| EF0099 | L-serine dehydratase, iron-sulfur-dependent, alpha subunit (*sdhA-1*) | -2.7 | 0.6 |
| EF0630 | glyoxalase family protein | -1.0 | 0.1 |
| EF0867 | glyoxalase family protein | -1.2 | 0.1 |
| EF1358 | glycerol dehydrogenase, putative | -1.6 | 0.5 |
| EF1617 | conserved hypothetical protein (*EutQ*) | -2.6 | 0.5 |
| EF1618 | ethanolamine utilization protein (*EutH*) | -2.7 | 0.6 |
| EF1619 | carbon dioxide concentrating mechanism protein CcmL, putative (*EutN*) | -2.8 | 0.3 |
| EF1620 | hypothetical protein (*EutX*) | -2.9 | 0.5 |
| EF1621 | conserved hypothetical protein (*EutY*) | -2.8 | 0.5 |
| EF1622 | conserved domain protein (*EutZ*) | -3.0 | 0.3 |
| EF1623 | microcompartment protein (*EutK*) | -2.6 | 0.4 |
| EF1624 | aldehyde dehydrogenase, putative (*EutE*) | -3.1 | 0.1 |
| EF1625 | microcompartment protein family (*EutM*) | -3.0 | 0.0 |
| EF1626 | ethanolamine utilization protein (*EutL*) | -3.1 | 0.1 |
| EF1627 | ethanolamine ammonia-lyase small subunit (*EutC*) | -3.3 | 0.1 |
| EF1629 | ethanolamine ammonia-lyase large subunit (*EutB*) | -3.4 | 0.1 |
| EF1632 | sensor histidine kinase (*HK*) | -1.1 | 0.1 |
| EF1633 | response regulator (*RR*) | -1.1 | 0.1 |
| EF1634 | propanediol utilization protein PduU (*EutS*) | -1.1 | 0.1 |
| EF1635 | propanol dehydrogenase PduQ, putative (*EutG*) | -2.1 | 0.0 |
| EF1637 | ATP:cob(I)alamin adenosyltransferase, putative (*EutT*) | -1.7 | 0.0 |
| EF1638 | propanediol utilization protein PduV (*eutP*) | -1.3 | 0.1 |
| EF1669 | glyoxylase family protein | -1.0 | 0.1 |
| Protein biosynthesis and fate | | | |
| EF0100 | seryl-tRNA synthetase (*serS-1*) | -3.4 | 0.1 |
| EF0650 | lipoate-protein ligase A (*lplA-1*) | -1.4 | 0.1 |
| DNA metabolism | | | |
| EF2114 | adenine methyltransferase, putative | -1.7 | 0.1 |
| Transport and binding protein | | | |
| EF0636 | Na+H+ antiporter (*nhaC-2*) | -1.1 | 0.2 |
| EF0986 | cation transporter | -1.0 | 0.1 |
| EF2623 | cadmium-translocating P-type ATPase (*cadA*) | -2.4 | 0.4 |
| Regulation and signal | | | |
| EF0097 | regulatory protein pfoR, putative | -2.0 | 0.3 |
| EF0110 | transcriptional regulator, ArsR family | -1.2 | 0.1 |
| EF0143 | transcriptional regulator, CroCI family | -1.2 | 0.5 |
| EF0923 | transcriptional regulator, LysR family | -1.2 | 0.2 |
| EF1668 | transcriptional regulator, MarR family | -1.4 | 0.1 |
| EF2141 | transcriptional regulator, CroCI family | -1.3 | 0.1 |
| Cell envelope and cell division | | | |
| EF0139 | FtsKSpoIIIE family protein | -1.1 | 0.0 |
| EF0153 | cell wall surface anchor family protein | -1.1 | 0.2 |

