Caractérisation de la réponse au stress digestif *in vitro* « batch » d'une bactérie à Gram-négatif et d'une bactérie à Grampositif par des outils moléculaires

1. Présentation de l'article 4 en construction

L'objectif de cette dernière partie de la thèse était de caractériser plus finement la réponse au stress digestif de deux micro-organismes, leur choix ayant été effectué en fonction des résultats obtenus dans les chapitres précédents. Des outils moléculaires ont été utilisés avec (i) de la transcriptomique, (ii) de la protéomique, et (iii) la validation de certaines hypothèses par la mise en évidence de métabolismes particuliers. Les stress majeurs auxquels sont soumis les microorganismes lors de la digestion sont la chute de pH, la présence de sels biliaires et la raréfaction puis la quasi absence d'oxygène (anoxie). Le stress éventuel de la rencontre avec le microbiote intestinal et ses métabolites n'a pas été abordé dans ces travaux.

Les travaux disponibles actuellement sur la réponse au stress digestif de bactéries, concernent surtout les micro-organismes probiotiques ou pathogènes. La résistance au stress acide des bactéries à Gram-positif a été revue de manière assez exhaustive par Cotter and Hill (2003) et, bien que cette revue ait plus de 10 ans, elle reste encore très pertinente. Une revue plus récente de Krulwich et al. (2011) complète les observations de la première revue, en y apportant un aspect mécanistique plus poussé traitant des bactéries à Gram-négatif. Citons aussi les travaux importants et de l'équipe de J. Slonczewski (2009) portant sur le maintien du pH intracellulaire lors de stress acide intense et, notamment, la mise en place de méthode permettant de mesurer ce pH. Les mécanismes de résistance aux sels biliaires ont été décrits dans la revue de Begley et al. (2005) et, surtout, bien caractérisés chez les entérobactéries ainsi que chez certains probiotiques comme les lactobacilles et le genre *Propionibacterium*.

Dans ce contexte de littérature ne traitant pas de micro-organismes « alimentaires » au sens technologique, notre premier choix s'est porté sur la bactérie à Gram-positif *Staphyloccocus equorum* Mu2, cette souche ayant montré un potentiel anti-inflammatoire (Chapitre 1) ainsi qu'une certaine sensibilité au stress gastrique batch, tout en résistant au passage à travers le DIDGI (Chapitre 2). Le second choix a été la bactérie à Gram-negatif *Hafnia alvei* GB001, cette bactérie s'étant montrée la plus résistante à toutes les conditions de digestion (Chapitre 1) et possédant un profil anti-inflammatoire (Chapitre 1).

Nous avons construit un plan d'expérience reprenant la démarche du stress batch utilisée dans le Chapitre 1, tout en modifiant les conditions de stress gastrique de manière à ce que *S.equorum* Mu2, qui ne survivait pas à ces conditions trop drastiques, conserve une viabilité suffisante pour permettre l'extraction de son ARN pour l'analyse RNAseq. Le stress duodénal a été conservé à l'identique. Le stress combiné a consisté en l'application du stress gastrique modifié, suivi du stress duodénal.

En parallèle, une extraction des protéines a été réalisée concernant les deux micro-organismes après application du stress acide seulement dans l'objectif d'une analyse protéomique ultérieure.

2. Faits marquants

- ✓ Nous avons développé une méthode de digestion *in vitro* statique en deux étapes
- ✓ Les bactéries à Gram-positif et à Gram-négatif ont différé par leurs réponses.
- ✓ Nous avons caractérisé la réponse transcriptomique au stress digestif in vitro d'une bactérie à Gram-positif et d'une bactérie à Gram-négatif
- La réponse au stress combiné de la bactérie à Gram-positif ressemble à la réponse au stress gastrique
- ✓ La réponse au stress combiné de la bactérie à Gram-négatif est intermédiaire entre la réponse au stress gastrique et a réponse au stress duodénal

Insights of the transcriptomic *in vitro* digestive stress response of a Grampositive and a Gram-negative bacterium from cheese origins.

Nadège Adouard^{ab}, Eric Dugat-Bony^b, **Pascal Bonnarme***^b

 ^a AgroParisTech, Centre de Biotechnologies Agroindustrielles, AgroParisTech INRA, UMR 782 Genie & Microbiologie des Procedes Alimentaires, F-78850 Thiverval Grignon, France
^b INRA, Centre de Biotechnologies Agroindustrielles, AgroParisTech INRA, UMR 782 Genie & Microbiologie des Procedes Alimentaires, F-78850 Thiverval Grignon, France

*Corresponding author pascal.bonnarme@grignon.inra.fr Phone: +33 130 815 388

Abstract

Although large numbers of viable microorganisms are ingested through ripened cheese consumption, little is known about the microorganisms' ability to withstand digestion. We investigated the resistance to digestive stress of a Gram-positive – Staphylococcus equorum Mu2 – and a Gram-negative bacterium – Hafnia alvei – constitutive of that ripened cheese microflora. The approach mimicked gastric and/or duodenal digestion. Transcriptional changes were measured using a global RNA-Seq transcriptomic approach and viability of both strains was assessed. S.equorum Mu2 was far more sensitive to gastric stress (viability decrease of 3 log CFU/mL) than H.alvei GB001 (no significant decrease of viability) and, therefore, more sensitive to a combined stress. Both strains were equally resistant to duodenal challenge. Overall, 1730, 1761 and 2308 genes in H. alvei GB001 and 573, 796 and 510 genes in S.equorum Mu2, were differentially modified following, respectively, gastric-like, duodenal-like and combined in vitro batch stress (p-value <0.05) among a total of 4692 CDS in H. alvei GB001 and a total of 2932 CDS in S. equorum Mu2. Following gastric-like stress, H. alvei GB001 exhibited up-regulated genes of the cysteine metabolism (cysND, cysC and cysIJ) along with glutamate decaboxylation metabolism. Hydrogenases encoding genes from the Hyd complexes were both up- (hyfFG) and down-regulated (hybABCO, hypBCD). S.equorum Mu2 up-regulated genes encoding diverse dehydrogenase, among them malate dehydrogenase (mdh and mqo), along with genes encoding enzymes involved in the polyamine biosynthesis (potAD). Both bacteria showed upregulated genes encoding transporters from the multidrug resistance family (e.g. emrB and acrAB). H. alvei GB001 transcriptomic profile in the combined challenge seems to be an intermediate between the individual stresses whereas S. equorum Mu2 displayed a combined stress response very much alike gastric stress.

Highlights

- ✓ We performed a two-steps *in vitro* batch digestive stress experiment.
- ✓ We assessed transcriptomic response to *in vitro* digestive stress of a Gram-positive and a Gram-negative bacteria from cheese origin
- ✓ Gram-positive and Gram-negative bacteria differed in their resistance to digestive challenge.
- ✓ Gram-positive bacteria response to combined challenge looked like gastric challenge
- ✓ Gram-negative bacteria response to combined challenge was intermediate between the individual stress

Keywords

Smear-ripened cheese microbiota, in vitro digestive model, digestive stress, transcriptome, RNA-Seq

1. Introduction

Cheese is one of the oldest ways of conserving milk: in Northern Europe, evidence of cheesemaking activity has been found at sites dating from the sixth millenium BC (Salque et al, 2012). At present, Europe produces around 9000 thousand tons of cheese per annum (Eurostat, 2013), and Europeans eat between 25 and 30 kg of cheese per capita per annum. Given that a gram of cheese contains 10⁸ to 10⁹ live microorganisms on average (Beresford et al. 2001), the annual intake of viable cells can be estimated at 10¹³ to 10¹⁴ per capita per annum. Thus, a fermented food product like cheese is an important source of diverse microorganisms in the human diet. However, few studies have investigated the survival of the cheese microflora in the gastrointestinal tract. A review of the literature shows that most of the research in this field has focused on Lactobacilli, Bifidobacteria and Propionibacteria (Cousin et al. 2011, Saarela et al. 2000) with a view to find new probiotics or using cheese as a carrier for known probiotics (Gardiner et al. 1999, Saxelin et al. 2010). It has been reported that pH is the major stress factor in the gastric compartment, whereas the presence of enzymes has a negligible effect on the microorganisms (Sumeri et al. 2012). The impact of the stomach's hydrochloric acid (HCl) on both Gram-positive and Gram-negative bacteria has been well characterized (mainly potential probiotics or pathogens) (Krulwich et al. 2011). In contrast, the impact of bile has been less documented (Begley et al. 2005) and has focused on food-borne pathogens (e.g. Escherichia coli and Salmonella typhimurium (Merrit et al. 2009)) or probiotic candidates such as Bifidobacteria and Lactobacilli (Ruiz et al. 2013). One of the few studies related to cheese-ripening bacteria found that the genus Corynebacterium survived passage through the gastrointestinal tract in human microbiota-associated rats (Lay et al. 2004). Cheese-ripening yeasts, such as Debaryomyces hansenii, Kluyveromyces lactis and Geotrichum candidum, were found to be able to survive in vitro challenges with acid and bile (Kumura et al. 2004, Lay et al. 2004, Psomas et al. 2001). However, to date, no study has been focusing on the response to digestive stress of ripening microorganisms, using high throughput molecular tools such as next generation sequencing. Given our previous work related to the ability of a selection of ripening microorganisms to withstand digestive stress along with the characterization of their basic immunomodulatory properties (Adouard et al., 2014 accepted, see Chap. 1; Adouard et al. unpublished data, see Chap.2), we selected for this study a Gram-positive bacterium (Staphylococcus equorum Mu2) and a Gramnegative bacterium (Hafnia alvei GB001) which are of importance in the ripening process of surfaceripened cheese (Irlinger et al., 2009) and whose genome has been sequenced (Irlinger, personal communication ; Irlinger et al., 2012).

We implemented a slightly modified version of the three-step *in vitro* digestive batch method used in the previous works (Adouard et al., unpublished data, see Chap.2), consisting of (i) a gastric-like challenge, (ii) a duodenal-like challenge and (iii) a gastric-like followed by a duodenal-like challenge (Adouard et al. 2014, submitted). We used a global transcriptomic analysis based on RNA sequencing to study the transcriptional response of both strains to each of the three *in vitro* stress conditions.

2. Materials & Methods

2.1. Microorganisms

Hafnia alvei GB001 and *Staphylococcus equorum* Mu2 are part of the GMPA strain collection and are both able to grow in cheese environment (Plé et al. 2014).

2.2. Growth and plate count media

Both bacteria were cultured in 100 mL of Brain Heart Infusion broth (BHI: Biokar Diagnosis, Beauvais, France) in 500 mL Erlenmeyer flasks at 25°C, with shaking at 200 rpm. Prior to use in the experiments described below, all strains were grown until they reached the same growth phase (the late stationary phase, as defined in prior growth kinetics experiments; data not shown). Microorganisms were counted on the BHI agar. Prior to plating, cultures were diluted in Maximum Recovery Diluent (MRD, 9 g/L) (Difco,Pessac, France).

The same incubation temperatures were used as for broth cultures.

2.3. In vitro gastric and duodenal batch challenges

The stress conditions and the stress medium's composition were adapted from our previous work (Adouard et al 2014, Chap.2). All digestive juice components were purchased from Sigma (Saint-Quentin-Fallavier, France) and diluted in MRD. Pepsin (P6887, EC 3.4.23.1, activity: 3300 U/mg of protein, calculated using hemoglobin as a substrate), pancreatin (P1750), and bile (B8631) were of porcine origin. The "gastric lipase" was a recombinant enzyme produced in the fungus *Rhizopus oryzae* (80612, EC 3.1.1.3, activity: \geq 30 U/mg). Simulated gastric and duodenal juices were made fresh daily. Gastric juice consisted in pepsin (0.025 g/L or 1.10^6 U/mL), lipase from *R. oryzae* (0.2 g/L or 6.10^4 U/L) and NaCl (2,75 g/L) suspended in a 0.020 M glycine-HCl buffer pH 4 at 37°C prior to experiments.

Duodenal juice was prepared by suspending pancreatin (9 g/L), bile (30 g/L) and NaCl (7 g/L) in 0.1 M phosphate buffer pH 6.5 at 37°C, prior to the experiments. In a 1000 mL sterile Erlenmeyer, 15 or 20 milliliters of a late-stationary phase culture were added to 135 mL or 130 mL of either gastric or duodenal juice for respectively *S. equorum* Mu2 or *H. alvei* GB001. The pH was checked again after inoculation and (if necessary) re-adjusted to either pH 4 or pH 6.5 using HCL 1M. Compared to the previous stress conditions (Chap.2), the pH of gastric-like conditions was changed from pH 3 for 1h to pH 4.5 for 45 min in such a way *S. equorum*'s Mu2 remaining viability was suitable for RNA extraction and further RNAseq analysis.The incubation times were respectively 45 min and 2 h for gastric stress followed by duodenal stress) was performed by adjusting the pH of 45-min gastric juice incubation to 6.5 with 1M Na₂CO₃, and then adding bile and pancreatin under sterile conditions for the subsequent 2 h duodenal incubation.

Control samples were made by incubating each species in MRD instead of the stress media, all else being equal (i.e. incubating time of 45 min, 120 min or 165 min, 37°C, final volume 150 mL, 100 rpm shaking table). Control and stressed samples were repeated 4 times. To assess cell survival, strains were counted on the corresponding agar-based media before and after each stress condition.

2.4. RNA extraction

Cells were harvested by centrifugation at 5000 x g for 5 min and pellets were immediately resuspended into 1.5 ml (*S. equorum* Mu2) or 2.1 ml (*H. alvei* GB001) of RLT Buffer containing β -mercaptoethanol (1% V/V) (Qiagen). RNA extraction was performed using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. DNase treatment was performed on 10 µg of total RNA using the TURBO DNA-free kit (Ambion, Grand Island, NY, USA) according to the manufacturer's instructions. Finally, RNA was purified using the RNA cleanup protocol of the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. Purified RNA was quantified at 260 nm using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The quality of the RNA was analyzed with a 2100 Bioanalyzer (Agilent, Palo Alto, CA,USA) using RNA 6000 NANO chips.

2.5. RNA sequencing and differential analysis

RNA samples were provided to Genome Quebec and McGill University Innovation Centre (Montréal, Québec, Canada) for an additional RNA quality control and for library preparation and sequencing. Briefly, cDNA libraries were prepared using the TruSeq stranded mRNA sample preparation kit and sequenced (100 bp single-end reads) on a HiSeq 2000 instrument (Illumina, San Diego, CA, USA) following the manufacturer's protocol. The quality of sequencing reads was checked with FastqC software V0.10.1 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Trimming, including the removal of adaptators and quality filtering, was performed using Trimmomatic V0.32 (Bolger et al., 2014) with the following parameters: crop = 85, headcrop = 10, leading = 10, trailing = 10, slidingwindow = 4:20, minlen = 60. Then, reads were mapped onto the sequenced genome of *H. alvei* GB001 (total coding DNA sequence (CDS) : 4692 ; accession number PRJEB6257) or *S. equorum* Mu2 (total coding DNA sequence (CDS) : 2932 ; accession number CAJL01000001-CAJL01000030) using the Bowtie mapping software (Langmead et al., 2009), allowing up to one mismatch in alignments. The number of reads uniquely mapped to each gene was determined using HTSeq (Anders et al., 2014).

Differential expression analysis was performed by comparing the number of mapped reads for each gene in the four replicates from two different conditions (stress versus control). First, the count table was filtered to select only data corresponding to CDS features exhibiting more than 200 mapped reads on average. Then, data were normalized by using R and the package DESeq2 (Love et al., 2014).

Finally, differentially expressed genes were identified according to the false discovery rate adjusted Benjamini Hochberg *p*-value (Benjamini and Hochberg, 1995) implemented within the DESeq2 package and using a cutoff of adjusted *p*-value < 0.05. Genes showing an adjusted *p*-value less than 0.05 were considered to have significantly different transcript levels between the stress and control conditions. Functional classification of the transcriptome datasets were performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations (Kanehisa et al., 2012).

3. Results and discussion

3.1. Survival analysis

We assessed the resistance to in vitro batch digestive stress of *H. alvei* GB001 and S. *equorum* Mu2 two surface-ripened cheese isolated microorganisms. The incubation under gastric-like stress conditions took place for 45 min with pepsin, at 37°C and pH 4.5 ; duodenal-like stress incubation conditions were 2 h with bile and pancreatic enzymes, at 37°C and pH 6.5. Incubation under gastric-like conditions followed by duodenal-like conditions was also performed.

The strain *H. alvei* GB001 displayed a loss of viability of 0.29 log CFU/mL, 0.51 log CFU/mL and 0.38 log CFU/mL under gastric, duodenal and combined challenge conditions, respectively (Table 1). *S. equorum* Mu2 showed a loss of viability of 3.06 log CFU/mL following exposure to gastric stress, 0.60 log CFU/mL after duodenal challenge and 3.15 log CFU/mL following the combination of stress. Overall, *S. equorum* Mu2 proved to be more sensitive to gastric stress than *H. alvei* GB001, which is consistent with previous works (Adouard et al., unpublished data, Chap.2).

