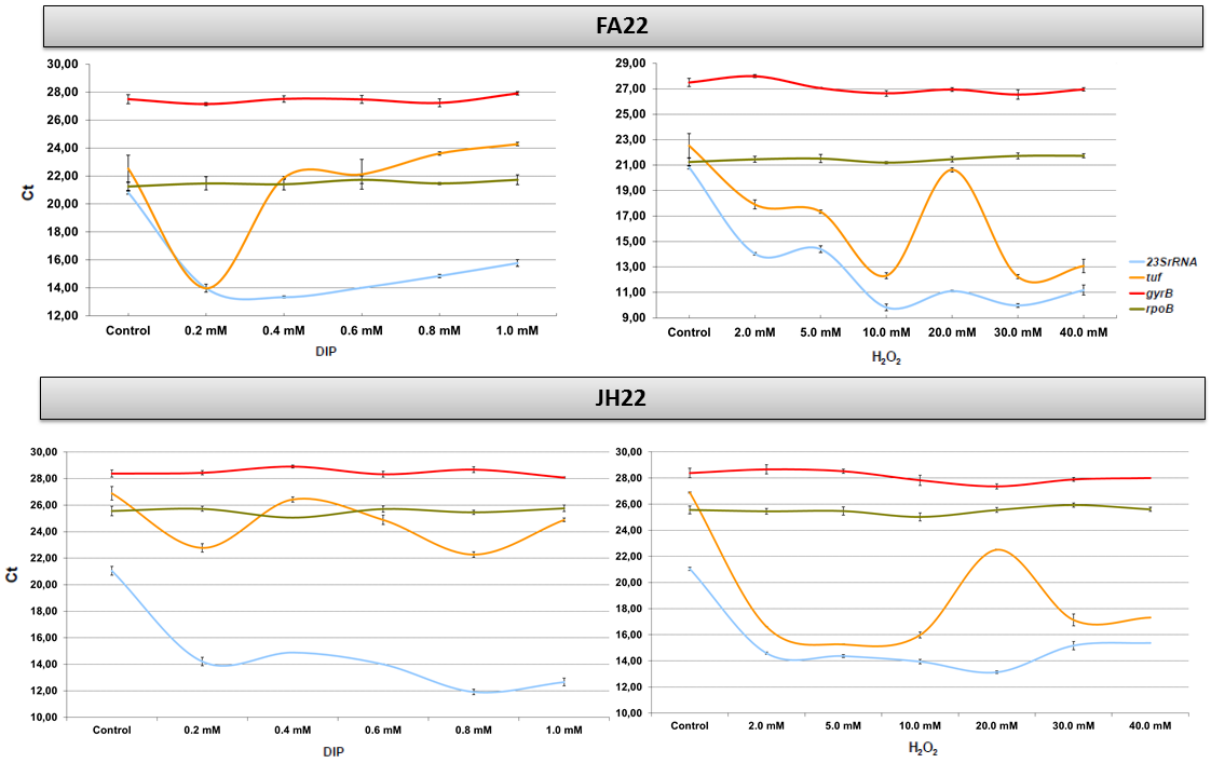
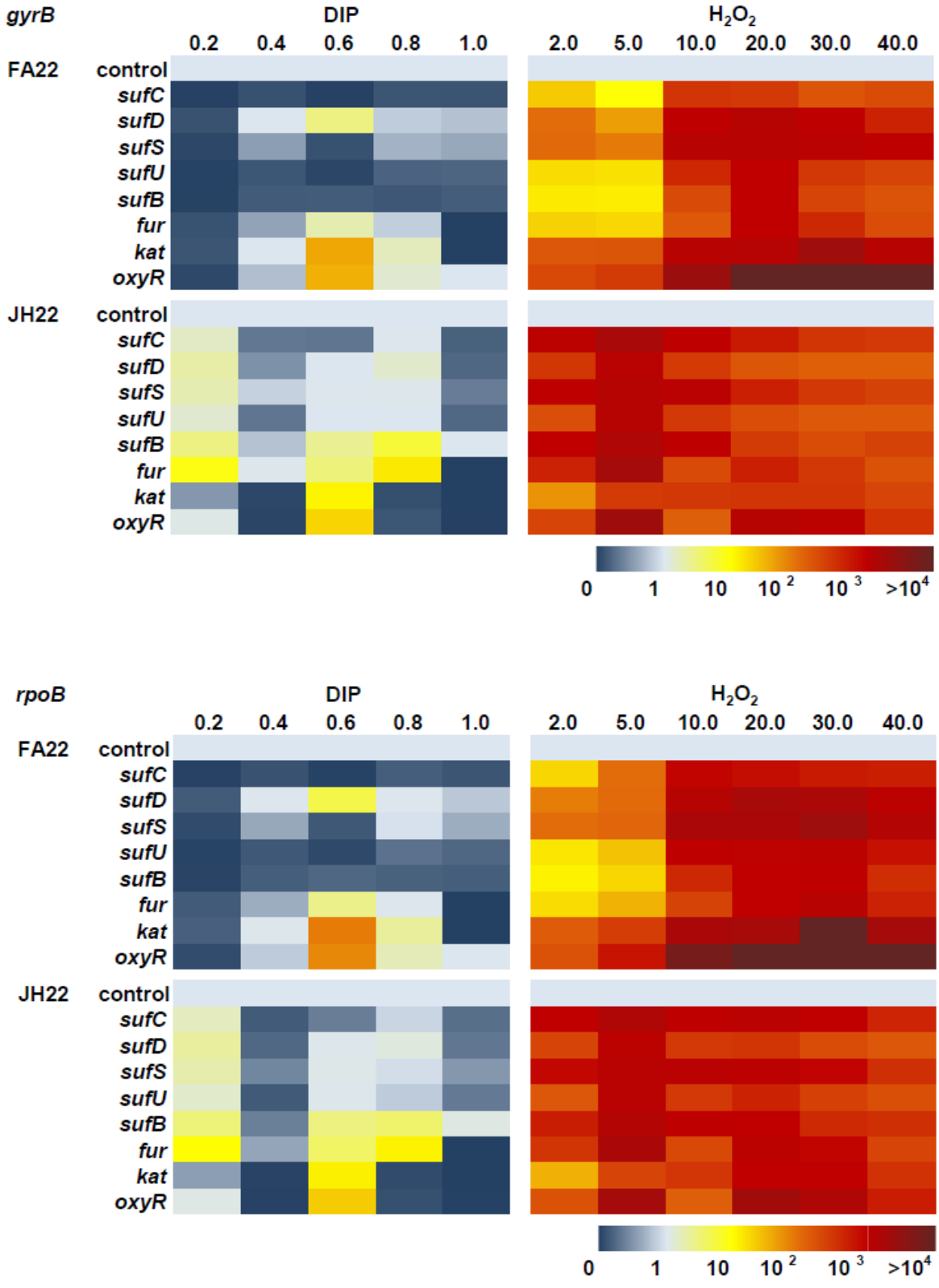