| | | | |
|---|---|---|---|
| Stress and virulence | | | |
| EF0149 | aggregation substance, putative | -1.4 | 0.2 |
| | | | |
| Mobile and extrachromosomal element functions | | | |
| EF0158 | conjugal transfer protein, putative | -1.3 | 0.4 |
| EF2096 | tail protein | -1.5 | 0.1 |
| | | | |
| Unknown | | | |
| EF0083 | hypothetical protein | -1.1 | 0.6 |
| EF0109 | ThiJPfpI family protein | -1.1 | 0.1 |
| EF0131 | conserved domain protein | -1.5 | 0.2 |
| EF0132 | hypothetical protein | -1.4 | 0.2 |
| EF0134 | hypothetical protein | -1.1 | 0.1 |
| EF0135 | conserved hypothetical protein | -1.0 | 0.1 |
| EF0136 | hypothetical protein | -1.1 | 0.1 |
| EF0138 | conserved domain protein | -1.1 | 0.0 |
| EF0142 | conserved hypothetical protein | -1.0 | 0.3 |
| EF0144 | conserved domain protein | -1.2 | 0.3 |
| EF0151 | hypothetical protein | -1.2 | 0.4 |
| EF0152 | hypothetical protein | -1.0 | 0.6 |
| EF0154 | conserved hypothetical protein | -1.7 | 0.1 |
| EF0155 | conserved hypothetical protein | -1.6 | 0.4 |
| EF0415 | conserved hypothetical protein | -1.4 | 0.4 |
| EF0519 | hypothetical protein | -1.0 | 0.1 |
| EF0971 | conserved hypothetical protein | -1.1 | 0.1 |
| EF1327 | BadFBadGBcrABcrD ATPase family protein | -1.4 | 0.5 |
| EF1359 | conserved hypothetical protein | -1.3 | 0.3 |
| EF1360 | dihydroxyacetone kinase family protein | -1.2 | 0.4 |
| EF1361 | dihydroxyacetone kinase family protein | -1.4 | 0.2 |
| EF1362 | conserved domain protein | -1.1 | 0.1 |
| EF2014 | coenzyme F420 hydrogenase domain protein | -1.0 | 0.1 |
| EF2065 | conserved hypothetical protein | -1.0 | 0.1 |
| EF2094 | hypothetical protein | -1.1 | 0.1 |
| EF2095 | hypothetical protein | -1.1 | 0.3 |
| EF2097 | hypothetical protein | -1.5 | 0.1 |
| EF2098 | hypothetical protein | -1.4 | 0.2 |
| EF2099 | hypothetical protein | -1.6 | 0.1 |
| EF2100 | hypothetical protein | -1.5 | 0.1 |
| EF2101 | hypothetical protein | -1.6 | 0.2 |
| EF2102 | hypothetical protein | -1.7 | 0.4 |
| EF2103 | hypothetical protein | -1.6 | 0.3 |
| EF2104 | hypothetical protein | -1.7 | 0.1 |
| EF2105 | hypothetical protein | -1.7 | 0.1 |
| EF2106 | conserved domain protein | -1.6 | 0.1 |
| EF2107 | hypothetical protein | -1.7 | 0.1 |
| EF2108 | hypothetical protein | -1.6 | 0.3 |
| EF2109 | conserved domain protein | -1.8 | 0.0 |
| EF2110 | hypothetical protein | -1.6 | 0.0 |
| EF2111 | hypothetical protein | -1.7 | 0.1 |
| EF2112 | hypothetical protein | -1.7 | 0.1 |
| EF2113 | conserved hypothetical protein | -1.6 | 0.3 |
| EF2120 | conserved hypothetical protein | -1.0 | 0.6 |
| EF2125 | hypothetical protein | -1.0 | 0.3 |
| EF2127 | hypothetical protein | -1.0 | 0.2 |

| | | | |
|---|---|---|---|
| EF2133 | hypothetical protein | -1.0 | 0.3 |
| EF2134 | hypothetical protein | -1.0 | 0.6 |
| EF2137 | hypothetical protein | -1.2 | 0.3 |
| EF2140 | conserved hypothetical protein | -1.2 | 0.2 |
| EF2606 | conserved hypothetical protein | -1.0 | 0.1 |
| EF2792 | conserved hypothetical protein | -1.0 | 0.0 |

*M is the mean Log2(fold ratio); SD is the standard deviation.