			Viability (log CFU/mL)
Hafnia alvei	G-like	Control	9.40 ± 0.15
GB001	stress	Stress	9. 11 ± 0.12
	D-like	Control	9.32 ± 0.13
	stress	Stress	8.81 ± 0.12 **
	Combined	Control	9.80 ± 0.08
_	stress	Stress	9.42 ± 0.14 **
Staphyloccocus	G-like	Control	9.56 ± 0.12
equorum	stress	Stress	6.50 ± 0.08 ***
IVIUZ	D-like	Control	9.65± 0.09
	stress	Stress	9.05 ± 0.14 *
	Combined	Control	9.49 ± 0.09
	stress	Stress	6.31 ± 0.06 ***

Table 1: Viability of Hafnia alvei GB001 and Staphylococcus equorum Mu2 before and after in vitro batchdigestive stress

Results are shown as the mean \pm SD, n=3. Viable counts (log CFU/mL) of each strain were compared with a control sample incubated at 37°C in Maximum Recovery Diluent for the same length of time i.e. 45 min, 120 min or 165 min for respectively G-like, D-like and Combine stress.

G-like stress : Gastric challenge ; D-like stress : Duodenal challenge and Combined stress : Gastric followed by duodenal challenge.

The statistical significance of differences between controls and challenged groups was established using a two-tailed Student's t-test. Results are indicated as follows: * p < 0.05; ** p < 0.01; *** p < 0.001

3.2. Overview of the transcriptomic dataset

The bacterial transcriptome modifications induced by gastric-like stress (G), duodenal-like stress (D) or a combination of both (C), were determined for a Gram-positive bacterium (*S. equorum* Mu2) and a Gram-negative bacterium (*H. alvei* GB001) by whole transcriptome sequencing (RNA-Seq). To this end, 48 RNA-Seq libraries (24 per bacterial species) were generated and sequenced for a total of >1 billion reads. Sequencing statistics are provided in (Table 2). The number of raw sequence reads per library varied between 16,990,785 and 34,458,232. Most sequences (>90%) passed the quality filtering and mapped the reference genomes.

	H. alvei GB001	S. equorum Mu2
Total number of reads	651,524,345	524,935,363
Average number of reads/library	27,146,848	21,872,307
Average number of filtered reads/library	25,006,194	20,894,352
Average number of unique mapped reads	24,683,814	20,425,363
Average expression (reads per CDS)	3,164	4,733

Table 2: Sequencing statistics and expression data.

Overall, the differential expression analysis allowed to identify 3168 genes for *H. alvei* GB001 (68% of the predicted CDS) and 2456 genes for *S. equorum* Mu2 (84% of the predicted CDS) whose expression was significantly different (adjusted *p*-value < 0.05) from the control in at least one stress condition (G, D or C). Such variations indicate dramatic metabolic changes under stress conditions for these two microorganisms. A hierarchical clustering (Single Linkage method) of the samples was performed using the correlation matrix built from the expression data corresponding to those genes (Figure 1). This analysis revealed a perfect clustering of biological replicates (4 per stress condition or the corresponding controls) and a clear segregation between stress conditions. Furthermore, it emphasizes the importance of using a set of specific controls for each stress conditions. Indeed, each stress condition had its set of specific controls, namely a sample in which microorganisms were kept in a minimal medium for as long as the stressed was performed in the sample group. The clustering of controls made for each stress condition points out the necessity to use control that are different from a sample taken just before the stress and that does not take into account the period of time during which microorganisms are stressed.





3.3. <u>Transcriptional response of *Hafnia alvei* GB001 and *Staphyloccocus equorum* Mu2 to *in vitro* batch digestive stress</u>

3.3.1. Overall response

In order to determine which genes were involved in bacterial Gram-negative and Grampositive response to gastric-like and/or duodenal-like batch digestive stress, we focused on pair-wise comparisons performed between stressed groups and controls. Overall, Table 3 exhibits 1730, 1761 and 2308 genes whose expression was significantly modified in response respectively to gastriclike,duodenal-like and combined *in vitro* batch stress (*p*-value <0.05) among a total of 4692 CDS in *H. alvei* GB001. Pair-wise comparisons performed between stressed groups and controls for the *S. equorum* Mu2 gave 573, 796 and 510 genes whose expression was significantly modified in response to G-like, D-like and combined *in vitro* batch stress, respectively (*p*-value<0.05); for a total of 2932 CDS in *S. equorum* Mu2 (Table 3). It is of note that, regarding *S. equorum* Mu2, around 50% to 60% of the differentially expressed genes encode for putative or unknown proteins. Such a lack of information makes the search of complete metabolisms very difficult to carry on.

	Hafnia alvei GB001 ⁽¹⁾			Staphylo	Staphylococcus equorum Mu2 ⁽¹⁾		
	Total genome : 4692 CDS			Tota	genome : 2932 CDS		
	Up-regulated Down-regulated %Total CDS Up-regulated				Down-regulated	%Total CDS	
	CDS	CDS	(up+down)	CDS	CDS	(up+down)	
Gc-like stress	838	892	37	721	558	44	
DI-like stress	827	934	38	899	906	62	
Combined stress	1174	1132	49	634	668	44	

Table 1: General view of up-regulation and down-regulation (adjusted p-value < 0.05) of Coding DNA Sequence (CDS) in *Hafnia alvei* GB001 and *Staphylococcus equorum* Mu2 when exposed to (i) G-like, (ii) Dlike and (iii) G-like followed by D-like – i.e. combined- stress

(1) Coding DNA Sequence of the [...] bacterial strain. Genomes available under accession number PRJEB6257 for *H. alvei* GB001 and CAJL01000001-CAJL01000030 for *S. equorum* Mu2.

Figure 2 and Figure 3 show the number of up- and down-regulated genes after each of the three stress conditions for respectively *H. alvei* GB001 and *S. equorum* Mu2. The global expression pattern was evaluated by classifying differentially-expressed genes according to KEGG annotations (Kanehisa et al. 2012). Overall, even though the ranking was slightly different among each stress and bacterial strain, the main functional categories with differentially expressed genes were carbohydrates metabolism, amino acid metabolism, metabolism of cofactors and vitamins, membrane transport, energy metabolism, signal transduction and translation.

From a global perspective, Table 4 and Table 5 showed that, respectively for *H. alvei* GB001 and *S. equorum* Mu2, general stress response genes were significantly up-regulated for the three stress conditions. Indeed, genes such as those encoding molecular chaperons (*e.g. dnaK, clpB, hscAB, htpG*) were up-regulated. Their role is to address and stabilize proteins, by preventing and reversing their aggregation or by degrading irreversibly damaged materials (Hartl et al. 1996). Heat-shock and cold-shock proteins coding genes (namely *ibpA* and *cspE* for *H. alvei* GB001 and *cspA* for *S. equorum* Mu2) were up-regulated as well. They are reported to collaborate with the previously mentioned chaperones to achieve protein sorting, repairing and ultimately degrading, if necessary (Thomas and Baneyx, 1998).





Figure 2: Functional classification of differentially expressed genes for *Hafnia alvei* GB001 after (A) gastric-like (B) duodenal-like and (C) Combined *in vitro* batch stress, , compared to control groups. Dark bars : amount of up-regulated genes ;

Light bars amount of downregulated genes - in each condition

(C) Combined challenge Hafnia alvei

(A) G-like challenge

Staphylococcus equourm







50

70

Amino acid metabolism Biosynthesis of other secondary metabolites Carbohydrate metabolism Cell growth and death Energy metabolism Folding. sorting and degradation Glycan biosynthesis and metabolism Lipid metabolism Membrane transport Metabolism of cofactors and vitamins Metabolism of other amino acids Metabolism of terpenoids and polyketides Nucleotide metabolism Replication and repair Signal transduction Transcription Translation Transport and catabolism

> > (C) Combined challenge Staphyloccocus equorum

Figure 3: Functional classification of differentially expressed genes for *Staphyloccocus equorum* Mu2 after (A) gastric-like (B) duodenal-like and (C)Combined stress, alone or in combination, compared to control groups. Dark bars : amount of up-regulated genes ;

Light bars amount of downregulated genes - in each conditions

-70

Amino acid metabolism Biosynthesis of other secondary metabolites Carbohydrate metabolism Cell growth and death Cellmotility Energy metabolism Folding. sorting and degradation Glycan biosynthesis and metabolism Lipid metabolism Membrane transport Metabolism of cofactors and vitamins Metabolism of other amino acids Metabolism of terpenoids and polyketides Nucleotide metabolism Replication and repair Signal transduction Transcription Translation Transport and catabolism

3.3.2.Gastric-like stress

Figure 2 (A) and Figure 3 (A) show the number of up- and down-regulated genes per functional categories for *H. alvei* GB001 and *S. equorum* Mu2, respectively, after the gastric-like challenge compared to controls.

As mentioned in the previous section, numerous genes involved in the general stress response were up-regulated (Table 4 and Table 5). For example, the gene encoding the cold shock protein (cspA) was up-regulated in both microbial strains. In the particular case of H. alvei GB001, the phage shock protein complex (PSP-complexe; encoded by pspABCD genes) was also highly upregulated. It has been reported that this complex helps preventing membrane damage in Escherichia coli, and in Gram-negative bacteria in general, in response to a wide range of environmental stresses (Darwin et al., 2006). Interestingly, Darwin et al. demonstrated a PSP-complex induction after the proton motrice force (PMF) was dissipated in the cell, and they reported that PSP-complex to be induced when a defect occurred in the assembly of cytochrome O oxidase and F1-F0ATPsynthase complex. A closer look to the energy metabolism of *H. alvei* GB001 showed that most of the oxidative phosphorylation main genes were repressed - *e.g.* F₁-F₀ATPsynthase complex (encoded by genes atpABEH), cytochrome O oxidase complex (encoded by genes cyoBCDE) and NADH dehydrogenase complexe (encoded by genes nuoCEFGHKN). cyoBCDE and nuoCEFGHKN genes have already been reported to be up-regulated in E. coli when submitted to acid stress (Krulwich et al. 2011). H. alvei is genetically and metabolically close to E. coli (Janda et al., 2002) but does not seem to be able to use its Cyo and Nuo complex in the same way when exposed to an acidic environment. Therefore, as mentioned in Darwin's review conclusion (2006), we can speculate that PSP-complex may have been up-regulated in order to counteract the dysfunctions related to PMF defect and functioned as a signal transduction-like system. As a whole, and for both microorganisms, genes involved in carbohydrates metabolism were generally repressed. As shown in Table 4 and Table 5, major genes coding for enzymes of the TCA cycle (e.g. sdhAB, sucABCD, frdABC) and glycolysis (e.g. pgi, pgk, pgm, acs, fba, fbp) were significantly down-regulated, clearly suggesting that the cells were not using the carbon resources as efficiently to produce energy. Along with the repression of oxidative phosphorylation related genes already mentioned for *H. alvei* GB001 and that occurred as well for *S.* equorum Mu2, we can state that both bacteria tend to limit the use of aerobic respiration in response to gastric-like stress conditions.

		Log ₂ expression ratio ⁽¹⁾		atio ⁽¹⁾
		G-like	D-like	Combined
Gene	Product	stress	stress	stress
General stress				
proteins				
clpA	ATP-dependent Clp protease ATP-binding subunit	0,85	-	-0,58
сlpВ	ATP-dependent chaperone protein ClpB	2,80	1,07	2,09
clpP	ATP-dependent Clp protease proteolytic subunit	2,05	-	0,80
clpS	ATP-dependent Clp protease adaptor protein ClpS	2,44	-	-
clpX	ATP-dependent serine protease specificity subunit of ClpX-ClpP	1,51	-0,51	-
cspA2	Major cold shock protein	1,97	-	4,54
cspD	Cold shock-like protein CspD	2,96	-1,84	-
cspE	Cold shock protein	1,48	1,57	1,18
dnaJ	Chaperone protein dnaJ	1,62	1,05	1,12
dnaK	Chaperone hsp70, autoregulated heat shock protein	3,03	0,55	1,65
hfq	RNA chaperone Hfq	2,11	-	-
hscA	chaperone protein HscA	1,44	-	-
hscB	co-chaperone protein HscB	1,63	-	-1,89
hslO	Heat shock protein Hsp33	2,34	-0,40	1,94
hslR	Heat shock protein	1,94	-0,44	2,61
htpG	Chaperone protein HtpG	3,07	-	1,62
, ibpA	Heat shock chaperone	5,42	1,33	1,90
ibpB	Heat shock chaperone	4.29	-0.75	2.26
pspA	Phage shock protein A	4.32	-0.42	-0.95
pspB	Phage shock protein B	3.93	-0.40	-1.45
nsnC	Phage shock protein C	3.59	-0.76	-0.88
nsnD	Phage shock protein D	2 80	-	-1 21
usnG1	Universal stress protein G	-1.04	2 1 7	
uspG2	Universal stress protein G		2,15	_
usp82 usn4	Universal stress protein	-1 48	2,13	_
aroEl	60 kDa chaneronin	1,40	2,10	0.53
gioll			2,05	0,55
Response to				
oxidative stress				
and reductase				
katE	Catalase	-1,72	1,89	-1,94
osmY1	Putative lipoprotein	-1,50	1,23	-2,95
sodA	Superoxide dismutase	-	-4,23	-
sodB	Superoxide dismutase	-	3,99	-
cbiJ	Cobalt-precorrin-6a reductase	-	2,06	-
cysH	phosphoadenosine phosphosulfate reductase	-	1,48	3,41
dmsC	Anaerobic dimethyl sulfoxide reductase, C subunit	-1,88	2,95	-
ghrA	Hydroxypyruvate reductase	-	1,38	0,49
metF	methylenetetrahydrofolate reductase (NAD(P)H)	0,87	1,75	1,52
nemA2	NADH:flavin oxidoreductase	0,75	1,15	, 1,50
nrdA	Ribonucleoside-diphosphate reductase	-0,82	1,54	-
nrdG	Anaerobic ribonucleoside-TP reductase activating protein	-1,45	2,01	0,79
torD1	Putative oxidoreductase component	-1,39	1,41	-
grdB	Glycine reductase complex component B	-	1,20	-

Table 4: Selection of genes (CDS) significantly up- or down-regulated in *H. alvei* GB001 after exposure to (i) G-like, (ii) D-like and (iii) G-like followed by D-like stress – i.e. combined- stress

		Log ₂ ex	pression I	atio
		G-like	D-like	Combined
Gene	Product	stress	stress	stress
ТСА				
acnA	aconitate hydratase	-0,62	0,29	-
acnB	aconitate hydratase 2	-1,18	1,40	-2,16
frdA	fumarate reductase flavoprotein subunit	-0,64	-	1,62
frdB	fumarate reductase iron-sulfur subunit	-1,18	-	0,73
frdC	fumarate reductase subunit C	-1,54	-	-
gltA	citrate synthase	-1,42	0,60	-4,97
mdh	malate dehydrogenase	-1,72	0,95	-1,52
sdhA	succinate dehydrogenase flavoprotein subunit	-0,89	-	-
sdhB	succinate dehydrogenase iron-sulfur subunit	-1,85	-	-
sucA	2-oxoglutarate dehydrogenase E1 component	-1,53	1,92	-1,42
sucB	2-oxoglutarate dehydrogenase E2 component	-2,59	-	-1,23
sucC	succinyl-CoA synthetase beta subunit	-3,51	-	-
sucD	succinyl-CoA synthetase alpha subunit	-3,46	-	-
Glycolyse				
	acetyl-CoA synthetase	-1 23	0.56	-2 79
fha	fructose-hisphosphate aldolase, class II	-1.09	0,50	-1 16
fhaB	fructose-bisphosphate aldolase, class l	-2.18	1 1 1	-2 1/
fhn	fructose 1 6-bisnbosnbatase I	-1 /19	1 37	_1 10
jop alnX	fructose 1.6 bisphosphatase I	-0.40	1,57	1 01
anml	2 3-hisnhosnhoglycerate	-1.02	1 33	0.35
nfkB	6-nhosnhofructokinase 2	-0.34	1 15	-0.56
pjkb	ducose-6-nhosnhate isomerase	-1 18	1 11	-0.98
pgi nak	nhosnhoglycerate kinase	-0.73	0.41	-1 04
nam	nhosnhoglucomutase	-0 59	1 23	1,04 0.86
pgin tni∆	triosenhosnhate isomerase (TIM)	-1 25	1 17	- 0,00
Ovudativa		1,20	-,-,	
phosphorvlation				
atpA	ATP synthase subunit alpha	-0,51	-	-
atpB	ATP synthase subunit a	-0,52	-	-
atpE	ATP synthase subunit c	-0,66	-	-
atpH	ATP synthase	-0,41	-	-
atpl	ATP synthase F0, I subunit	-0,98	-	-
cydA1	Cytochrome D ubiquinol oxidase subunit I	-	1,79	-
cydB	Cytochrome D ubiquinol oxidase subunit II	-1,22	2,48	-
суоВ	Cytochrome o ubiquinol oxidase subunit I	-2,24	2,64	-
суоС	Cytochrome o ubiquinol oxidase, subunit III	-2,39	-	-2,39
cyoD	Cytochrome o ubiquinol oxidase subunit IV	-2,69	-	-2,25
суоЕ	Protoheme IX farnesyltransferase	-2,44	-	-1,68
nuoC	NADH:ubiquinone oxidoreductase, chain C,D	-0,66	1,62	-
nuoE	NADH dehydrogenase I chain E	-0,74	-	-
nuoF	NADH:ubiquinone oxidoreductase, chain F	-0,60	-	-
nuoG	NADH-quinone oxidoreductase	-0,53	-	-
nuoH	NADH:ubiquinone oxidoreductase, membrane subunit H	-0,49	-	
nuoJ	NADH dehydrogenase I chain J		1,20	-0,42
пиоК	NADH:ubiquinone oxidoreductase, membrane subunit K	-0,96	1,34	-
nuoL	NADH dehydrogenase I chain L	-	1,52	-
nuoM	NADH:ubiquinone oxidoreductase, membrane subunit M	-	1,97	-
nuoN	NADH:ubiquinone oxidoreductase, membrane subunit N	-1,44	2,08	0,73