Quantitative polymerase chain reaction critical threshold (Ct) values for gyrase beta chain (*gyrB*), RNA polymerase beta chain (*rpoB*), elongation factor for transporter RNA (*tuf*) and 23S ribosomal RNA (*23SrRNA*) genes of *Enterococcus faecalis* strains FA22 and JH22 exposed to increasing conditions of iron depletion [2',2'-dipyridyl (DIP)] and oxidative stress [hydrogen peroxide (H_2O_2)].

$^{\Delta\Delta}$Ct data following normalisation with the constitutive genes gyrase beta chain (*gyrB*) or RNA polymerase beta chain (*rpoB*). Graphics are relative to experiments performed on *Enterococcus faecalis* strains FA22 and JH22 in the presence of increasing conditions of iron depletion [2',2'-dipyridyl (DIP): 0.2-1.0 mM] and oxidative stress [hydrogen peroxide (H_2O_2): 2.0-40 mM], as depicted in the labels. Error bars indicate data variance.



General relative gene expressions ($2^{-\Delta\Delta Ct}$) of *Enterococcus faecalis* strains FA22 and JH22 *sufCDSUB*, *fur*, *kat* and *oxyR* genes normalised to the expression patterns of the constitutive genes gyrase beta chain (*gyrB*) (upper panels) or RNA polymerase beta chain (*rpoB*) (lower panels). Cellular stress conditions comprehended increasing conditions of iron depletion [2',2'-dipyridyl (DIP): 0.2-1.0 mM] and oxidative stress [hydrogen peroxide (H_2O_2) 2.0-40 mM], as indicated. Colours relate to the degree of relative gene expression from absent (blue) to high expression (dark red), as depicted in the label.

Bioinformatics procedures

Enterococcus faecalis V583 and *Escherichia coli K-12* genome annotations were used for all *in silico* analyses. The Database of Prokaryotic Operons - DOOR (csbl1.bmb.uga.edu/OperonDB/DOOR.php) (Dam et al. 2007), Microbesonline (microbesonline.org/) (Dehal et al. 2010) and PREDetector (montefiore.ulg.ac.be/~hiard/PreDetector) (Hiard et al. 2007) repositories were used for identification of operons, regulons and *cis*-acting DNA elements.

Analysis of the E. faecalis sufcdsub promoter region - In order to characterise the sulfur assimilation (*SUF*) machinery in *E. faecalis,* its operon promoter regions and *cis*-elements were determined Bioinformatics analyses using the *SUF* genes of *E. faecalis* V583 were also performed to verify whether *SUF* genes are expressed as an operon. Genome annotators and operon predictors showed that *sufCDSUB* could indeed be grouped in an operon unit, as predicted by the annotations ID9111 (Dam et al. 2007) and VIMSS356533 (Dehal et al. 2009). The *E. coli sufABCDSE* genes were similarly grouped as operon, as demonstrated by codes ID429364 and VIMSS5496861.