Table 4. Comparison of gene expression analyzed by real-time quantitative PCR and Microarray.

| ***Locus*** | ***(Gene symbol)* Function** | **$H_2O_2$/GM17** | |
|---|---|---|---|
| | | RT qPCR | Microarray |
| *ef0188* | iron compound ABC transporter, substrate-binding protein | 9.24±1.95 | 3.94±1.20 |
| *ef0191* | ferrichrome ABC transporter, ATP-binding protein | 3.33±0.70 | 2.20±0.44 |
| *ef0475* | (feoA) ferrous iron transport protein A | 3.16±0.99 | 2.48±0.03 |
| *ef0567* | (kdpA) potassium-transporting ATPase, subunit A | 2.72±1.27 | 2.27±0.34 |
| *ef0570* | (kdpD) sensor histidine kinase KdpD | 1.67±0.38 | 1.99±0.12 |
| *ef0575* | cationic ABC transporter, ATP-binding protein | 2.37±0.77 | 1.92±0.25 |
| *ef1120* | amino acid ABC transporter, ATP-binding protein | 6.44±0.25 | 3.52±0.03 |
| *ef3085* | iron compound ABC transporter, permease protein | 18.1±7.18 | 11.2±3.81 |
| *ef0630* | glyoxalase family protein | -3.92±0.91 | -2.00±0.14 |
| *ef0867* | glyoxalase family protein | -2.67±0.40 | -2.33±0.12 |
| *ef1617* | (eutQ) conserved hypothetical protein | -8.16±0.48 | -6.10±1.41 |
| *ef1620* | (eutX) hypothetical protein | -8.62±0.50 | -7.50±1.41 |
| *ef1621* | (eutY) conserved hypothetical protein | -3.41±0.95 | -7.00±1.41 |
| *ef1624* | (eutE) aldehyde dehydrogenase, putative | -9.18±0.60 | -8.60±1.07 |
| *ef1627* | (eutC) ethanolamine ammonia-lyase small subunit | -10.1±0.46 | -9.80±1.07 |
| *ef1629* | (eutB) ethanolamine ammonia-lyase large subunit | -7.76±0.45 | -10.6±1.07 |
| *ef1633* | (RR17) response regulator | -4.13±0.24 | -2.10±1.07 |
| *ef1635* | (eutG) propanol dehydrogenase PduQ, putative | -11.7±0.85 | -4.30±1.07 |
| *ef1638* | (eutP) propanediol utilization protein PduV | -2.82±0.26 | -2.50±1.07 |
| *ef1669* | glyoxylase family protein | -2.90±1.15 | -2.04±0.04 |

Values are given as means of the fold ratios ± standard deviation (SD).

463 Table 5. Expression of all the genes encoding glyoxalase family protein analyzed by

464 Microarray.

| ***Locus*** | **Function** | **Level of gene expression** | | | | **Ratio** |
|---|---|---|---|---|---|---|
| | | **GM17** | **$H_2O_2$** | **GM17** | **$H_2O_2$** | **$H_2O_2$/GM17** |
| *ef0358* | glyoxalase family protein | 330.7 | 164.6 | 408.5 | 224.2 | -1.92±0.10 |
| *ef0939* | methylglyoxal synthase | 1627.9 | 961 | 1806.1 | 909.7 | -1.84±0.15 |
| *ef0630* | glyoxalase family protein | 1152.1 | 541.5 | 945.8 | 508.4 | -2.00±0.14 |
| *ef0656* | glyoxalase family protein | 138.8 | 112.8 | 236.1 | 178.6 | -1.28±0.04 |
| *ef0666* | glyoxalase family protein | 2717 | 1834 | 2571.2 | 1811.8 | -1.45±0.03 |
| *ef0745* | glyoxalase family protein | 1003.8 | 974.3 | 1216.9 | 1119.1 | -1.06±0.03 |
| *ef0867* | glyoxalase family protein | 253.7 | 114.8 | 292.7 | 119.8 | -2.33±0.12 |
| *ef1669* | glyoxylase family protein | 260.3 | 125.4 | 314.8 | 161 | -2.04±0.04 |
| *ef2591* | glyoxalase family protein | 3065.4 | 3012.3 | 3317.9 | 2821.4 | -1.10±0.08 |
| *ef3092* | glyoxalase family protein | 1302.2 | 1004.4 | 1238.1 | 937.8 | -1.31±0.01 |