		Log ₂ expression rat		itio ⁽¹⁾	
		G-like	D-like	Combined	
Gene	Product	stress	stress	stress	
Hydrogonasos					
hyfE	Hydrogenase-1 component F	2 61	_		
hyfG	Hydrogenase-4 component G	2,01	_		
hyj0 hyh0	Hydrogenase-2 small chain	-2.12	_	2 74	
hyb0 hyb4	Hydrogenase 2 oneron protein HybA	-3 19	_	1 96	
hybR	Ni/Fe-hydrogengse 2 B-type cytochrome subunit	-3 70	_	1,50	
fdhE	Formate dehydrogenase formation protein	-1.83	0.76	-U 90	
fdol	Formate dehydrogenase, cytochrome b556 protein	-2 13	-	-1 69	
hvhC	Hydrogengse-2 large chain	-3.48	_	1,05	
hybe	Hydrogenase expression/formation protein	-1 87	-	-	
hynB	Hydrogenase nickel incorporation protein	-1 75	_	-	
hyp	Putative hydrogenase formation protein	-2.16	_	-	
hype	Formate hydrogenlyase subunit F	1 29	-	0 99	
nyez		1,25		0,55	
Formate					
dehydrogenase		4.05	4.00	2.26	
faoG	Aerobic formate denydrogenase, alpha subunit	-1,05	1,98	-2,36	
јаон († С	Formate dehydrogenase-O, iron-sulfur subunit	-1,67	-	-	
fdoG	Formate dehydrogenase-O, selenocysteine-containing	-1,/2	2,05	-	
fdol	Formate dehydrogenase, cytochrome b556 protein	-2,13	-	-1,69	
Glutamate/GABA					
metabolism					
gadB	Glutamate decarboxylase beta	2,84	-	1,02	
aspB	Glutamate synthase [NADPH] large chain	0,57	0,95	-	
aspB	Glutamate synthase [NADPH] small chain.	0,57	0,95	-	
gdhA	Glutamate dehydrogenase	-0,68	-0,65	-	
gabT	4-aminobutyrate aminotransferase, PLP-dependent	-1,27	-3,47	-	
arg	Carbamoyl-phosphate synthase, large subunit	-0,84	-	-	
carA	Carbamoyl-phosphate synthase, small subunit	-3,11	-	2,24	
glmS	L-glutamine:D-fructose-6-phosphate aminotransferase	-0,36	1,44	1,58	
Decarboxylase					
adi	arginine decarboxylase	1 35	-0 38	-1 96	
Sulfur		1,00	0,50	1,50	
Sullur metabolism and					
transport					
cvsA	sulfate/thiosulfate import ATP-binding protein cvsA	4.20	-	-	
cvsP	thiosulfate-binding protein	6.47	-	-	
cvsU	sulfate transport system permease protein cysT	5,75	-	-	
cvsW	sulfate transport system permease protein cysW	5.42	-	-	
cvsC	adenvlvl-sulfate kinase	4.41	-	2.61	
, cysD	sulfate adenylyltransferase subunit 2	7,07	-	-	
cysl	sulfite reductase [NADPH] flavoprotein alpha-component	2,81	1,33	-	
cvsJ	sulfite reductase [NADPH] flavoprotein alpha-component	4.05	0.72	-	
cvsN	sulfate adenvlvltransferase subunit 1	5.06	-	-	
iscS	cysteine desulfurase	1,85	-	-1,43	
sbp	sulfate-binding protein	4,09	-	-	
Nitrate					
metabolism					
narK	Nitrite extrusion protein (MFS-family transporter)	1,68	-	-0,93	
narH	Nitrate reductase 1, beta subunit	1,41	-	-	
narG	Respiratory nitrate reductase 1 alpha chain	0,91	-	-	

		Log ₂ expression ratio ⁽¹⁾		
		G-like	D-like	Combined
Gene	Product	stress	stress	stress
Transporter (ABC, PTS, Others)				
	ABC transporter, substrate-binding component	7,25	-	-
	ABC transporter, inner membrane subunit	6,17	0,66	2,36
	ABC transporter, inner membrane subunit	4,00	0,77	1,79
malX	PTS system, maltose and glucose-specific llabc component	2,07	-	3,99
fliY	Putative amino-acid ABC transporter	4,85	-	1,29
	Sigma-54 dependent transcriptional regulator/ABC transporter	2,77	-	-
yecS	Extracellular solute-binding protein-amino acid abc transporter	2,86	-2,14	-
H^{\dagger} co-transport				
kdpA	P-type ATPase, high-affinity potassium transport system, A chain	1,82	-	2,99
kdpB	Putative potassium-transporting ATPase B chain	2,79	-	2,09
kdpC	K+-transporting ATPase, C subunit	1,82	-	-
trkA	Potassium uptake protein	-0,43	-	-1,09
trkH	Trk system potassium uptake protein TrkH	-0,85	-	-
mgtB	Magnesium transport ATPase	2,53	-	1,84
mgtC1	Mg2+ transport ATPase	4,41	-	2,64
sapB	Putative membrane protein	0,53	-0,87	-2,31
Efflux pumps /				
Porines				
emrB	multidrug resistance protein B	0,87	2,35	2,59
tolC	Outer membrane protein TolC	-1,11	1,32	-
acrA	Acriflavin resistance protein A	-0,36	0,56	0,34
acrD	putative aminoglycoside efflux pump	2,36	-0,75	-1,60
sdeY	Multidrug resistance efflux pump	-	1,39	0,39
ybhR	ABC-type multidrug transport system, permease component	1,39	1,16	0,62
mdIB	Multidrug ABC transporter, permease/ATP-binding protein	-	1,02	1,61
отрН	Cationic 19 kDa outer membrane protein	-	1,40	1,40
mdlB	Outer membrane pore protein N, non-specific	-	1,02	1,61
ompA2	Putative exported protein	1,28	1,24	-
galF	UTP-glucose-1-phosphate uridylyltransferase, GalF protein		1,37	-
теоА	Outer membrane protein C, porin	-1,91	1,85	-2,39