The similarities between the *SUF* promoter regions of *E. coli* and *E. faecalis* were also assessed. Potential upstream *cis*-elements within the promoter region of the *E. faecalis SUF* genes were screened and compared to the known *E. coli sufABCDSE* promoter sequences. Four *cis*-acting DNA sites were biochemically described in *E. coli*, which were associated to alterations in the *SUF* transcriptional response to oxidative stress (IscR and OxyR-binding elements) and cellular iron depletion (Fur-binding element). Putative transcription factor-binding DNA sequences were predicted according to the method of Hiard et al. (2007). Comparison matrices were constructed using DNA regions that were previously biochemically characterised in *E. coli* and homologues in the regulatory regions of *E. faecalis V583* were investigated. Matrices containing specific *E. coli K12* and *E. faecalis V583* genomic regions were used as positive standardisation controls and the cut-off range was tested (Supplementary data). Putative high-scoring *cis*-elements for the Fur regulator (*fur* binding box) were identified, as well as an ORE II-like region (corresponding to the IHF torsion region, also linked to control of the *SUF* promoter region (Fig. 1). Although *E. faecalis* was predicted to encode putative coding sequences for the OxyR (ORE I), IscR (ORE III) and Fur regulators, no homolog to the *E. coli iscR cis*-element was identified. The putative ORE I element had the smallest prediction score and this might have been due to the size of the matrix used, given the large size of this *cis*-element. In summary, these *in silico* prediction support the presence of *cis*-elements in the regulatory region of the *E. faecalis sufCDSUB* operon, similar to those identified in the promoter region of the *E. coli sufABCDSE* gene cluster.

List of the putative regulatory *cis*-elements distributed in the *Enterococcus faecalis* sulfur assimilation (*SUF*) operon promoter region

				Cut-off				
Region	Matrix	Target	*cis*-element	Min	Max	Score	Gene id	Position
IHF	IHF_*coli*	*E. coli* K12	Tcattgttagat	-13.2	11.94	11.94	ECDH10B_1818c, sufA	-181
IHF	IHF_coli_faecalis	*E. coli* K12	Tcattgttagat	-13.2	10.02	8.94	ECDH10B_1818c, sufA	-181
IHF	IHF_*coli_faecalis*	*E. faecalis* V583	Tcctccctacac	-13.2	10.02	10.02	EF_2394c, sufC	-197
IHF	IHF_*faecalis*	*E. faecalis* V583	Tcctccctacac	-13.2	13.02	13.02	EF_2394c, sufC	-197
ORE I_Fur	ORE I_*coli*	*E. coli* K12	Agtgataatgattatcagttcaacccag	-30.8	29.84	29.84	ECDH10B_1818c, sufA	-70
ORE I_Fur	ORE I_*coli_faecalis*	*E. coli* K12	Agtgataatgattatcagttcaacccag	-30.8	24.68	22.24	ECDH10B_1818c, sufA	-70
ORE I_Fur	ORE I_*coli_faecalis*	*E. faecalis* V583	Attgagaatgaatcgcaaattacaattg	-30.8	24.68	15.74	EF_2394c, sufC	-86
ORE I_Fur	ORE I_*faecalis*	*E. faecalis* V583	Attgagaatgaatcgcaaattacaattgc	-31.9	30.17	30.17	EF_2394c, sufC	-87
ORE II_IscR	ORE II_*coli*	*E. coli* K12	Cagttcaacccagcaaacgcaggggcttta	-33.01	32.88	32.88	ECDH10B_1818c, sufA	-87
ORE II_IscR	ORE II_*coli_faecalis*	*E. coli* K12	Cagttcaacccagcaaacgcaggggcttta	-33	25.74	25.24	ECDH10B_1818c, sufA	-87
ORE II_IscR	ORE II_*coli_faecalis*	*E. faecalis* V583	Aaattacaattgctaaaaataacaactctt	-33	25.74	19.74	EF_2394c, sufC	-104
ORE II_IscR	ORE II_*faecalis*	*E. faecalis* V583	Caaattacaattgctaaaaataacaactctta	-35.2	30.77	30.77	EF_2394c, sufC	-105
ORE III_OxyR	ORE III_*coli*	*E. coli* K12	gttgtattgaaaatgactatttaagagataggtaaaaaagtgcagcgt	-52.8	49.46	49.46	ECDH10B_1818c, sufA	-276
ORE III_OxyR	ORE III_*coli_faecalis*	*E. coli* K12	gttgtattgaaaatgactatttaagagataggtaaaaaagtgcagcgt	-52.8	49.46	49.46	ECDH10B_1818c, sufA	-276
ORE III_OxyR	ORE III_*coli_faecalis*	*E. faecalis* V583	gcgttttcttaaataattactttagaatgacttttgcaaagagagcgt	-52.8	49.46	2.45	EF_2394c, sufC	-288
ORE III_OxyR	ORE III_*faecalis*	*E. faecalis* V583	gcgttttcttaaataattactttagaatgacttttgcaaagagagcgt	-52.8	49.46	37.67	EF_2394c, sufC	-288