465 Values are given as means of the fold ratios ± standard deviation (SD).

466

467

468 Table 6. Expression of the genes known to be involved in enterococcal adaptation to oxidative

469 stress.

| ***Locus*** | **Protein (*Gene*)** | **Expression of genes** | | **Ratio** |
|---|---|---|---|---|
| | | **GM17** | **GM17 +$H_2O_2$** | **$H_2O_2$/GM17** |
| Oxidative stress proteins | | | | |
| *ef0453* | Organic hydroperoxide resistance protein (*ohr*) | 3712.9 | 3449.6 | -1.07 |
| *ef0463* | Superoxide dismutase (*sodA*) | 4697.8 | 4342.7 | -1.08 |
| *ef0606* | DNA-binding protein (*dps*) | 384.5 | 370 | -1.04 |
| *ef1211* | NADH peroxidase (*npr*) | 4228.5 | 3577.3 | -1.18 |
| *ef1338* | Thioredoxine reductase (*trx*) | 3292.4 | 2649.6 | -1.24 |
| *ef1405* | Thioredoxine reductase (*trx*) | 1787.7 | 1772.6 | -1.01 |
| *ef1586* | NADH oxidase (*nox*) | 5842.3 | 6058.5 | 1.04 |
| *ef1597* | Catalase (*katA*) | 2734.8 | 2806.2 | 1.03 |
| *ef1681* | Peptide methionine-S-sulfoxide reductase (*msrA*) | 2144 | 1908.5 | 1.12 |
| *ef2738* | Peroxiredoxine reductase (*ahpF*) | 3974 | 3984.6 | 1.00 |
| *ef2739* | Alkyl hydroperoxide reductase (*ahpC*) | 4536.4 | 4583 | 1.01 |
| *ef3164* | Peptide methionine-S-sulfoxide reductase (*msrB*) | 2449 | 1478.1 | -1.65 |
| *ef3233* | DNA-binding protein (*dps*) | 4553.2 | 4202.9 | -1.08 |
| *ef3270* | Glutathione reductase (*gor*) | 2707.7 | 2879.4 | 1.06 |
| Oxidative stress related transcriptional regulators | | | | |
| *ef1525* | (*fur*) | 2925.4 | 2397.8 | -1.22 |
| *ef1585* | (*perR*) | 2741.6 | 2304.2 | -1.19 |
| *ef2063* | (*soxS*) | 1847.9 | 1969.6 | 1.07 |
| *ef2417* | (*zur*) | 1017.1 | 804.2 | -1.26 |
| *ef2958* | (*hypR*) | 245.1 | 256.9 | 1.05 |