Gene Product G-like D-like Combined stress Translation/ Traduction Translation/ Traduction stress stress stress rpsQ 305 ribosomal protein S17 1,80 - 2,69 - - 4,96 rpsF 305 ribosomal protein S19 1,72 - 4,96 - 2,03 4,96 - 2,03 4,96 - 2,03 4,96 - 2,03 4,96 - 2,03 4,96 - 2,03 4,96 - 3,95 505 ribosomal subult protein S10 0,96 2,07 - 3,96 - 3,98 - 2,41 4,85 - 3,39 7,77 - 3,96 - 3,39 7,77 - 3,59 - 3,59 - 3,59 - 3,59 - 3,59 - 1,74 - 2,52 - 3,59 - - 1,74 - 2,52 - 1,76 2,11 <			Log ₂ expression ratio ⁽¹⁾		ratio ⁽¹⁾
Gene Product stress stress stress stress stress Translation/ Traduction Traduction - 2,49 -0,50 - rpsQ 305 ribosomal protein S19 1,72 - 4,95 - 2,03 - 4,95 - 2,03 4,95 - 2,03 4,95 - 2,03 4,95 - 2,03 4,95 - 2,03 4,95 - 2,03 4,95 - 2,03 3,95 - 2,03 3,95 - 3,05 ribosomal subunit protein s18 - 2,41 4,85 - 3,63 ribos - 3,05 7,05 3,05 ribosomal protein 116 1,85 - 3,24 - 3,64 ribos - 3,05 ribosomal protein 129 1,74 - 2,29 rpmC 505 ribosomal protein 129 1,74 - 2,29 rpmE 505 ribosomal protein 121 1,15 2,44 6,60 - 1,70 2,11 rpiE 505 ribosomal subunit protein 124 <td< th=""><th></th><th></th><th>G-like</th><th>D-like</th><th>Combined</th></td<>			G-like	D-like	Combined
Translation/ Traduction Ribosomal RNA large subunit methyltransferase J 2,49 0.50 - rpsQ 305 ribosomal protein S17 1,80 - 2,60 rpsF 305 ribosomal protein S19 1,72 - 4,96 rpsF 305 ribosomal protein S6 - 2,03 4,96 rpsF 305 ribosomal subunit protein s10 0,96 2,07 5,37 rpsR 305 ribosomal subunit protein s13 - 2,41 4,85 rpsF 505 ribosomal protein L19 1,74 - 3,53 rplB 505 ribosomal protein 12 1,74 - 3,53 rplB 505 ribosomal protein 13 1,15 2,41 6,60 rplC 505 ribosomal protein 13 1,15 2,41 6,60 rplD 505 ribosomal protein 13 1,15 2,41 6,60 rplD 505 ribosomal subunit protein 14 1,39 2,64 6,21 rplD 505 ribosomal subunit protein 19 - 2,64 3,83 rplD 505 ribos	Gene	Product	stress	stress	stress
Traduction 2,49 -0,50 rpsQ 305 ribosomal protein S17 1.80 - 2,60 rpsF 305 ribosomal protein S19 1,72 - 4,96 rpsF 305 ribosomal protein S10 0,96 2,07 6,37 rpsF 305 ribosomal subunit protein s10 0,96 2,07 6,37 rpsK 305 ribosomal subunit protein s13 1,77 - 3,96 rpsK 305 ribosomal protein L16 1,85 - 3,63 rplF 505 ribosomal protein L19 1,74 - 5,29 rpmC 505 ribosomal protein L29 1,82 - 3,64 rplE 505 ribosomal protein L3 1,15 2,12 rplE 505 ribosomal protein L3 1,15 2,12 rplE 505 ribosomal protein L3 1,80 1,55 2,12 rplE 505 ribosomal protein L3 1,80 1,55 2,12 rplE 505 ribosomal protein L4 1,39 2,64 6,621 rplL rplE 505 ribosomal subunit protein L4 1,39 -	Translation/				
Initial Relation Notating estabulation 2, -9 2, -9 2, -9 2, -9 2, -9 2, -9 2, -9 2, -9 2, -9 2, -9 1, -7 - 4, -96 - 2, -03 4, -96 - 2, -03 4, -96 - 2, -03 4, -96 - 2, -03 4, -96 - 2, -03 4, -96 - 2, -03 4, -96 - 2, -03 4, -96 - 2, -03 4, -96 - 2, -03 - 4, -14 4, -85 - 3, -66 - 2, -03 - - 2, -14 4, -85 - 3, -65 - 3, -63 - - 2, -3 - 3, -64 - 2, -13 - - 3, -14 - 3, -25 - - 3, -16 - - - - 3, -12 - - - - - - - - - - - - - - - - - -	I raduction	Dibecomel DNA large subunit methyltransferess I	2.40	0 5 0	
μpst 305 Thosonial protein S19 1,20 - 4,96 rpsF 305 ribosonial protein S19 1,72 - 4,96 rpsF 305 ribosonial subunit protein s10 0,96 2,07 6,37 rpsR 305 ribosonial subunit protein s13 - 2,41 4,85 rpsR 305 ribosonial protein 116 1,85 - 3,69 pDF 505 ribosonial protein 119 1,74 - 3,39 rplS 505 ribosonial protein 129 1,82 - 3,44 rplC 505 ribosonial protein 129 1,82 - 3,44 rplC 505 ribosonial protein 129 1,82 - 3,44 rplC 505 ribosonial protein 120 - 1,70 2,11 rplD 505 ribosonial protein 121 - 1,70 2,11 rplD 505 ribosonial protein 124 1,39 2,64 6,21 rplI 505 ribosonial protein 129 - 2,64 3,63 rplI 505 ribosonial protein 129 -	rrmu rnsQ	RIDOSOMAI RNA large subunit methyltransferase J	2,49	-0,50	
Jps/s Job Hobsonial protein 119 1/2 - <t< td=""><td>rpsQ</td><td>20S ribosomal protein S17</td><td>1,00</td><td>-</td><td>2,00</td></t<>	rpsQ	20S ribosomal protein S17	1,00	-	2,00
pps/ 305 Hobosomal subunit protein s10 0,96 2,07 4,30 rps/ 305 ribosomal subunit protein s18 - 2,41 4,85 rps/ 305 ribosomal protein L16 1,85 - 3,63 rpl/ 505 ribosomal protein L19 1,74 - 3,39 rpl/E 505 ribosomal protein L29 1,82 - 3,64 rpl/C 505 ribosomal protein L3 1,15 2,41 6,40 rpm/C 505 ribosomal protein L3 1,15 2,41 6,40 rpl/E 505 ribosomal protein L3 1,15 2,41 6,40 rpm/E 505 ribosomal protein L3 1,55 2,12 6,62 rpl/D 505 ribosomal subunit protein L4 1,39 2,64 6,21 rpl/D 505 ribosomal subunit protein L4 1,39 2,64 6,21 rpl/A Ribosomal arge subunit protein Sa - 1,70 - rpl/A Ribosomal arge subunit protein Sa - 1,60 - 1,31 1,66 -	rps5	205 ribosomal protein 519	1,72	2 02	4,90
ps/R 305 inbosomal subunit protein 330 0,30 2,40 0,37 rps/R 305 ribosomal subunit protein 33 1,77 - 3,63 rpl/P 505 ribosomal protein 116 1,85 - 3,63 rpl/S 505 ribosomal protein 12 1,74 - 5,29 rpmC 505 ribosomal protein 12 1,74 - 5,29 rpmC 505 ribosomal protein 13 1,15 2,41 6,40 rpmE 505 ribosomal protein 13 1,15 2,41 6,40 rpmE 505 ribosomal protein 15 - 1,70 2,11 rpl/D 505 ribosomal subunit protein 14 1,39 2,64 3,83 fmt Methionyl-tRNA formyltransferase -1,08 -1,24 0,33 fmt Methionyl-tRNA formyltransferase 1,07 2,58 - rpl/A Ribosomal protein 11 - 1,90 - rpl/A Ribosomal protein 12 - 1,60 - 1,616 rpl/A Ribosomal protein 13	rpsi	30s ribosomal subunit protein s10	0.96	2,05	4,90
Josh Josh Budsmit protein 320 Image: Constrained and the protein 320 Image: Constrained and the protein 320 rpsC 305 ribosomal suburit protein 33 1,77 - 3,96 rplB 505 ribosomal protein L19 1,74 - 3,39 rplB 505 ribosomal protein L29 1,74 - 5,29 rpmC 505 ribosomal protein L3 1,15 2,41 6,40 rpmE 505 ribosomal protein L3 1,80 1,55 2,12 rplL 505 ribosomal protein L3 1,15 2,41 6,40 rplE 505 ribosomal protein L3 1,15 2,41 6,40 rplL 505 ribosomal protein L3 1,27 2,11 7/0 2,11 rplD 505 ribosomal subunit protein L4 1,33 2,64 6,21 rplK Ribosomal and protein L3 1,07 2,58 - rplA Ribosomal rprotein L1 - 1,90 - rpsN Ribosomal protein L1 - 1,90 - rpoD RNA polyme	rpsp	20s ribosomal subunit protein s10	0,90	2,07	1 85
Ipsic 305 Interstant studing protein LS 1,77 1 3,90 rplP SOS ribosomal protein L16 1,85 - 3,61 rplS SOS ribosomal protein L19 1,74 - 3,39 rplR SOS ribosomal protein L29 1,82 - 3,64 rplC SOS ribosomal protein L3 1,15 2,41 6,40 rplE SOS ribosomal protein L3 1,25 2,12 6,46 rplE SOS ribosomal protein L4 1,39 2,64 6,21 rplI SOS ribosomal subunit protein L9 - 2,64 3,83 fmt Methionyl-tRNA formyltransferase -1,08 -1,22 0,33 rplA Ribosomal protein S14p - 1,90 - rpsN Ribosomal protein S14p - 1,80 - rpoD RNA chaperone Hfq 2,03 -0,59 - rpoD RNA chaperone Hose 1,62 - - rpoC tRNA (guanine-N(1)-)-methyltransferase 1,60 1,31 <td>rpsh</td> <td>205 ribosomal subunit protein 52</td> <td>- 1 77</td> <td>2,41</td> <td>4,65</td>	rpsh	205 ribosomal subunit protein 52	- 1 77	2,41	4,65
Ipin 30.01 Indestinal protein 110 1,63 1 30,03 rpl/S S05 ribosomal protein 119 1,74 - 3,39 rpl/B S05 ribosomal protein 129 1,74 - 3,29 rpl/C S05 ribosomal protein 129 1,82 - 3,64 rpl/E S05 ribosomal protein 13 1,15 2,41 6,40 rpl/E S05 ribosomal protein 14 1,39 2,64 6,21 rpl/D S05 ribosomal subunit protein 19 - 2,64 3,83 fmt Methionyl-tRNA formyltransferase 1,08 1,24 0,33 rpl/A Ribosomal and protein 19 - 2,64 3,83 rpl/A Ribosomal and protein 19 - 2,64 3,83 rpl/A Ribosomal and protein 19 - 2,64 3,83 rpl/A Ribosomal protein 11 - 1,90 - rpl/A Ribosomal protein 11 - 1,90 - rpl/A Ribosomal protein 129 2,211 0,93	rpsc	505 ribosomal protoin 116	1,77	-	2,50
Ippin Sost industrial protein L29 1,74 - 5,59 rp/B Sost indosomal protein L29 1,74 - 5,29 rpmC 50S ribosomal protein L3 1,15 2,41 6,40 rpmE 50S ribosomal protein L3 1,80 1,55 2,12 rplE 50S ribosomal protein L3 - 1,74 - 2,64 6,21 rplI 50S ribosomal subunit protein L4 1,39 2,64 6,21 7,70 2,11 rplD 50S ribosomal subunit protein L3 - - 2,64 3,83 fmt Methonyl-tRNA formyltransferase -0,63 -1,73 1,16 rplA Ribosomal protein 11 - 1,90 - 1,80 - rplA Ribosomal protein 11 - 1,90 - 1,80 - - 1,80 - - 1,80 - - 1,90 - 1,80 - - 1,80 - - 1,80 - - 1,80 <td>rpir</td> <td>EQS ribosomal protein L10</td> <td>1,85</td> <td>-</td> <td>3,03</td>	rpir	EQS ribosomal protein L10	1,85	-	3,03
Type Sign Hodsonia protein L2 1,7,4 - 5,25 rpmC 505 ribosomal protein L2 1,82 - 3,64 rplC 505 ribosomal protein L3 1,15 2,41 6,40 rpmE 505 ribosomal protein L3 1,80 1,55 2,12 rplE 505 ribosomal protein L4 1,39 2,64 6,21 rplI 505 ribosomal subunit protein L4 1,39 2,64 6,21 rplI 505 ribosomal aubunit protein L9 - 2,64 3,83 fmt Methionyl-tRNA formyltransferase -0,63 -1,73 1,16 rluA Ribosomal protein L1 - 1,90 - rplA Ribosomal protein S14p - 1,80 - yheL Ribosomal protein S14p - 1,80 - rpoD RNA chaperone Hfq 2,03 -0,59 - rmD RNA polymerase sigma factor 1,65 1,20 - rpoC RNA (guanine-N(1)-)-methyltransferase 1,60 -	rpIB	EQS ribosonial protein L19	1,74	-	5,59
Tp/Inc. Sos Rubsonia protein L3 1,82 - 3,64 rp/IC Sos ribosomal protein L3 1,15 2,41 6,40 rp/IE Sos ribosomal protein L3 1,80 1,55 2,12 rp/IE Sos ribosomal subunit protein L4 1,39 2,64 6,21 rp/I Sos ribosomal subunit protein L9 - 2,64 3,83 fmt Methionyl-tRNA formyltransferase -1,08 -1,24 0,33 yibk Predicted rRNA methylase -0,63 -1,73 1,16 rluA Ribosomal protein L1 - 1,90 - rpsN Ribosomal protein S14p - 1,80 - rpoD RNA chaperone Hfq 2,03 -0,59 - rpoD RNA chaperone Hfq 2,03 -0,59 - rpoC tRNA (guanine-N(1)-)-methyltransferase 1,60 - 1,31 DNA synthethis - - - - - and reparation - - - <td< td=""><td>трів тала С</td><td></td><td>1,74</td><td>-</td><td>5,29</td></td<>	трів тала С		1,74	-	5,29
IP/C SOS Hoosomal protein L3 1,15 2,41 6,40 rpmE SOS ribosomal protein L3 1,80 1,55 2,12 rp/E SOS ribosomal protein L4 1,39 2,64 6,21 rp/I SOS ribosomal subunit protein L9 - 2,64 6,21 rp/I SOS ribosomal subunit protein L9 - 2,64 3,83 fmt Methionyl-tRNA formyltransferase -1,08 -1,24 0,33 yib/K Predicted rRNA methylase -0,63 -1,73 1,16 rluA Ribosomal protein L1 - 1,90 - rpsN Ribosomal protein S14p - 1,80 - rpoD RNA chaperone Hfq 2,03 -0,59 - rpoD RNA chaperone Hfq 2,03 -0,59 - rpoC tRNA (guanine-N(1)-)-methyltransferase 1,60 - 1,31 DNA synthethis and reparation - - - - - - - - - - <td>rpmc</td> <td>505 ribosomal protein L29</td> <td>1,82</td> <td>-</td> <td>3,64</td>	rpmc	505 ribosomal protein L29	1,82	-	3,64
Tpmic SUS Ribosomal protein L31 1,80 1,55 2,12 rp/E SOS ribosomal protein L5 - 1,70 2,11 rp/D SOS ribosomal subunit protein L4 1,39 2,64 6,21 rp/I SOS ribosomal subunit protein L9 - 2,64 3,83 fmt Methionyl-tRNA formyltransferase -1,008 -1,24 0,33 yibK Predicted rRNA methylase -0,63 -1,73 1,16 rluA Ribosomal protein L1 - 1,90 - rp/A Ribosomal protein S14p - 1,80 - rpsN Ribosomal RNA large subunit methyltransferase J 2,21 -0,93 - rpoD RNA chaperone Hfq 2,03 -0,59 - trmD RNA polymerase signa factor 1,65 1,20 - luxS S-ribosylhomocysteine lyase 1,60 - 1,31 DNA synthethis - - - - - and reparation - - 0	rpic	505 ribosomai protein L3	1,15	2,41	6,40
rpit SUS Ribosomal protein LS - 1,70 2,11 rpiD 50S ribosomal subunit protein L4 1,39 2,64 6,21 rpiI 50S ribosomal subunit protein L9 - 2,64 3,83 fmt Methionyl-tRNA formyltransferase -1,08 -1,24 0,33 yibk Predicted rRNA methylase -0,63 -1,73 1,16 rluA Ribosomal protein 11 - 1,90 - rpsN Ribosomal protein 11 - 1,90 - rpsN Ribosomal protein 14 - 1,90 - rpsN Ribosomal protein 14 - 1,90 - rpsN Ribosomal protein 14 - 1,80 - rpsN Ribosomal actor 1,65 1,20 - rpoD RNA chaperone Hfq 2,03 -0,59 - rpoC tRNA (guanine-N(1)-)-methyltransferase 1,60 - 1,31 DNA synthethis - - - - <t< td=""><td>rpmE</td><td>505 ribosomai protein L31</td><td>1,80</td><td>1,55</td><td>2,12</td></t<>	rpmE	505 ribosomai protein L31	1,80	1,55	2,12
rpiD Sub mossional subunit protein L4 1,39 2,64 6,21 rpll SOS ribosomal subunit protein L9 - 2,64 3,83 fmt Methionyl-tRNA formyltransferase -1,08 -1,24 0,33 yibK Predicted rRNA methylase -0,63 -1,73 1,16 rluA Ribosomal protein L1 - 1,90 - rpsN Ribosomal protein S14p - 1,80 - rpoD RNA chaperone Hfq 2,03 -0,59 - rpoD RNA chaperone Hfq 2,03 -0,59 - rpoC tRNA polymerase sigma factor 1,65 1,20 - rpoC tRNA (guanine-N(1)-)-methyltransferase 1,60 - 1,31 DNA synthethis and reparation - - - - mutM DNA glycosylase 4,11 -0,43 1,89 - - hupB DNA topoisomerase 1,81 - 1,67 - - fmtD InAguantie epsilon -0,45 0,87 -3,13 - <t< td=""><td>rpie</td><td>505 ribosomai protein L5</td><td>-</td><td>1,70</td><td>2,11</td></t<>	rpie	505 ribosomai protein L5	-	1,70	2,11
rpli Sub moseomar suburit protein L9 - 2,64 3,83 fmt Methionyl-tRNA formyltransferase - 0,63 -1,72 0,33 fmt Ribosomal large subunit pseudouridine synthase A 1,07 2,58 - r/µA Ribosomal protein L1 - 1,90 - rpsN Ribosomal protein S14p - 1,80 - yheL Ribosomal protein S14p - 1,80 - rpDD RNA chaperone Hfq 2,03 -0,59 - trmD RNA polymerase sigma factor 1,65 1,20 - rpoC tRNA (guanine-N(1)-)-methyltransferase 1,60 - 1,31 DNA synthethis - 1,80 - - - and reparation - 1,60 - 1,31 - mutM DNA glycosylase 4,11 -0,43 1,89 - - dnaG DNA polymerase III subunit epsilon -1,04 - 2,58 - <td< td=""><td>rpiD</td><td>505 ribosomai subunit protein L4</td><td>1,39</td><td>2,64</td><td>6,21</td></td<>	rpiD	505 ribosomai subunit protein L4	1,39	2,64	6,21
JmtMethionyltransferase-1,08-1,240,33yibKPredicted rRNA methylase-0,63-1,731,16rluARibosomal large subunit pseudouridine synthase A1,072,58-rplARibosomal protein L1-1,90-rpsNRibosomal protein S14p-1,80-yheLRibosomal RNA large subunit methyltransferase J2,21-0,93-rpDDRNA chaperone Hfq2,03-0,59-trmDRNA polymerase sigma factor1,651,20-luxSS-ribosylhomocysteine lyase1,62rpoCtRNA (guanine-N(1)-)-methyltransferase1,60-1,31DNA synthethisand reparationmutMDNA glycosylase4,11-0,431,89dnaGDNA binding protein HU-betadnaQDNA binding protein HU-betadnaQDNA polymerase III subunit epsilonholEDNA-binding proteinogtmethylated-DNAprotein-cysteine methyltransferaseogtmethylated-DNAprotein-cysteine methyltransferasefinJDNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA repair protein RecO0,49finJDNA-directed RNA polymerase, alpha	rpii	505 ribosomal subunit protein L9	-	2,64	3,83
ylbkPredicted rKNA methylase40,63-1,731,16rluARibosomal large subunit pseudouridine synthase A1,072,58-rplARibosomal protein L1-1,90-rpsNRibosomal protein S14p-1,80-yheLRibosomal RNA large subunit methyltransferase J2,21-0,93-rpoDRNA chaperone Hfq2,03-0,59-trmDRNA polymerase sigma factor1,651,20-luxSS-ribosylhomocysteine lyase1,62rpoCtRNA (guanine-N(1)-)-methyltransferase1,60-1,31DNA synthethisand reparationmutMDNA glycosylase4,11-0,431,89hupBDNA-binding protein HU-beta-1,04-2,58dnaQDNA polymerase III subunit epsilon-0,05-0,860,94tusInhibitor of replication at Ter, DNA-binding protein-1,14DpsDNA-binding protein-1,32-0,60ogtmethylated-DNAprotein-cysteine methyltransferase-1,34-0,570,55comEADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA repair protein RecO0,49-1,17-dinJDNA-drage-inducible protein J0,51-1,21-1,48	fmt	Methionyl-tRNA formyltransferase	-1,08	-1,24	0,33
HUARibosomal large subunit pseudouridine synthase A1,072,58-rplARibosomal protein L1-1,90-rpsNRibosomal protein S14p-1,80-yheLRibosomal RNA large subunit methyltransferase J2,21-0,93-rpoDRNA chaperone Hfq2,03-0,59-trmDRNA polymerase sigma factor1,651,20-luxSS-ribosylhomocysteine lyase1,62rpoCtRNA (guanine-N(1)-)-methyltransferase1,60-1,31DNA synthethisand reparationmutMDNA glycosylase4,11-0,431,89hupBDNA-binding protein HU-beta-1,04-2,58dnaQDNA polymerase III subunit epsilon-1,05-0,860,94tusInhibitor of replication at Ter, DNA-binding protein-1,14DpSDNA-binding protein-1,320,60-0,620,62ogtmethylated-DNAprotein-cysteine methyltransferase-1,34-0,570,55comEADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA repair protein RecO0,49-1,17-dinJDNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA repair protein RecO0,49-1,17-dinJDNA-drage-inducible protein J0,51-1,21-1,48	yibk	Predicted rRNA methylase	-0,63	-1,/3	1,16
rpIARibosomal protein L1-1,90-rpsNRibosomal protein S14p-1,80-yheLRibosomal RNA large subunit methyltransferase J2,21-0,93-rpoDRNA chaperone Hfq2,03-0,59-trmDRNA polymerase sigma factor1,651,20-luxSS-ribosylhomocysteine lyase1,62rpoCtRNA (guanine-N(1)-)-methyltransferase1,60-1,31DNA synthethisand reparationmutMDNA glycosylase4,11-0,431,89dnaGDNA primase3,07topADNA topoisomerase1,81-1,89hupBDNA-binding protein HU-beta-1,04dnaQDNA polymerase III subunit epsilon-1,04dpsDNA-binding protein-0,450,87-3,13holEDNA polymerase III theta subunit-1,320,60-0,62ogtmethylated-DNAprotein-cysteine methyltransferase-1,34-0,570,55comEADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA-damage-inducible protein J0,51-1,21-1,42dinJDNA-damage-inducible protein J0,51-1,21-1,42	rluA	Ribosomal large subunit pseudouridine synthase A	1,07	2,58	-
rpsNRibosomal protein S14p-1,80-yheLRibosomal RNA large subunit methyltransferase J2,21-0,93-rpoDRNA chaperone Hfq2,03-0,59-trmDRNA polymerase sigma factor1,651,20-lwSS-ribosylhomocysteine lyase1,62rpoCtRNA (guanine-N(1)-)-methyltransferase1,60-1,31DNA synthethis and reparationmutMDNA glycosylase4,11-0,431,89dnaGDNA primase3,07topADNA topoisomerase1,81-2,58dnaQDNA polymerase III subunit epsilon-1,05-0,860,94tusInhibitor of replication at Ter, DNA-binding protein-1,41DpsDNA-binding protein-0,450,87-3,13holEDNA polymerase III theta subunit-0,320,60-0,62ogtmethylated-DNAprotein-cysteine methyltransferase-1,34-0,570,55comEADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA-damage-inducible protein J0,61-1,21-1,44	rpIA	Ribosomal protein L1	-	1,90	-
yheLRibosomal RNA large subunit methyltransferase J2,21-0,93-rpoDRNA chaperone Hfq2,03-0,59-trmDRNA polymerase sigma factor1,651,20-luxSS-ribosylhomocysteine lyase1,62rpoCtRNA (guanine-N(1)-)-methyltransferase1,60-1,31DNA synthethisand reparationmutMDNA glycosylase4,11-0,431,89dnaGDNA primase3,07topADNA topoisomerase1,81-1,89hupBDNA-binding protein HU-beta-1,04-2,58dnaQDNA polymerase III subunit epsilon-1,05-0,860,94tusInhibitor of replication at Ter, DNA-binding protein-1,141,67DpsDNA-binding protein-1,320,60-0,62ogtmethylated-DNAprotein-cysteine methyltransferase-1,34-0,570,55comEADNA uptake protein-1,460,66-0,95rpoADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA-dimage-inducible protein J0,51-1,21-1,48wdDDNA-damage-inducible protein J0,51-1,21-1,48vdDDNA-damage-inducible protein J0,51-1,21-1,48	rpsN	Ribosomal protein S14p	-	1,80	-
rpoDRNA chaperone Hfq2,03-0,59-trmDRNA polymerase sigma factor1,651,20-luxSS-ribosylhomocysteine lyase1,62rpoCtRNA (guanine-N(1)-)-methyltransferase1,60-1,31DNA synthethis and reparation-1,31-mutMDNA glycosylase4,11-0,431,89dnaGDNA primase3,07topADNA topoisomerase1,81-1,89hupBDNA-binding protein HU-beta-1,04-2,58dnaQDNA polymerase III subunit epsilon-1,05-0,860,94tusInhibitor of replication at Ter, DNA-binding protein-1,141,67DpsDNA-binding protein-0,450,87-3,13holEDNA polymerase III theta subunit-1,320,60-0,62ogtmethylated-DNAprotein-cysteine methyltransferase-1,34-0,570,55comEADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA-damage-inducible protein J0,61-1,17-dinJDNA-damage-inducible protein J0,51-1,21-1,48	yheL	Ribosomal RNA large subunit methyltransferase J	2,21	-0,93	-
trmDRNA polymerase sigma factor1,651,20-luxSS-ribosylhomocysteine lyase1,62rpoCtRNA (guanine-N(1)-)-methyltransferase1,60-1,31DNA synthethis and reparationmutMDNA glycosylase4,11-0,431,89dnaGDNA primase3,07topADNA topoisomerase1,81-1,89hupBDNA-binding protein HU-beta-1,04-2,58dnaQDNA polymerase III subunit epsilon-1,05-0,860,94tusInhibitor of replication at Ter, DNA-binding protein-1,141,67DpsDNA-binding protein-1,320,60-0,62ogtmethylated-DNAprotein-cysteine methyltransferase-1,34-0,570,55comEADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA-damage-inducible protein J0,51-1,21-1,48urdDDNA-damage-inducible protein J0,51-1,21-1,48	rpoD	RNA chaperone Hfq	2,03	-0,59	-
IuxSS-ribosylhomocysteine lyase1,62-rpoCtRNA (guanine-N(1)-)-methyltransferase1,60-1,31DNA synthethis and reparationmutMDNA glycosylase4,11-0,431,89dnaGDNA primase3,07topADNA topoisomerase1,81-1,89hupBDNA-binding protein HU-beta-1,04-2,58dnaQDNA polymerase III subunit epsilon-1,05-0,860,94tusInhibitor of replication at Ter, DNA-binding protein-1,141,67DpsDNA-binding protein-1,320,60-0,62ogtmethylated-DNAprotein-cysteine methyltransferase-1,34-0,570,55comEADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA-damage-inducible protein J0,51-1,21-1,48urdDDNA-damage-inducible protein J0,51-1,21-1,48	trmD	RNA polymerase sigma factor	1,65	1,20	-
rpoCtRNA (guanine-N(1)-)-methyltransferase1,60-1,31DNA synthethis and reparationnd reparation4,11-0,431,89mutMDNA glycosylase4,11-0,431,89dnaGDNA primase3,07topADNA topoisomerase1,81-1,89hupBDNA-binding protein HU-beta-1,04-2,58dnaQDNA polymerase III subunit epsilon-1,05-0,860,94tusInhibitor of replication at Ter, DNA-binding protein-1,141,67DpsDNA-binding protein-1,320,60-0,62ogtmethylated-DNAprotein-cysteine methyltransferase-1,34-0,570,55comEADNA uptake protein-1,460,66-0,99rpoADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA repair protein RecO0,49-1,17-dinJDNA-damage-inducible protein J0,51-1,21-1,48	luxS	S-ribosylhomocysteine lyase	1,62	-	-
DNA synthethis and reparationmutMDNA glycosylase4,11-0,431,89dnaGDNA primase3,07-topADNA topoisomerase1,81-1,89hupBDNA-binding protein HU-beta-1,04-2,58dnaQDNA polymerase III subunit epsilon-1,05-0,860,94tusInhibitor of replication at Ter, DNA-binding protein-1,141,67DpsDNA-binding protein-1,320,60-0,62ogtmethylated-DNAprotein-cysteine methyltransferase-1,34-0,570,55comEADNA uptake protein-1,460,66-0,95recODNA repair protein RecO0,49-1,17-dinJDNA-damage-inducible protein J0,51-1,21-1,48	rpoC	tRNA (guanine-N(1)-)-methyltransferase	1,60	-	1,31
mutMDNA glycosylase4,11-0,431,89dnaGDNA primase3,07topADNA topoisomerase1,81-1,89hupBDNA-binding protein HU-beta-1,04-2,58dnaQDNA polymerase III subunit epsilon-1,05-0,860,94tusInhibitor of replication at Ter, DNA-binding protein-1,141,67DpsDNA-binding protein-0,450,87-3,13holEDNA polymerase III theta subunit-1,320,60-0,62ogtmethylated-DNAprotein-cysteine methyltransferase-1,460,66-0,95rpoADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA-damage-inducible protein J0,51-1,21-1,48wdDDNA-tanage-inducible protein J0,51-1,21-1,48	DNA synthethis and reparation				
InduktDiktrgited protein1,110,131,03dnaGDNA primase3,07topADNA topoisomerase1,81-1,89hupBDNA-binding protein HU-beta-1,04-2,58dnaQDNA polymerase III subunit epsilon-1,05-0,860,94tusInhibitor of replication at Ter, DNA-binding protein-1,141,67DpsDNA-binding protein-0,450,87-3,13holEDNA polymerase III theta subunit-1,320,60-0,62ogtmethylated-DNAprotein-cysteine methyltransferase-1,460,66-0,95rpoADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA repair protein RecO0,49-1,17-dinJDNA-damage-inducible protein J0,51-1,21-1,48	mutM		4 11	-0.43	1 89
topADNA topoisomerase1,81-1,89hupBDNA-binding protein HU-beta-1,04-2,58dnaQDNA polymerase III subunit epsilon-1,05-0,860,94tusInhibitor of replication at Ter, DNA-binding protein-1,141,67DpsDNA-binding protein-0,450,87-3,13holEDNA polymerase III theta subunit-1,320,60-0,62ogtmethylated-DNAprotein-cysteine methyltransferase-1,460,66-0,95rpoADNA directed RNA polymerase, alpha subunit0,741,552,93recODNA repair protein RecO0,49-1,17-dinJDNA-damage-inducible protein J0,51-1,21-1,48	dnaG	DNA primase	3.07	- 0,+5	- 1,05
hupBDNA-binding protein HU-beta-1,04-2,58dnaQDNA polymerase III subunit epsilon-1,05-0,860,94tusInhibitor of replication at Ter, DNA-binding protein-1,141,67DpsDNA-binding protein-0,450,87-3,13holEDNA polymerase III theta subunit-1,320,60-0,62ogtmethylated-DNAprotein-cysteine methyltransferase-1,460,66-0,95comEADNA uptake protein-1,460,66-0,95rpoADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA repair protein RecO0,49-1,17-dinJDNA-damage-inducible protein J0,51-1,21-1,48	tonA	DNA tonoisomerase	1 81	_	1 89
ImpleDividenting protein frontent2,50dnaQDNA polymerase III subunit epsilon-1,05-0,860,94tusInhibitor of replication at Ter, DNA-binding protein-1,141,67DpsDNA-binding protein-0,450,87-3,13holEDNA polymerase III theta subunit-1,320,60-0,62ogtmethylated-DNAprotein-cysteine methyltransferase-1,34-0,570,55comEADNA uptake protein-1,460,66-0,95rpoADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA repair protein RecO0,49-1,17-dinJDNA-damage-inducible protein J0,51-1,21-1,48wrdDDutativo DNA tangicomerace-0,23-0,23	hunB	DNA-binding protein HII-beta	-1.04	_	2 58
IndexDistribution1,030,0000,04tusInhibitor of replication at Ter, DNA-binding protein-1,141,67DpsDNA-binding protein-0,450,87-3,13holEDNA polymerase III theta subunit-1,320,60-0,62ogtmethylated-DNAprotein-cysteine methyltransferase-1,34-0,570,55comEADNA uptake protein-1,460,66-0,95rpoADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA repair protein RecO0,49-1,17-dinJDNA-damage-inducible protein J0,51-1,21-1,48wdDDutativo DNA tangicomerace-2,27	dnaO	DNA polymerase III subunit ensilon	-1.05	-0.86	0.94
DpsDNA-binding protein-0,450,87-3,13holEDNA polymerase III theta subunit-1,320,60-0,62ogtmethylated-DNAprotein-cysteine methyltransferase-1,34-0,570,55comEADNA uptake protein-1,460,66-0,95rpoADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA repair protein RecO0,49-1,17-dinJDNA-damage-inducible protein J0,51-1,21-1,48	tus	Inhibitor of replication at Ter DNA-binding protein	-1 1/	0,00	-1 67
bpsbox soluting proteinco,43co,47co,47co,47holEDNA polymerase III theta subunit-1,320,60-0,62ogtmethylated-DNAprotein-cysteine methyltransferase-1,34-0,570,55comEADNA uptake protein-1,460,66-0,95rpoADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA repair protein RecO0,49-1,17-dinJDNA-damage-inducible protein J0,51-1,21-1,48	Dns	DNA-binding protein	-0.45	0.87	_2 12
IndicDNA polymerase in theta subunit-1,320,000,02ogtmethylated-DNAprotein-cysteine methyltransferase-1,34-0,570,55comEADNA uptake protein-1,460,66-0,95rpoADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA repair protein RecO0,49-1,17-dinJDNA-damage-inducible protein J0,51-1,21-1,48wrdDPutativo DNA tangicomerace-2,27	bolE	DNA polymerase III theta subunit	-0,45	0,87	-3,13
comEADNA uptake protein-1,460,66-0,95rpoADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA repair protein RecO0,49-1,17-dinJDNA-damage-inducible protein J0,51-1,21-1,48	oat	methylated_DNA_protein_cysteine methyltransferase	-1,32	-0.57	-0,02
rpoADNA-directed RNA polymerase, alpha subunit0,741,552,93recODNA repair protein RecO0,49-1,17-dinJDNA-damage-inducible protein J0,51-1,21-1,48wdDPutativo DNA tapair parage0,23	comEA	DNA uptako protoin	-1,54	0,57	0,05
rec0DNA repair protein Rec00,49-1,17dinJDNA-damage-inducible protein J0,51-1,21-1,48wd0Putativo DNA tapoiromerase-1,20-2,27	rnoA	DNA-directed RNA polymerase alpha subunit	0.74	1 55	20,95 2 D2
dinJ DNA-damage-inducible protein J 0,51 -1,21 -1,48 wrdD Putativo DNA tapairomeraco 2,27	recO	DNA repair protain RecO	0,74	1,55	2,33
$\frac{1}{2}$	dinl	DNA-damage_inducible protein l	0,49	1_7	- 1 / 9
	vrdD		0,51	-1.21	-1,40

The metabolism of amino acids appeared to be of importance in both bacteria. Decarboxylation of amino acids to help bacteria fight against acid stress has been nicely described in the literature, especially in the Enterobacteriacea family (for review see Krulwich et al. 2001). A focus on the glutamate metabolism (Figure 4) show that H. alvei GB001 up-regulated the L-Glutamate conversion to GABA while down-regulating the other pathways involving glutamate as a substrate. It is of note that the gene encoding the GadC transporter (exporting GABA while importing Glutamate) was not found in the annotated genome of H. alvei GB001 and might be explained by the poor composition of the stress medium. Indeed, if no extra-cellular glutamate is available to import, the cell shall not waste its GABA pool and last molecule could be used in other part of the microorganism metabolism. The arginine decarboxylase encoding gene, adi, along with fliY and yecS encoding two amino acids transporters were up-regulated as well, which add some more proof of the ability of H. alvei GB001 to use decarboxylation of amino acid to increase its internal pH. Decarboxylation of amino acid was up-regulated as well in S. equorum Mu2 with the over-expression of a putative lysine/arginine/ornithine decarboxylase encoding gene (unamed). Such phenomenon has been documented in gram-positive bacteria like Lactococcus lactis (Cotter et al. 2003). We also observed the up-regulation of genes potAD involved in polyamines metabolism in S. equorum Mu2. In agreement with this, spermidine and putrescine have been identified as agent regulating pH homeostasis among other functions in the bacterial cell (Pegg and Casero, 2009).



Figure 4 : Glutamate metabolism and conversion to GABA. Blue:Up-regulated genes. Red:down-regulated genes 1.4.1.13, glutamate synthase (NADPH) ; 1.4.1.4, glutamate dehydrogenase (NADP+) ; 2.6.1.16, glutamine-fructose-6-phosphate transaminase (isomerizing) ; 2.6.1.19, 4-aminobutyrate-2-oxoglutarate transaminase ; 6.3.5.5, Carbamoyl-phosphate synthase (glutamine-hydrolysing) ; 4.1.1.15, Glutamate decarboxylase A

		Log ₂ ex	pression r	atio ⁽¹⁾
		G-like	D-like	Combined
Gene	Product	stress	stress	stress
General stress				
proteins				
	universal stress family domain-containing protein	7,09	-1,57	1,60
hslO	33 kDa chaperonin		1,61	-
asp23	alkaline shock protein 23	3,43	-1,51	-
clpC	ATP-dependent Clp protease ATP-binding subunit		3,63	-
clpX	ATP-dependent Clp protease ATP-binding subunit ClpX	-2,74	3,63	1,81
clpP	ATP-dependent Clp protease proteolytic subunit ClpP	-	4,20	-
сlpВ	chaperone protein ClpB	-	4,52	-2,04
dnaJ	chaperone protein DnaJ	-	2,05	-1,46
dnaK	chaperone protein DnaK		1,81	-1,40
groEL	chaperonin GroEL	-1,79	3,33	-1,04
groES	co-chaperonin GroES	-2,10	3,69	-
cspA	cold shock protein CspA	4,36	-0,69	1,54
	general stress protein	3,76	-1,18	0,72
hfq	RNA chaperone Hfq	1,00	-	1,80
	universal stress protein	6,12	-3,63	-
Reductase /				
oxidoreductase		_		
panE	2-dehydropantoate 2-reductase	4,02	-0,58	-1,39
	aldehyde dehydrogenase	2,06	1,54	-
arsC	arsenate reductase	1,26	2,08	-1,15
cdr	coenzyme A disulfide reductase	2,41	0,71	0,97
guaC	GMP reductase	-	1,97	-
hisD	histidinol dehydrogenase	2,64	-1,90	-
guaB	IMP dehydrogenase	2,11	-1,98	-
mdh	malate dehydrogenase	2,49	0,37	-
mqo	malate dehydrogenase (acceptor)	3,09	-0,84	-
	malate dehydrogenase (oxaloacetate-decarboxylating)	1,71	1,27	-1,35
nfrA	NADPH-dependent oxidoreductase	5,75	-2,85	-
	nitroreductase family protein	4,06	-0,38	1,21
msrA	peptide-methionine (S)-S-oxide reductase	2,17	-0,48	1,66
putA	proline dehydrogenase	2,85	-0,35	-
dkgA	putative 2,5-didehydrogluconate reductase	1,52	1,72	0,68
	putative arsenate reductase	1,63	-1,42	1,28
	putative oxidoreductase	2,74	-	-
	putative thioredoxin reductase	-1,18	2,47	-
	putative Zn-dependent alcohol dehydrogenase	1,80	-3,18	-0,89
	pyridine nucleotide-disulfide oxidoreductase	3,47	-3,93	-
	short-chain dehydrogenases/reductases family protein	3,91	7,06	0,92
	short-chain dehydrogenases/reductases family protein	3,91	7,06	0,92
	short-chain dehydrogenases/reductases family protein	2,20	7,82	0,92
	short-chain dehydrogenases/reductases family protein	1,82	7,06	0,89
	short-chain dehydrogenases/reductases family protein	1,79	2,19	0,90
Deen en este				
Response to				
codd		1 00		
300A	superovide districtase	1,09	-	-

Table 5: Selection of genes (CDS) significantly up- or down-regulated in *S. equorum* Mu2 after exposure to (i) G-like, (ii) D-like and (iii) G-like followed by D-like – i.e. combined- stress

			.og ₂ expres	sion ratio ⁽¹⁾
		G-like	D-like	Combined
Gene	Product	stress	stress	stress
Oxydative phosphorylation				
ubiE	demethylmenaquinone methyltransferase	-0,89	-	-
atpA	H(+)-transporting two-sector ATPase, alpha chain	-3,56	0,79	-
atpD	H(+)-transporting two-sector ATPase, beta chain	-3 <i>,</i> 38	0,62	-
atpE	H(+)-transporting two-sector ATPase, chain C	-4,00	-	-0,97
atpH	H(+)-transporting two-sector ATPase, delta chain fragment	-3,88	0,60	-
atpH	H(+)-transporting two-sector ATPase, delta chain fragment	-3,88	0,60	-
atpC	H(+)-transporting two-sector ATPase, epsilon chain	-4,23	0,71	-
atpG	H(+)-transporting two-sector ATPase, gamma chain	-3,88	0,64	-
ctaA/cyoB	heme A synthase	-2,41	-0,68	-
	probable NADH-quinone oxidoreductase subunit	-2,15	-	-
ctaB/cyoB	protoheme IX farnesyltransferase	-1,49	-0,59	-
qoxB	quinol oxidase subunit 1	-1,68	-1,96	-
qoxA	quinol oxidase subunit 2	-2,24	-2,00	-
sdhA	succinate dehydrogenase flavoprotein subunit	-1,18	-	-
sdhC	succinate dehydrogenase, cytochrome b558 subunit	-1,16	-0,64	-
TCA Cycle				
lpdA	dihydrolipoyl dehydrogenase	-1,46	1,45	-
pdhC	dihydrolipoyllysine-residue acetyltransferase	-2,31	1,55	-
рус	pyruvate carboxylase	-1,72	0,44	1,23
pdhA	pyruvate dehydrogenase E1 component subunit alpha	-2,24	1,43	-
pdhB	pyruvate dehydrogenase E1 component subunit beta	-2,24	1,58	-
sdhA	succinate dehydrogenase flavoprotein subunit	-1,18	-	-
sdhC	succinate dehydrogenase, cytochrome b558 subunit	-1,16	-0,64	-
sucD	succinyl-CoA synthetase (ADP-forming) alpha subunit	-1,88	-0,48	-
sucC	succinyl-CoA synthetase beta subunit	-1,37	-0,46	-
Glycolyse				
acs	acetyl-CoA synthetase	-1,56	-	-
galM	aldose 1-epimerase	-0,76	-	-
рскА	phosphoenolpyruvate carboxykinase (ATP)	-2,51	-0,98	-1,67
panA malla B	pyruvate denydrogenase E1 component subunit alpha	-2,24	1,43	-
panB radh C	pyruvate denydrogenase E1 component subunit beta	-2,24	1,58	-
panc pdhD	dihydrolipoamide dehydrogenase	-2,31 -1,46	1,55	-
Amino acid decarboxylases				
	putative lysine decarboxylase fragment	1,77	0,53	-
	arginine/lysine/ornithine decarboxylase	1,15	-	-
Polyamines				
	spermidine/putrescine ABC transporter, inner membrane subunit	2,30	-2,80	-
potD	spermidine/putrescine ABC transporter, substrate-binding protein	2,24	-2,72	0,95
potA	spermidine/putrescine ABC transporter, ATP-binding subunit	2,01	-]	-1,67
Sulfur metabolism				
cysK	putative cysteine desulfurase	-	1,77	0,97
	cysteine synthase	-	1,28	-1,66
	putative iron-sultur cluster assembly accessory protein	-1,11	1,83	1,28