470

471

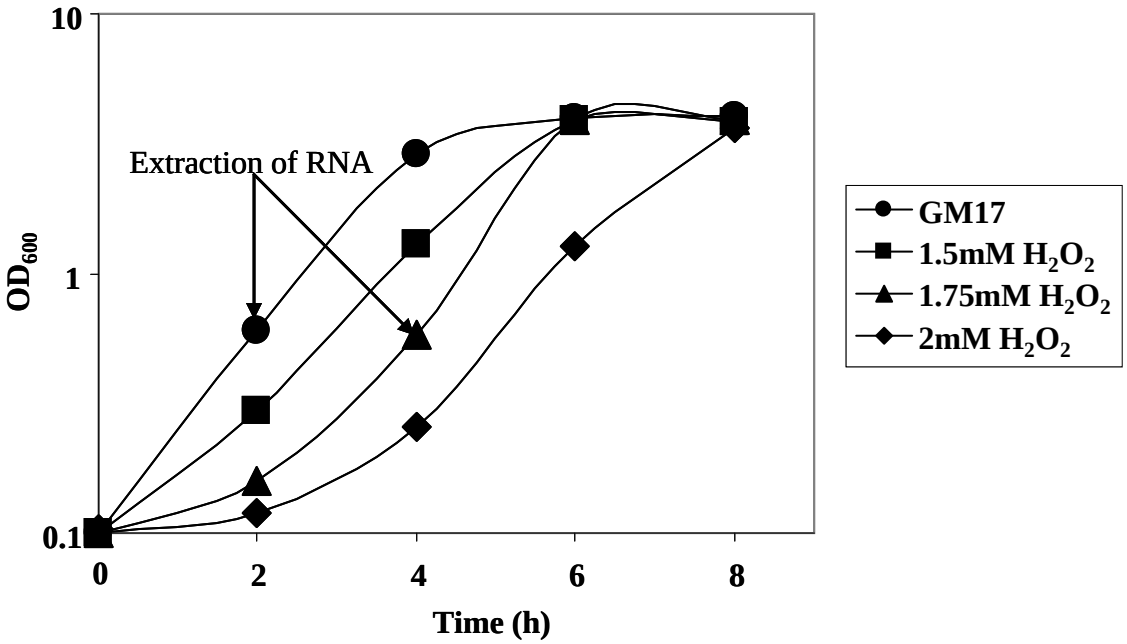
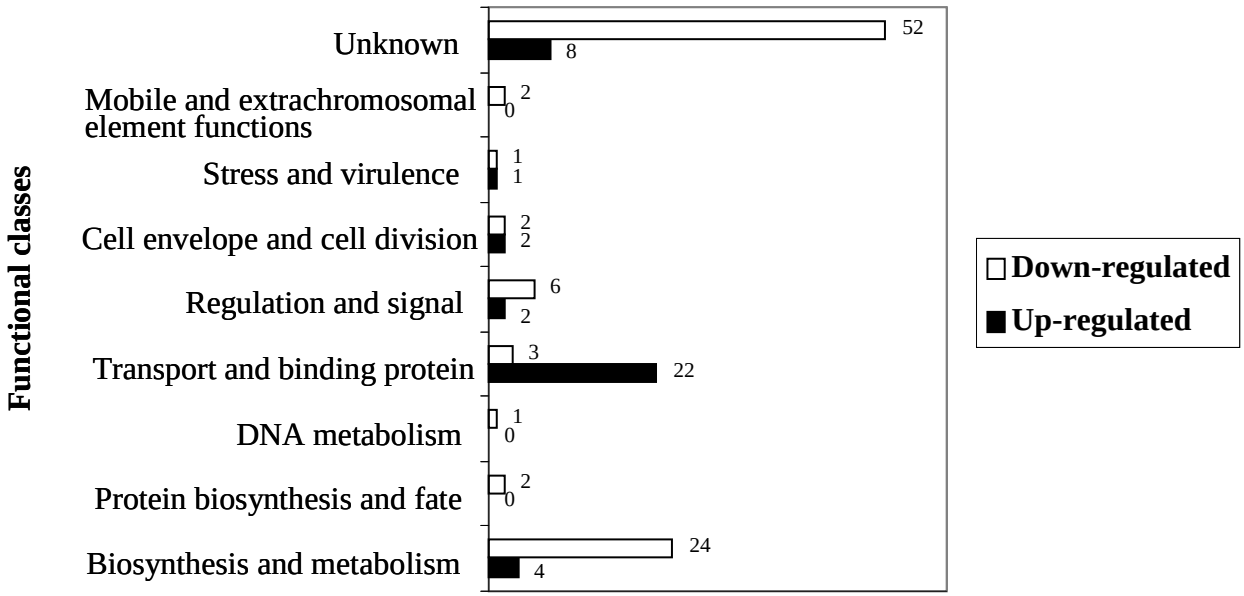

10
1
0.1
OD600
Extraction of RNA
0
2
4
6
8
Time (h)
GM17
1.5mM H2O2
1.75mM H2O2
2mM H2O2

Fig. 1.


Functional classes
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52
8
Mobile and extrachromosomal element functions
2
0
Stress and virulence
1
1
Cell envelope and cell division
2
2
Regulation and signal
6
2
Transport and binding protein
3
22
DNA metabolism
1
0
Protein biosynthesis and fate
2
0
Biosynthesis and metabolism
24
4
Down-regulated
Up-regulated

Fig. 2.