		Lo	og ₂ expres	sion ratio ⁽¹⁾
		G-like	D-like	Combined
Gene	Product	stress	stress	stress
Transporter (ABC, PTS, other)				
	ABC transporter, ATP-binding subunit	4,41	2,38	-1,12
	ABC transporter, inner membrane and binding protein subunit	1,73	-	-1,38
	ABC transporter, inner membrane subunit	5,34	2,42	-0,95
	amino acid ABC transporter, binding protein subunit	1,99	-	-1,93
	amino acid ABC transporter, inner membrane subunit	1,40	-	-1,69
	amino acid transporter	1,89	0,48	1,36
aluA	BUCT family osmoprotectant transporter	-	2,01	-
ушА	iron-siderophore ABC transporter ATP-binding subunit	2,00	-	- 0.84
	iron-siderophore ABC transporter substrate-binding subdim	2.26	0,50	-0.48
metN	methionine ABC transporter. ATP-binding subunit MetN	1.82	- 0,05	
	MFS superfamily transporter	1,73	3,23	-1,71
	glycine/carnitine/choline/L-proline ABC transporter	-1,84	1,94	1,03
	polar amino acid ABC transporter, ATP-binding subunit	1,47	0,84	-1,53
	possible betaine/carnitine/choline transporter	2,26	-2,93	-
	putative ABC transporter component	1,84	-	-1,52
	putative nitrate/nitrite transporter	1,66	-	-1,66
	putative transporter	3,09	1,69	0,70
	RHBT family amino acid transporter	3,03	-	-1,50
rbsA	ribose ABC transporter, ATP-binding subunit	1,93	-0,90	-2,30
sacs	sodium-dependent dicarboxylate transporter SdcS	1,83	-1,73	- 1.20
	TRAP dicarboxylate transporter substrate-binding component	1,99	-2,04 -0.90	-1,39
	The dealboxylate transporter, substrate smalling component	1,75	0,50	1,05
transport				
trkA	Trk system potassium uptake protein TrkA	2.00	0.50	-
mnhC	Na(+)/H(+) antiporter subunit C	-1,20	-0,53	-1,83
mnhB	Na(+)/H(+) antiporter subunit B	-1,48	-0,63	-1,95
mnhG	Na(+)/H(+) antiporter subunit G	-1,50	-	-0,85
mnhA	Na(+)/H(+) antiporter subunit A	-1,51	-0,66	-1,41
mnhE	Na(+)/H(+) antiporter subunit E	-1,60	-	-1,26
mnhD	Na(+)/H(+) antiporter subunit D	-1,85	-0,33	-1,61
mnhF	Na(+)/H(+) antiporter subunit F	-1,89	-	-1,16
Metal transport				
	putative heavy metal/cadmium-transporting ATPase	8,09	-1,81	-
	iron-siderophore ABC transporter, ATP-binding subunit	1,77	0,90	0,84
	iron-siderophore ABC transporter, substrate-binding protein	2,26	0,85	-0,48
	iron-siderophore ABC transporter, substrate-binding protein	2,26	0,85	-0,48
	metal ion transporter	2,42	0,47	0,95
in a d A	metal-dependent hydrolase	2,84	-1,32	1,41
modA	nutative metal untake regulation protein	1,61	- 1 96	- 2 1 9
	putative metal-dependent amidase/carboxynentidase	5,00 <u> </u>	-1,80	2,10
Multi-drug resistance transporter		1,05	0,50	0,04
	MFS superfamily transporter	1,73	3,23	- <u>1,71</u>
	putative MFS superfamily transporter	-1,24	3,66	-0,92
EmrB	EmrB/QacA subfamily drug resistance transporter		1,74	-
	putative drug exporter of the RND superfamily	1,24	2,79	-0,82
	putative drug resistance ATP-binding protein	1,09	3,91	-
	putative EmrB/QacA subfamily drug resistance transporter	-	1,21	-1,00
	quaternary ammonium compound-resistance protein	-	4,72	-