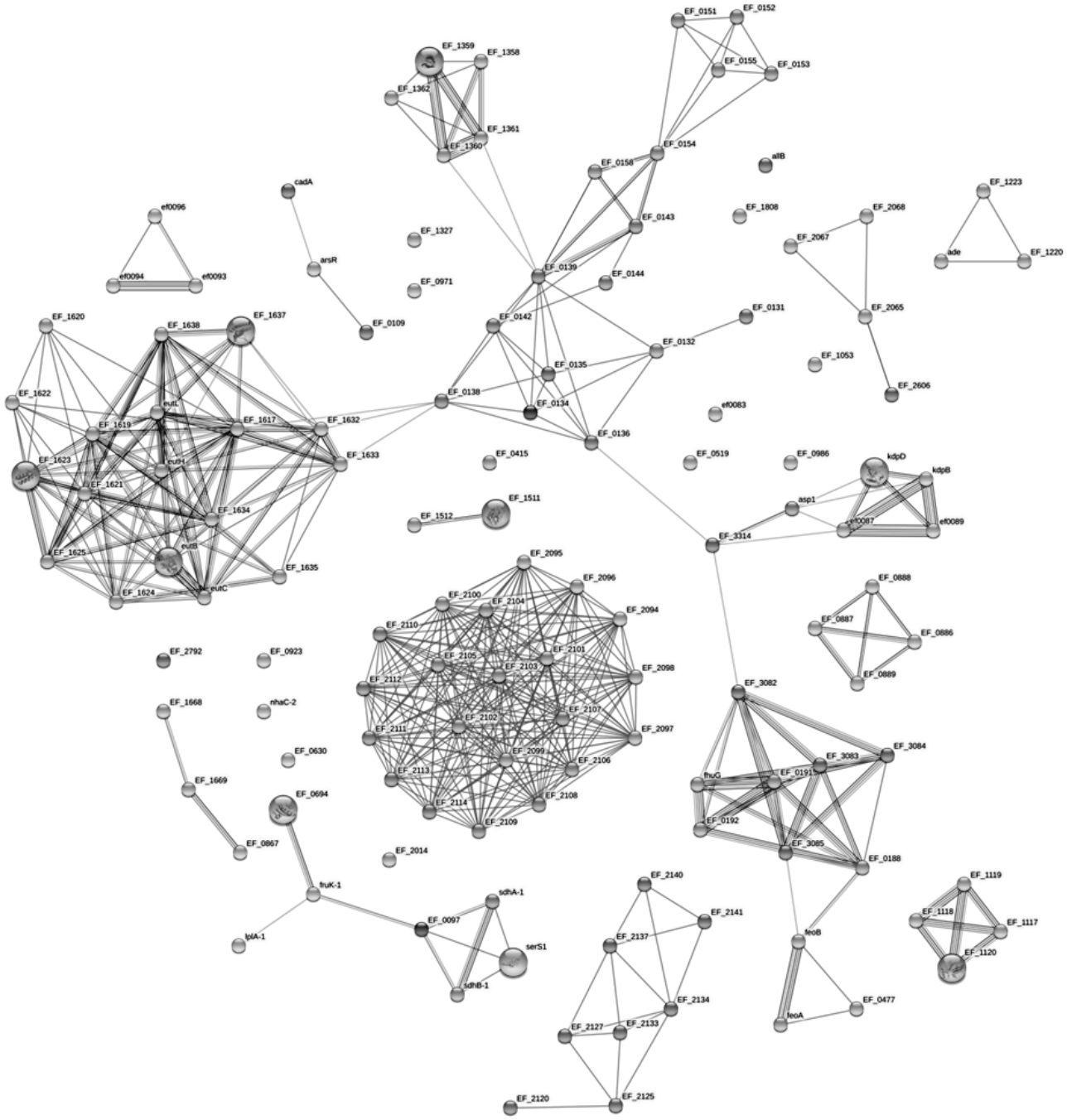


Fig. 3