Gene Product G-like stress D-like stress Combined stress Translation/ Traduction Intervention Intervention Intervention Intervention fr ribosomal protein L11 methyltransferase 1.124 1.96 1.52 fr ribosoma protein S10 2.97 2.33 0.84 gal 305 ribosomal protein S11 -3.06 1.51 1.52 gal 305 ribosomal protein S3 -3.33 3.81 - gal 305 ribosomal protein S5 -3.33 3.81 - gal 305 ribosomal protein S19 -3.52 1.91 1.20 gal 305 ribosomal protein S19 -3.52 1.91 1.20 gal 305 ribosomal protein S17 -3.35 1.51 1.20 gal 305 ribosomal protein S13 -2.57 2.06 0.63 gamt/ 505 ribosomal protein S14 -4.35 2.06 0.63 gamt/ 505 ribosomal protein S2 -3.67 1.11 -1.77 gal 505 ribos			Log ₂ expression ratio ⁽¹⁾		sion ratio ⁽¹⁾
Gene Product stress stress stress stress stress stress Traduction pmA Ribosomal protein L11 methyltransferase -1,24 1,96 -1,32 0,84 0,84 0,84 0,84 0,84 0,84 0,84 0,84 0,84 0,84 0,85 1,56 1,56 1,56 1,56 1,56 1,56 1,56 1,55 1,58 2,39 - 7,94 0,83 0,85 1,55 1,58 2,39 - 7,95 30,57 1,55 1,51 1,32 1,09 7,95 30,57 1,55 1,51 1,32 1,09 7,95 30,57 1,95 3,52 1,91 -1,120 7,95 30,57 3,55 1,51 1,32 1,99 -1,27 7,97 3,55 1,51 1,32 1,99 -1,27 7,97 3,55 1,51 1,32 1,91 -1,20 7,97 7,97 3,55 5,55 1,51 1,32 1,91 1,12 1,91 <th></th> <th></th> <th>G-like</th> <th>D-like</th> <th>Combined</th>			G-like	D-like	Combined
Translation/ Traduction Instruction prmA Ribosomal protein L11 methyltransferase 1,24 1,96 1,52 fr ribosome-recycling factor -1,56 1,34 0,86 grsM 305 ribosomal protein S10 -2,07 2,39 - grsK 305 ribosomal protein S3 -3,35 1,68 -1,25 grsD 305 ribosomal protein S4 -4,33 3,81 - grsF 305 ribosomal protein S5 -3,33 1,59 - grsF 305 ribosomal protein S19 -5,52 1,91 -1,20 grsG 305 ribosomal protein S19 -5,52 1,51 - grsD 305 ribosomal protein S19 -5,52 1,51 - grsD 305 ribosomal protein S13 -2,29 2,06 0,63 grmH 505 ribosomal protein 133 -2,29 2,06 0,63 grmH 505 ribosomal protein 135 -3,16 1,58 -1,30 grmH 505 ribosomal protein 136 -3,67 2,72 -	Gene	Product	stress	stress	stress
prmA Ribosomal protein L11 methyltransferase 1.24 1.96 1.52 frr ribosoma-recycling factor 1.56 1.13 0.86 rpsK 305 ribosomal protein S10 2.97 2.39 - rpsK 305 ribosomal protein S1 3.05 1.51 - rpsC 305 ribosomal protein S3 3.15 1.68 1.23 rpsC 305 ribosomal protein S5 3.33 1.53 - rpsG 305 ribosomal protein S6 -4.44 3.09 - rpsG 305 ribosomal protein S1 -3.52 1.91 -1.20 rpsG 305 ribosomal protein S1 -3.52 1.91 -1.27 rpsG 305 ribosomal protein S1 -3.52 1.91 -1.27 rpsG 305 ribosomal protein S12 -4.44 4.33 2.96 1.11 rpsG 305 ribosomal protein S12 -4.41 1.11 -1.11 rpsG 305 ribosomal protein S12 -4.41 3.61 2.25 - rplH 505 r	Translation/ Traduction				
fr rbssome-recycling factor 1.50 1.34 0.86 rmM 165 rhk-Aprocessing protein RimM 3.74 1.08 0.84 rpsJ 305 ribosomal protein S10 2.97 2.39 - rpsC 305 ribosomal protein S3 -3.15 1.68 -1.22 rpsD 305 ribosomal protein S4 -3.33 3.81 - rpsE 305 ribosomal protein S5 -3.33 3.81 - rpsG 305 ribosomal protein S7 -3.99 0.94 - rpsG 305 ribosomal protein S17 -3.55 1.51 -1.20 rpsG 305 ribosomal protein S17 -3.55 1.51 -1.20 rpsG 305 ribosomal protein S12 -4.41 1.11 - rpmG 505 ribosomal protein 1.33 -2.55 1.51 -1.20 rpmH 505 ribosomal protein 1.3 -2.66 -1.11 - rpmH 505 ribosomal protein 1.3 -3.06 2.25 - rpHE 505 ribosomal protein 1.5 -3.16	prmA	Ribosomal protein L11 methyltransferase	-1,24	1,96	-1,52
rimM 165 rthNA-processing protein NImM 3,74 1.08 4.08 rpsK 305 rthosomal protein S11 3,06 1.51 rpsC 305 rthosomal protein S3 3,15 1.68 1.25 rpsC 305 rthosomal protein S4 3,33 3,81 - rpsC 305 rthosomal protein S5 3,43 1,32 1.09 rpsG 305 rthosomal protein S5 4,44 1,32 1.09 rpsG 305 rthosomal protein S1 3,55 1,51 1.27 rpsG 305 rthosomal protein S1 3,55 1,51 1.27 rpsG 305 rthosomal protein S1 3,55 1,51 1.27 rpsG 305 rthosomal protein S1 3,56 1,51 1.27 rpsG 305 rthosomal protein S1 3,66 2,26 1,11 rpmH 505 rthosomal protein S2 2,96 1,11 1.9 rpmH 505 rthosomal protein L3 3,67 2,72 1.9 rpMF 505 rthosomal protein L4 3,35 1.95	frr	ribosome-recycling factor	-1,56	1,34	0,86
rps/ 305 ribosomal protein 510 2,07 2,39 rps/ 305 ribosomal protein 53 3,15 1,66 1,225 rps/ 305 ribosomal protein 54 3,33 3,81 rps/ 305 ribosomal protein 55 3,33 1,51 1,02 rps/ 305 ribosomal protein 57 3,99 0,94 rps/ 305 ribosomal protein 517 3,52 1,51 1,27 rps/ 305 ribosomal protein 517 3,55 1,51 1,27 rps/ 305 ribosomal protein 517 3,55 1,51 1,27 rps/ 305 ribosomal protein 133 2,50 2,06 0,63 rpm/ 505 ribosomal protein 124 4,43 2,96 1,11 rps/ 305 ribosomal protein 124 4,35 2,96 1,11 rpm/ 505 ribosomal protein 124 4,35 2,96 1,11 rpm/ 505 ribosomal protein 124 3,67 2,72 - rpl/ 505 ribosomal protein 124 3,61 1,53 1,30 rpl/ 505 ribosomal protein 15 3,61	rimM	16S rRNA-processing protein RimM	3,74	-1,08	-0,84
rpsf: 305 ribosomal protein S1	rpsJ	30S ribosomal protein S10	-2,97	2,39	-
rpsC 305 ribosomal protein S3 -3.15 1.68 -1.25 rpsC 305 ribosomal protein S5 -3.33 3.84 - rpsG 305 ribosomal protein S5 -3.33 3.84 - rpsG 305 ribosomal protein S7 -4.49 9.09 - rpsG 305 ribosomal protein S17 -3.52 -9.99 0.94 - rpsG 305 ribosomal protein S17 -3.55 -3.51 1.37 -1.27 rpsG 305 ribosomal protein S17 -3.55 -3.67 3.16 - rpmG 505 ribosomal protein 133 -2.59 2.06 0.63 rpmH 505 ribosomal protein 133 -2.59 2.06 0.63 rpmH 505 ribosomal protein 135 -3.67 2.72 - rpMH 505 ribosomal protein 135 -3.67 2.72 - - rpMI 505 ribosomal protein 14 -3.67 2.72 - - - - - - - - - - - - - - - - - -	rpsK	30S ribosomal protein S11	-3,06	1,51	-
prb2 305 ribosomal protein S4 3,33 1.33 1.09 rps6 305 ribosomal protein S5 3,33 1.03 rps7 305 ribosomal protein S19 3,22 1.91 rps7 305 ribosomal protein S19 3,22 1.91 rps7 305 ribosomal protein S19 3,22 1.91 rps7 305 ribosomal protein S17 3,35 1.51 rps7 305 ribosomal protein S16 3,67 2,29 rps7 305 ribosomal protein 134 4,35 2,26 0,63 rpm6 505 ribosomal protein 134 4,35 2,26 0,61 rpm1 505 ribosomal protein 134 4,35 2,26 0,61 rpm1 505 ribosomal protein 135 3,67 2,27 - rp10 505 ribosomal protein 15 -3,16 1,58 -1,30 rp11 505 ribosomal protein 15 -3,16 1,58 -1,30 rp12 505 ribosomal protein 12 -4,41 1,40 -1,41 rp14 505 ribosomal protein 12 -3,45 1,58 -1,30 rp16 505 ribosomal pr	rpsC	30S ribosomal protein S3	-3,15	1,68	-1,25
rpsF 305 ribosomal protein S5 4,34 3,23 1,33 1,03 rpsF 305 ribosomal protein S7 3,99 0,94 - rpsS 305 ribosomal protein S19 3,52 1,91 -1,20 rpsG 305 ribosomal protein S17 -3,55 1,51 -1,37 rpsF 305 ribosomal protein S16 -3,67 3,16 - rpsF 305 ribosomal protein 133 -2,59 2,06 0,63 rpmH 505 ribosomal protein 134 -4,35 2,96 0,11 rpmH 505 ribosomal protein 132 -4,41 1,11 - rpmH 505 ribosomal protein 135 -3,67 2,72 - rpID 505 ribosomal protein 135 -3,67 2,72 - rpID 505 ribosomal protein 14 -3,61 1,88 -1,40 rpIE 505 ribosomal protein 15 -3,16 1,88 -1,40 rpIE 505 ribosomal protein 16 - - - - rpIE 505 ribosomal protein 17/1.22 -4,74 1,76 - - rpIE	rpsD	30S ribosomal protein S4	-3,33	3,81	-
rpsF 305 ribosomal protein S7 3,99 0,94 - rpsG 305 ribosomal protein S19 -3,52 1,91 -1,20 rpsH 305 ribosomal protein S1 -3,55 1,51 -1,27 rpsG 305 ribosomal protein 13 -2,59 2,06 0,63 rpmH 505 ribosomal protein 13 -4,41 1,11 - rpmH 505 ribosomal protein 134 -4,35 2,96 1,11 rpmH 505 ribosomal protein 14 -3,61 1,27 - rpiE 505 ribosomal protein 15 -3,16 1,89 - - rpiE 505 ribosomal protein 16 -3,45 1,40 -1,17 rpiE 505 ribosomal protein 15 -3,51 1,55 - rpiE 505 ribosomal protein 16 <t< td=""><td>rpsE</td><td>30S ribosomal protein S5</td><td>-3,33</td><td>1,53</td><td>-1,09</td></t<>	rpsE	30S ribosomal protein S5	-3,33	1,53	-1,09
rpsC 305 ribosomal protein S19	rpsF	30S ribosomal protein S6	-4,44	3,02	-
grbs/ 305 ribosomal protein S19 -3,52 1,91 -1,20 rps/L 305 ribosomal protein S17 -3,55 1,51 1,37 rps/D 305 ribosomal protein S16 -3,64 1,39 1,37 rps/D 305 ribosomal protein S16 -3,67 3,16 - rpm/D 505 ribosomal protein 133 -2,59 2,06 0,63 rpm/H 505 ribosomal protein 122 -4,41 1,11 - rpm/H 505 ribosomal protein 123 -3,67 2,72 - rpi/L 505 ribosomal protein 135 -3,67 2,72 - rpi/L 505 ribosomal protein 14 -3,61 2,25 - rpi/L 505 ribosomal protein 15 -3,16 1,58 -1,39 argin/me	rpsG	30S ribosomal protein S7	-3,99	0,94	-
Tpsh 305 ribosomal protein 517 3,51 1,53 1,51 1,37 rpsQ 305 ribosomal protein 517 3,55 1,51 1,51 1,37 rpmG 505 ribosomal protein 133 -2,59 2,06 0,63 rpmH 505 ribosomal protein 134 4,35 2,96 1,11 rpmH 505 ribosomal protein 134 4,35 2,96 1,11 rpmH 505 ribosomal protein 134 4,35 2,96 1,11 rpmH 505 ribosomal protein 14 -3,67 2,72 - rplD 505 ribosomal protein 15 -3,16 1,58 -1,30 rplL 505 ribosomal protein 15 -3,16 1,58 -1,30 rplL 505 ribosomal protein 17/L12 -4,74 1,95 - org argtinine-tNNA ligase -1,36 1,55 - gotA aspartyl/glutamyl-tRNA amidotransferase subunit A -2,78 2,44 0,68 rpoA DNA-directed RNA polymerase alpha chain -3,16 1,55 - <t< td=""><td>rpsS</td><td>305 ribosomal protein S19</td><td>-3,52</td><td>1,91</td><td>-1,20</td></t<>	rpsS	305 ribosomal protein S19	-3,52	1,91	-1,20
Ipsic Soin Housenial protein S16 3,71 1,73 <t< td=""><td>rpsH</td><td>305 ribosomal protein S8</td><td>-3,54</td><td>1,59</td><td>-1,27</td></t<>	rpsH	305 ribosomal protein S8	-3,54	1,59	-1,27
pps/F Soci Industrial protein 133 2,50 3,10 - prmG S05 ribosomal protein 133 2,53 2,06 0,63 rpmH 505 ribosomal protein 134 4,33 2,96 1,11 rpmH 505 ribosomal protein 134 4,33 2,96 1,11 rpmH 505 ribosomal protein 135 3,67 2,72 - rplD 505 ribosomal protein 14 3,61 2,25 - rplE 505 ribosomal protein 15 3,16 1,38 -1,30 rplF 505 ribosomal protein 17/112 -4,74 1,95 - gatA aspartyl/glutamyl-tRNA amidotransferase subunit A -2,78 2,47 0,76 gatB aspartyl/glutamyl-tRNA amidotransferase subunit B -3,01 2,44 0,68 rpoA DNA-directed RNA polymerase alpha chain -3,46 1,55 - gitQS glycineRNA ligase -1,89 0,82 -1,24 tmfO methyltransferase -2,43 1,99 - rpiA sadenosy	rpsQ	305 ribosonial protein 517	-3,55	1,51	-1,37
pmm Dot industrial protein 134 2,35 2,06 0,03 rpsJ 305 ribosomal protein 134 4,35 2,96 1,11 rpmH 505 ribosomal protein 134 4,35 2,96 1,11 rpmH 505 ribosomal protein 135 3,67 2,72 - rplE 505 ribosomal protein 14 3,61 2,25 - rplE 505 ribosomal protein 15 3,16 2,25 - rplE 505 ribosomal protein 16 - 3,45 1,40 -	rpsp	505 ribosomal protein 122	-3,07	2,10	-
Imm Sol Indosemia fraction S12 44,01 1,11 - rpmH 50S ribosomal protein I34 4,35 2,96 1,111 rpmH 50S ribosomal protein I35 -3,67 2,72 - rplD 50S ribosomal protein I4 -3,66 1,22 - rplE 50S ribosomal protein I5	rnmH	505 ribosomal protein L33	-2,35	2,00	0,05
prod 503 holocamil protein 134 44,35 2,96 1,11 rpmH 505 ribosomal protein 135 3,67 2,72 - rpID 505 ribosomal protein 135 3,61 2,25 - rpIE 505 ribosomal protein 15 -3,16 1,58 -1,30 rpIF 505 ribosomal protein 16 -3,45 1,40 -1,17 rpIL 505 ribosomal protein 17/112 -4,74 1,95 - arg5 argininetRNA ligase -1,96 - - gotA aspartyl/glutamyl-tRNA amidotransferase subunit A -2,78 2,47 0,76 gotB aspartyl/glutamyl-tRNA amidotransferase subunit B -3,01 1,55 - gl/QS glycinetRNA ligase -1,89 0,82 -2,05 2,04 - trmFO methyltransferase -2,05 2,04 - - - gueA S-adenosylmethionine:tRNA ribosyltransferase -3,32 -0,49 -0,75 - - trmFO methyltransferase fragment	rnsl	305 ribosomal protein 512	-4,35	2,50	1,11
pmml Sos Rubosomal protein L35 -0.56 2,75 2,12 rplD 505 ribosomal protein L3 -3,67 2,72 - rplE 505 ribosomal protein L4 -3,61 2,25 - rplE 505 ribosomal protein L5 -3,16 1,58 -1,30 rplF 505 ribosomal protein L7/L12 -4,74 1,95 - args arginine-tRNA ligase -1,96 - - gdtA aspart/V/glutamyl-tRNA amidotransferase subunit A -2,78 2,47 0,76 gdtB aspart/V/glutamyl-tRNA amidotransferase subunit A -2,78 2,47 0,67 gdtA aspart/V/glutamyl-tRNA amidotransferase subunit B -3,16 1,55 - glvCS glvcmtRNA ligase -3,35 0,51 - ileS isoleucine-tRNA ligase -3,35 0,51 - ileY uethyltransferase -3,31 1,84 1,28 rtmFO methyltransferase -3,32 0,49 -0,75 rimA tRNA isopentenylt	rnmH	505 ribosomal protein 134	-4 35	2 96	1 1 1
prilo 505 ribosomal protein L4 -3,61 2,25 - rplF 505 ribosomal protein L5 -3,16 1,58 -1,30 rplF 505 ribosomal protein L6 -3,45 1,40 -1,17 rplL 505 ribosomal protein L7/L12 -4,74 1,95 - arg5 arginine-tRNA ligase -1,96 - - gttB aspartyl/glutamyl-tRNA amidotransferase subunit A -2,78 2,47 0,76 gttB aspartyl/glutamyl-tRNA amidotransferase subunit B -3,01 2,44 0,68 rpoA DNA-directed RNA polymerase alpha chain -3,16 1,55 - glvQ3 glv(rine-tRNA ligase -1,89 0,82 -2,04 - trmFO methyltransferase -2,05 2,04 - - gu4 queuine RNA-ligase -1,59 0,83 - - trmD methyltransferase fragment 1,60 0,65 1,17 trp gu4 S-adenosylmethioninetRNA-libosyltransferase 2,24 1,20 <td>rpml</td> <td>505 ribosomal protein L35</td> <td>-3.67</td> <td>2.72</td> <td></td>	rpml	505 ribosomal protein L35	-3.67	2.72	
rplE 50S ribosomal protein L5 -3,16 1,58 -1,30 rplF 50S ribosomal protein L6 -3,45 1,40 -1,17 rplL 50S ribosomal protein L6 -3,45 1,40 -1,17 rplL 50S ribosomal protein L7/122 -4,74 1,95 - gatA asparty//glutamyl-tRNA amidotransferase subunit A - - - gatB asparty//glutamyl-tRNA amidotransferase subunit B -3,01 2,44 0,68 groA DNA-directed RNA polymerase alpha chain -3,15 1,55 - gl/QS glycinetRNA ligase -1,89 0,82 -1,24 tmFO methyltransferase -2,05 2,04 - tgt queuine tRNA-ribosyltransferase -2,05 2,04 - tgt queuine tRNA-ribosyltransferase -2,05 2,04 - tgt queuine tRNA-ribosyltransferase -3,33 1,84 1,28 rlmD tRNA (guanine-N(1)-)-methyltransferase isomerase 2,41 1,19 - <	rpID	505 ribosomal protein L4	-3.61	2.25	-
rpl/F 50S ribosomal protein L6 -3,45 1,40 -1,17 rplL 50S ribosomal protein L7/L12 -4,74 1,95 - argS arginine-tRNA ligase -1,96 - - gtA aspartyl/glutamyl-tRNA amidotransferase subunit A 2,278 2,47 0,76 gtB aspartyl/glutamyl-tRNA amidotransferase subunit B -3,01 2,44 0,68 rpoA DNA-directed RNA polymerase alpha chain -3,16 1,55 - glvQCS glvcine-tRNA ligase -3,35 -0,51 - ileS isoleucine-tRNA ligase -1,89 0,82 -1,24 trmfO methyltransferase -2,05 2,04 - rgt queuine tRNA-ribosyltransferase -3,33 1,64 1,28 rlmN ribosomal RNA large subunit methyltransferase-isomerase -2,43 1,19 - trmD tRNA (guanine-N(1)-)-methyltransferase fragment 1,62 0,90 1,16 DNA synthethis - - - - - -<	rpIE	50S ribosomal protein L5	-3.16	1.58	-1.30
rpl. 50S ribosomal protein L7/L12 -4,74 1,95 - arg5 arginineRNA ligase -1,96 - - gatA aspartyl/glutamyl-tRNA amidotransferase subunit A -2,78 2,47 0,76 gatB aspartyl/glutamyl-tRNA amidotransferase subunit B -3,01 2,44 0,68 rpoA DNA-directed RNA polymerase alpha chain -3,16 1,55 - glyQ5 glycinetRNA ligase -3,35 -0,51 - ileS isoleucine-tRNA ligase -3,35 -0,51 - iles isoleucine-tRNA ligase -3,35 -0,51 - igex queuine tRNA-ribosyltransferase -2,05 2,04 - tgt queuine tRNA-ribosyltransferase isomerase -2,33 1,84 1,28 rlmN ribosomal RNA large subunit methyltransferase-isomerase -2,33 1,19 - trmD tRNA (guanine-N(1)-)-methyltransferase-isomerase -2,33 -0,64 -0,75 miaA tRNA isopentenyltransferase fragment 1,62 0,90	rplF	50S ribosomal protein L6	-3,45	1,40	-1,17
argS argInine-tRNA ligase1.96-gatA aspartyl/glutamyl-tRNA amidotransferase subunit A2,782,470,76gatB aspartyl/glutamyl-tRNA amidotransferase subunit B3,012,440,68gotAaspartyl/glutamyl-tRNA amidotransferase subunit B3,012,440,68gotAspartyl/glutamyl-tRNA polymerase alpha chain3,161,55-glyQSglycine-tRNA ligaseglyQSglycine-tRNA ligaseileSisoleucine-tRNA ligasetmFOmethyltransferase-2,05queuine tRNA-ribosyltransferasequeAS-adenosylmethionine:tRNA ribosyltransferase-isomerasequeAS-adenosylmethionine:tRNA ribosyltransferase-isomeraseminAtRNA isopentenyltransferase fragment1,860,651,17trp5tryptophan-tRNA ligase1,620,901,16ONA synthethisand repairand repairrecARecA bacterial DNA recombination protein2,741,390,87mutYputative A/G-specific DNA glycosylase2,010,35-0.54mutYputative A/G-specific DNA glycosylase2,010,35-0.54mutLDNA mismatch repair protein MutL1,201,00pcrADNA	, rpIL	50S ribosomal protein L7/L12	-4,74	1,95	-
gatA aspartyl/glutamyl-tRNA amidotransferase subunit A 2,78 2,47 0,76 gatB aspartyl/glutamyl-tRNA amidotransferase subunit B 3,01 2,44 0,68 rpoA DNA-directed RNA polymerase alpha chain 3,16 1,55 - glvGS glv(cine-tRNA ligase 3,38 0,51 - ileS isoleucine-tRNA ligase 1,89 0,82 -1,24 trmFO methyltransferase 2,05 2,04 - tgt queuine tRNA-ribosyltransferase 3,43 1,84 1,28 queA S-adenosylmethionine:tRNA ribosyltransferase-isomerase 2,43 1,19 - trmD tRNA (guanine-N(1)-)-methyltransferase 3,32 0,49 0,75 miaA tRNA isopentenyltransferase fragment 1,62 0,90 1,16 DNA synthethis and repair - -0.64 - -0.64 mutY putative A/G-specific DNA glycosylase 2,01 0,35 -0.54 dnal primosomal protein Dnal 1,99 0,25	argS	argininetRNA ligase	-1,96	-	-
gatB aspartyl/glutamyl-tRNA amidotransferase subunit B -3,01 2,44 0,68 rpoA DNA-directed RNA polymerase alpha chain -3,16 1,55 - glyQS glycine-tRNA ligase -3,35 -0,51 - ileS isoleucine-tRNA ligase -1,89 0,82 -1,24 trmFO methyltransferase -2,05 2,04 - tgt queuine tRNA-ribosyltransferase -3,43 1,84 1,28 rlmN ribosomal RNA large subunit methyltransferase isomerase -2,43 1,19 - queA S-adenosylmethionine:tRNA ribosyltransferase-isomerase -2,43 1,19 - trmD tRNA (guanine-N(1)-methyltransferase -3,32 -0,49 -0,75 miaA tRNA isopentenyltransferase fragment 1,86 0,65 1,17 trpS tryptophantRNA ligase 1,62 0,90 1,16 DNA synthethis - - -0,64 - - mutY putative A/G-specific DNA glycosylase 2,01 0,35 <	gatA	aspartyl/glutamyl-tRNA amidotransferase subunit A	-2,78	2,47	0,76
rpoADNA-directed RNA polymerase alpha chain-3,161,55glyQSglycinetRNA ligase-3,350,51-ileSisoleucine-tRNA ligase-1,890,82-1,24trmFOmethyltransferase-2,052,04-tgtqueuine tRNA-ribosyltransferase-3,431,841,28rlmNribosomal RNA large subunit methyltransferase N-1,590,83-queAS-adenosylmethionine:tRNA ribosyltransferase-isomerase-2,431,19-trmDtRNA (guanine-N(1)-)-methyltransferase-3,320,490,75miaAtRNA isopentenyltransferase fragment1,860,651,17trpStryptophantRNA ligase1,620,901,16DNA synthethisand repairrecARecA bacterial DNA recombination protein2,741,390,87mutYputative A/G-specific DNA glycosylase2,010,35-0,54dnalprimosomal protein Dnal1,990,25-mutLDNA mismatch repair protein MutL-1,20pcrAATP-dependent DNA helicase PCA-1,202,080,82recODNA repair protein MutS-1,261,701,31mutSDNA mismatch repair protein MutS-1,260,73-dnaBreplicative DNA helicase PCA-1,200,44-recGATP-dependent DNA helicase RuvB-1,720,84-0,39ruvBHolliday	gatB	aspartyl/glutamyl-tRNA amidotransferase subunit B	-3,01	2,44	0,68
glyQS glycinetRNA ligase -3,35 -0,51 ileS isoleucine-tRNA ligase -1,89 0,82 -1,24 trmFO methyltransferase -2,05 2,04 - tgt queuine tRNA-ribosyltransferase -3,43 1,84 1,28 queA S-adenosylmethionine:tRNA ribosyltransferase isomerase -2,43 1,19 - trmD tRNA (guanine-N(1)-)-methyltransferase-isomerase -2,43 1,17 - trpS tryptophan-tRNA ligase 1,62 0,90 1,16 DNA synthethis -	rpoA	DNA-directed RNA polymerase alpha chain	-3,16	1,55	-
ileSisoleucine-tRNA ligase-1,890,82-1,24trmFOmethyltransferase2,052,04-tgtqueuine tRNA-ribosyltransferase-3,431,841,28tgtqueuine tRNA-ribosyltransferase-3,431,841,28queAS-adenosylmethionine:tRNA ribosyltransferase-isomerase-2,431,19-trmDtRNA (guanine-N(1)-)-methyltransferase-isomerase-2,431,19-trmDtRNA (guanine-N(1)-)-methyltransferase3,32-0,49-0,75miaAtRNA isopentenyltransferase fragment1,860,651,17trpStryptophantRNA ligase1,620,901,16DNA synthethisand repairrecARecA bacterial DNA recombination protein2,741,390,87mutYputative A/G-specific DNA glycosylase2,010,35-0.54dnalprimosomal protein Dnal1,990,25-mutLDNA mismatch repair protein MutL-1,20pcrAATP-dependent DNA helicase PcrA-1,261,701,31mutSDNA mismatch repair protein MutS-1,260,73-nutBHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-nutBHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-nutBHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-nuAHolliday junction ATP-dependent DNA	glyQS	glycinetRNA ligase	-3,35	-0,51	-
trmFOmethyltransferase-2,052,04tgtqueuine tRNA-ribosyltransferase-3,431,541,28rlmNribosomal RNA large subunit methyltransferase N-1,590,83-queAS-adenosylmethionine:tRNA ribosyltransferase-isomerase-2,431,19-trmDtRNA (guanine-N(1)-)-methyltransferase3,32-0,490.75miaAtRNA isopentenyltransferase fragment1,860,651,17trpStryptophantRNA ligase1,620,901,16DNA synthethis and repairrecARecA bacterial DNA recombination protein2,741,390,87mutYputative A/G-specific DNA glycosylase2,010,35-0,54dnalprimosomal protein Dnal1,990,25-mutLDNA mismatch repair protein MutL-1,20pccODNA repair protein MutS-1,260,73-mutSDNA mismatch repair protein MutS-1,260,73-mutSDNA mismatch repair protein MutS-1,260,73-mutSDNA nismatch repair protein MutS-1,260,73-mutSDNA dipricin ATP-dependent DNA helicase RuvB-1,720,840,39nutAHolliday junction ATP-dependent DNA helicase RuvB-1,220,45-mutSDNA-directed RNA polymerase alpha chain-3,160,99-nutADNA-directed RNA polymerase alpha chain-3,160,35-nutS </td <td>ileS</td> <td>isoleucine-tRNA ligase</td> <td>-1,89</td> <td>0,82</td> <td>-1,24</td>	ileS	isoleucine-tRNA ligase	-1,89	0,82	-1,24
tgtqueuine tRNA-ribosyltransferase-3,431,841,28rlmNribosomal RNA large subunit methyltransferase N1,590,83-queAS-adenosylmethionine:tRNA ribosyltransferase-isomerase2,431,19-trmDtRNA (guanine-N(1)-)-methyltransferase-isomerase2,431,19-trpStryptophan-tRNA ligase3,32-0,49-0,75miaAtRNA isopentenyltransferase fragment1,860,651,17trpStryptophantRNA ligase1,620,901,16DNA synthethisand repairrecARecA bacterial DNA recombination proteinputative A/G-specific DNA glycosylase2,010,35-0,54dnalprimosomal protein Dnal1,990,25-mutLDNA mismatch repair protein MutL-1,20pcrAATP-dependent DNA helicase PcrA-1,202,08-0,82recODNA repair protein MutS-1,260,73-nutSDNA mismatch repair protein MutS-1,260,73-nutSDNA mismatch repair protein MutS-1,260,73-nuthDNA-(apurinic or apyrimidinic site) lyase-1,431,850,91nuthDNA-(apurinic or apyrimidinic site) lyase-1,431,85-nuthDNA-dipected RNA polymerase alpha chain-3,161,55-nuthDNA-directed RNA polymerase alpha chain-3,161,55-nuth <td>trmFO</td> <td>methyltransferase</td> <td>-2,05</td> <td>2,04</td> <td>-</td>	trmFO	methyltransferase	-2,05	2,04	-
r/mNribosomal RNA large subunit methyltransferase N-1,590,83-queAS-adenosylmethionine:tRNA ribosyltransferase-isomerase2,431,19-trmDtRNA (guanine-N(1)-)-methyltransferase-isomerase3,32-0,49-0,75miaAtRNA isopentenyltransferase fragment1,860,651,17trpStryptophantRNA ligase1,620,901,16DNA synthethisand repairrecARecA bacterial DNA recombination protein2,741,390,87mutYputative A/G-specific DNA glycosylase2,010,35-0,54dnalprimosomal protein Dnal1,990,25-mutLDNA mismatch repair protein MutL-1,201,04pcrAATP-dependent DNA helicase PcrA-1,261,701,31mutSDNA mismatch repair protein MutS-1,260,73-trubHolliday junction ATP-dependent DNA helicase RuvB-1,431,850,91nthDNA-(apurinic or apyrimidnic site) lyase-1,431,85-0,33ruvAHolliday junction ATP-dependent DNA helicase RuvB-1,720,45-rucGATP-dependent DNA helicase RecG-2,360,89-rucADNA-directed RNA polymerase alpha chain-3,161,55-nuADNA-hinding rontein IDNA helicase RuvA-2,200,45-nubDNA-hinding rontein MUL-1,22-1,70-0,31rucAHolli	tgt	queuine tRNA-ribosyltransferase	-3,43	1,84	1,28
queAS-adenosylmethionine:tRNA ribosyltransferase-isomerase-2,431,19trmDtRNA (guanine-N(1)-)-methyltransferase3,32-0,49-0,75miaAtRNA isopentenyltransferase fragment1,860,651,17trpStryptophantRNA ligase1,620,901,16DNA synthethis and repairrecARecA bacterial DNA recombination protein2,741,390,87mutYputative A/G-specific DNA glycosylase2,010,35-0,54dnalprimosomal protein Dnal1,990,25-mutLDNA mismatch repair protein MutL-1,201,04pcrAATP-dependent DNA helicase PcrA-1,260,73-dnaBreplicative DNA helicase-1,431,850,91nthDNA-(apurinic or apyrimidinic site) lyase-1,492,000,44ruvAHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-ruvAHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-ruvAHolliday polymerase alpha chain-3,24-1,55-proADNA-directed RNA polymerase alpha chain-3,24-1,55-	rlmN	ribosomal RNA large subunit methyltransferase N	-1,59	0,83	-
trmDtRNA (guanine-N(1)-)-methyltransferase3,32-0,49-0,75miaAtRNA isopentenyltransferase fragment1,860,651,17trpStryptophantRNA ligase1,620,901,16DNA synthethis and repairrecARecA bacterial DNA recombination protein2,741,390,87muYputative A/G-specific DNA glycosylase2,010,35-0,54dnalprimosomal protein Dnal1,990,25-mutLDNA mismatch repair protein MutL-1,201,04pcrAATP-dependent DNA helicase PcrA-1,202,08-0,82recODNA repair protein MutS-1,261,701,31mutSDNA mismatch repair protein MutS-1,260,73-nthDNA-(apurinic or apyrimidinic site) lyase-1,431,850,91nthDNA-(apurinic or apyrimidinic site) lyase-1,492,000,44ruvAHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-proADNA-directed RNA polymerase alpha chain-3,161,55-nubDNA-directed RNA polymerase alpha chain-3,161,55-	queA	S-adenosylmethionine:tRNA ribosyltransferase-isomerase	-2,43	1,19	-
miaAtRNA isopentenyltransferase fragment1,860,651,17trpStryptophantRNA ligase1,620,901,16DNA synthethis and repairrecARecA bacterial DNA recombination protein2,741,390,87ImpB/MucB/SamB family DNA-damage repair protein2,68-0,64mutYputative A/G-specific DNA glycosylase2,010,35-0,54dnalprimosomal protein Dnal1,990,25-mutLDNA mismatch repair protein MutL-1,201,04pcrAATP-dependent DNA helicase PcrA-1,202,08-0,82recODNA repair protein RecO-1,261,701,31mutSDNA mismatch repair protein MutS-1,260,73-dnaBreplicative DNA helicase-1,431,850,91nthDNA-(apurinic or apyrimidinic site) lyase-1,492,000,44ruvBHolliday junction ATP-dependent DNA helicase RuvB-1,720,84-0,39ruvAHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-poADNA-dipreendent DNA helicase RecG-2,360,89-recGATP-dependent DNA helicase RecG-2,360,89-rpoADNA-bindinge protein H1-3,161,55-	trmD	tRNA (guanine-N(1)-)-methyltransferase	3,32	-0,49	-0,75
trpstryptopnantRNA ligase1,620,901,16DNA synthethis and repairrecARecA bacterial DNA recombination protein ImpB/MucB/SamB family DNA-damage repair protein2,741,390,87mutYputative A/G-specific DNA glycosylase2,010,35-0,64dnalprimosomal protein Dnal1,990,25-mutLDNA mismatch repair protein MutL-1,201,04pcrAATP-dependent DNA helicase PcrA-1,202,08-0,82recODNA repair protein RecO-1,261,701,31mutSDNA mismatch repair protein MutS-1,260,73-dnaBreplicative DNA helicase1,431,850,91nthDNA-(apurinic or apyrimidinic site) lyase-1,492,000,44ruvAHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-DNA topoisomerase1,221,170,91recGATP-dependent DNA helicase RecG-2,360,89-ruvADNA-directed RNA polymerase alpha chain-3,241,52-	miaA	tRNA isopentenyltransferase fragment	1,86	0,65	1,17
DNA synthethis and repairrecARecA bacterial DNA recombination protein2,741,390,87ImpB/MucB/SamB family DNA-damage repair protein2,680,64mutYputative A/G-specific DNA glycosylase2,010,35-0,54dnalprimosomal protein Dnal1,990,25-mutLDNA mismatch repair protein MutL-1,20pcrAATP-dependent DNA helicase PcrA-1,202,08-0,82recODNA repair protein RecO-1,261,701,31mutSDNA mismatch repair protein MutS-1,260,73-dnaBreplicative DNA helicase-1,431,850,91nthDNA-(apurinic or apyrimidinic site) lyase-1,492,000,44ruvAHolliday junction ATP-dependent DNA helicase RuvB-1,220,45-recGATP-dependent DNA helicase RuvA-2,200,45-nuA topoisomerase1,221,170,91-recGATP-dependent DNA helicase RuvA-2,260,89-nuApONA-topoisomerase alpha chain-3,160,55-nuADNA-directed RNA polymerase alpha chain-3,161,55-nuADNA-direction HU-3,24-1,58-	trpS	tryptophantRNA ligase	1,62	0,90	1,16
recARecA bacterial DNA recombination protein ImpB/MucB/SamB family DNA-damage repair protein2,741,390,87mutYputative A/G-specific DNA glycosylase2,010,35-0,64mutYprimosomal protein Dnal1,990,25-mutLDNA mismatch repair protein MutL-1,201,04pcrAATP-dependent DNA helicase PcrA-1,202,08-0,82recODNA repair protein RecO-1,261,701,31mutSDNA mismatch repair protein MutS-1,260,73-dnaBreplicative DNA helicase-1,431,850,91nthDNA-(apurinic or apyrimidinic site) lyase-1,492,000,44ruvAHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-nuvAHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-nuvADNA-directed RNA polymerase alpha chain-3,161,55-hunDNA-directed RNA polymerase alpha chain-3,161,55-	DNA synthethis and repair				
ImpB/MucB/SamB family DNA-damage repair protein2,68-0,64mutYputative A/G-specific DNA glycosylase2,010,35-0,54dnalprimosomal protein Dnal1,990,25-mutLDNA mismatch repair protein MutL-1,201,04pcrAATP-dependent DNA helicase PcrA-1,202,08-0,82recODNA repair protein RecO-1,261,701,31mutSDNA mismatch repair protein MutS-1,260,73-dnaBreplicative DNA helicase-1,431,850,91nthDNA-(apurinic or apyrimidinic site) lyase-1,492,000,44ruvAHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-DNA topoisomerase1,221,170,91-recGATP-dependent DNA helicase RecG-2,360,89-rpoADNA-directed RNA polymerase alpha chain-3,161,55-hunDNA-binding protein HU-3,24-1,58-	recA	RecA bacterial DNA recombination protein	2,74	1,39	0,87
mutYputative A/G-specific DNA glycosylase2,010,35-0,54dnalprimosomal protein Dnal1,990,25-mutLDNA mismatch repair protein MutL-1,201,04pcrAATP-dependent DNA helicase PcrA-1,202,08-0,82recODNA repair protein RecO-1,261,701,31mutSDNA mismatch repair protein MutS-1,260,73-dnaBreplicative DNA helicase-1,431,850,91nthDNA-(apurinic or apyrimidinic site) Iyase-1,492,000,44ruvBHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-DNA topoisomerase1,221,170,91-recGATP-dependent DNA helicase RecG-2,360,89-rpoADNA-directed RNA polymerase alpha chain-3,161,55-hunDNA-binding protein HU-3,24-1,58-		ImpB/MucB/SamB family DNA-damage repair protein	2,68	-	-0,64
dnalprimosomal protein Dnal1,990,25-mutLDNA mismatch repair protein MutL-1,201,04pcrAATP-dependent DNA helicase PcrA-1,202,08-0,82recODNA repair protein RecO-1,261,701,31mutSDNA mismatch repair protein MutS-1,260,73-dnaBreplicative DNA helicase-1,431,850,91nthDNA-(apurinic or apyrimidinic site) lyase-1,492,000,44ruvBHolliday junction ATP-dependent DNA helicase RuvB-1,720,84-0,39ruvAHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-DNA topoisomerase1,221,170,91-recGATP-dependent DNA helicase RecG-2,360,89-rpoADNA-directed RNA polymerase alpha chain-3,161,55-hunDNA-binding protein HU-3,24-1,58-	mutY	putative A/G-specific DNA glycosylase	2,01	0,35	-0,54
mutLDNA mismatch repair protein MutL-1,201,04pcrAATP-dependent DNA helicase PcrA-1,202,08-0,82recODNA repair protein RecO-1,261,701,31mutSDNA mismatch repair protein MutS-1,260,73-dnaBreplicative DNA helicase-1,431,850,91nthDNA-(apurinic or apyrimidinic site) lyase-1,492,000,44ruvBHolliday junction ATP-dependent DNA helicase RuvB-1,720,84-0,39ruvAHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-DNA topoisomerase1,221,170,91.recGATP-dependent DNA helicase RecG-2,360,89-rpoADNA-directed RNA polymerase alpha chain-3,161,55-hunDNA-binding protein HU-3,24-1,58-	dnal	primosomal protein Dnal	1,99	0,25	-
pcrAATP-dependent DNA helicase PcrA-1,202,08-0,82recODNA repair protein RecO-1,261,701,31mutSDNA mismatch repair protein MutS-1,260,73-dnaBreplicative DNA helicase-1,431,850,91nthDNA-(apurinic or apyrimidinic site) lyase-1,492,000,44ruvBHolliday junction ATP-dependent DNA helicase RuvB-1,720,84-0,39ruvAHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-DNA topoisomerase1,221,170,91.recGATP-dependent DNA helicase RecG-2,360,89-rpoADNA-directed RNA polymerase alpha chain-3,161,55-hunDNA-binding protein HU-3,24-1,58-	mutL	DNA mismatch repair protein MutL	-1,20	-	-1,04
rec0DNA repair protein Rec0-1,261,701,31mutSDNA mismatch repair protein MutS-1,260,73-dnaBreplicative DNA helicase-1,431,850,91nthDNA-(apurinic or apyrimidinic site) lyase-1,492,000,44ruvBHolliday junction ATP-dependent DNA helicase RuvB-1,720,84-0,39ruvAHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-DNA topoisomerase1,221,170,91recGATP-dependent DNA helicase RecG-2,360,89-rpoADNA-directed RNA polymerase alpha chain-3,161,55-hunDNA-binding protein HU-3,24-1,58-	pcrA	ATP-dependent DNA helicase PcrA	-1,20	2,08	-0,82
mutSDNA mismatch repair protein MutS-1,260,73-dnaBreplicative DNA helicase-1,431,850,91nthDNA-(apurinic or apyrimidinic site) lyase-1,492,000,44ruvBHolliday junction ATP-dependent DNA helicase RuvB-1,720,84-0,39ruvAHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-DNA topoisomerase1,221,170,91recGATP-dependent DNA helicase RecG-2,360,89-rpoADNA-directed RNA polymerase alpha chain-3,161,55-hunDNA-binding protein HU-3,24-1,58-	recO	DNA repair protein RecO	-1,26	1,70	1,31
dnaBreplicative DNA helicase-1,431,850,91nthDNA-(apurinic or apyrimidinic site) lyase-1,492,000,44ruvBHolliday junction ATP-dependent DNA helicase RuvB-1,720,84-0,39ruvAHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-DNA topoisomerase1,221,170,91recGATP-dependent DNA helicase RecG-2,360,89-rpoADNA-directed RNA polymerase alpha chain-3,161,55-hunDNA-binding protein HU-3,24-1,58-	mutS	DNA mismatch repair protein MutS	-1,26	0,73	-
nthDNA-(apurinic or apyrimidinic site) lyase-1,492,000,44ruvBHolliday junction ATP-dependent DNA helicase RuvB-1,720,84-0,39ruvAHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-DNA topoisomerase1,221,170,91recGATP-dependent DNA helicase RecG-2,360,89-rpoADNA-directed RNA polymerase alpha chain-3,161,55-hunDNA-binding protein HU-3,24-1,58-	dnaB	replicative DNA helicase	-1,43	1,85	0,91
ruvBHolliday junction ATP-dependent DNA helicase RuvB-1,720,84-0,39ruvAHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-DNA topoisomerase1,221,170,91recGATP-dependent DNA helicase RecG-2,360,89-rpoADNA-directed RNA polymerase alpha chain-3,161,55-hunDNA-binding protein HU-3,24-1,58-	nth	DNA-(apurinic or apyrimidinic site) lyase	-1,49	2,00	0,44
ruvAHolliday junction ATP-dependent DNA helicase RuvA-2,200,45-DNA topoisomerase1,221,170,91recGATP-dependent DNA helicase RecG-2,360,89-rpoADNA-directed RNA polymerase alpha chain-3,161,55-hunDNA-binding protein HU-3,24-1,58-	ruvB	Holliday junction ATP-dependent DNA helicase RuvB	-1,72	0,84	-0,39
DNA topoisomerase1,221,170,91recGATP-dependent DNA helicase RecG-2,360,89-rpoADNA-directed RNA polymerase alpha chain-3,161,55-hunDNA-binding protein HU-3,24-1,58-	ruvA	Holliday junction ATP-dependent DNA helicase RuvA	-2,20	0,45	-
recGATP-dependent DNA nelicase RecG-2,360,89-rpoADNA-directed RNA polymerase alpha chain-3,161,55-hunDNA-binding protein HU-3,24-1,58-		UNA topoisomerase	1,22	1,17	0,91
Image: https://www.amage.org/and/org/	recu	ATP-dependent DNA nellcase Recg	-2,36	0,89	-
	hun	DNA-unecteu Niva poryinerase alpita chaili DNA-binding protein HII	-3,10	1,55 _1 <u>59</u> _	-

A detailed observation of H. alvei GB001 sulfur metabolism (Table 4) showed a clear upregulation of the genes cysND, cysC and cysIJ respectively coding for a transferase, a kinase and a reductase which are involved in the conversion of sulfate into sulfide. These enzymes allow the conversion of a +6 oxidation state molecule (sulfate) to a -2 oxidation state molecule (sulfide). Aside from the putative role of the sulfide molecule itself, the reduction process is H (+) dependent and is likely to contribute to decrease the intracellular concentration of protons in the intracellular compartment of H. alvei. Genes involved in the sulfate uptake are up-regulated as well (cysA, cysT and cysW respectively coding for an ATP-binding protein and two permeases). It has been documented that acidophilic organisms can use sulfur as an energy source (Johnson et al., 2008). Based on the convergent up-regulation of both metabolism and sulfur uptake, it would appear that sulfur is a core molecule in *H. alvei* GB001 ability to withstand gastric stress and should be further investigated. To pursue with H. alvei's metabolism that can be linked to reduction of molecules, the genes involved in nitrate metabolism were up-regulated, namely narH and narK coding respectively for a nitrate reductase and a nitrite extrusion protein; both participate to the reduction of a nitrogen source and the same hypothesis can be drawn regarding the link between reduction and H(+)consumption as in the sulfur metabolism.

In S. equorum Mu2, genes encoding reductases and dehybrogenases, e.g. mdh and mgo encoding malate dehydrogenases, were up-regulated. These families of enzymes are thus possibly involved in pH homeostasis in this bacterium. Indeed, dehydrogenases are very often NAD+ dependent and take one proton to complete their dehydrogenation. Oxidized coenzymes need to be subsequently reduced which might explain the co-functioning of hydrogenases and reductases during gastric stress. In addition, still in S. equorum Mu2, sodA, encoding superoxide dismutase A, was upregulated in case of gastric challenge, which participated to reduce the oxidative consequences of that stress. In the case of H. alvei GB001, the hydrogenases complexes drew our attention. These enzymes, widely described among gram-negative bacteria (Noguchi et al. 2010), give the ability to either produce or degrade dihydrogen. The Hyd-1, Hyd-2, Hyd-3 and Hyd-4 complexes, have been described to use dihydrogen and produce H(+) and CO_2 and, conversely, this being linked to the aeration conditions (Hayes et al. 2006, Noguchi et al. 2010). Part of the genes coding for these complexes are either up- (hyfFG) or down-regulated (hybABCO, hypBCD). Therefore, we will need to further investigate which groups of genes are involved in the formation of which hydrogenase complex in order to draw hypothesis about the role of this family of enzymes in the resistance of H. alvei to gastric stress. Moreover, formate metabolism, especially formate dehydrogenase, is linked to Hyd-n complexes (Noguchi et al. 2010). Still in H. alvei GB001, The down-regulation of fdoGHI genes coding for such enzymes might be linked to intracellular pH regulation as well.