## ORIGINAL PAPER

Sarah W. Harcum · Fu'ad T. Haddadin

# Global transcriptome response of recombinant *Escherichia coli* to heat-shock and dual heat-shock recombinant protein induction

Received: 12 October 2005 / Accepted: 28 February 2006 / Published online: 6 May 2006 © Society for Industrial Microbiology 2006

Abstract Recombinant Escherichia coli cultures are used to manufacture numerous therapeutic proteins and industrial enzymes, where many of these processes use elevated temperatures to induce recombinant protein production. The heat-shock response in wild-type E. coli has been well studied. In this study, the transcriptome profiles of recombinant E. coli subjected to a heat-shock and to a dual heat-shock recombinant protein induction were examined. Most classical heat-shock protein genes were identified as regulated in both conditions. The major transcriptome differences between the recombinant and reported wild-type cultures were heavily populated by hypothetical and putative genes, which indicates recombinant cultures utilize many unique genes to respond to a heat-shock. Comparison of the dual stressed culture data with literature recombinant protein induced culture data revealed numerous differences. The dual stressed response encompassed three major response patterns: induced-like, in-between, and greater than either individual stress response. Also, there were no genes that only responded to the dual stress. The most interesting difference between the dual stressed and induced cultures was the amino acid-tRNA gene levels. The amino acid-tRNA genes were elevated for the dual cultures compared to the induced cultures. Since, tRNAs facilitate protein synthesis via translation, this observed increase in amino acid-tRNA transcriptome levels, in concert with elevated heat-shock chaperones, might account for improved productivities often observed for thermo-inducible systems. Most importantly,

S. W. Harcum (🖂)

Department of Bioengineering, Clemson University, 401 Rhodes Engineering Research Center, Clemson SC 29634-0905, USA E-mail: harcum@clemson.edu Tel.: +1-864-6566865 Fax: +1-864-6560567

F. T. Haddadin

Department of Chemical and Biomolecular Engineering, Clemson University, 127 Earle Hall, Clemson SC 29634-0910, USA the response of the recombinant cultures to a heat-shock was more profound than wild-type cultures, and further, the response to recombinant protein induction was not a simple additive response of the individual stresses.

**Keywords** Heat-shock response · Gene regulation · Chloramphenicol acetyltransferase · Transcriptome · DNA microarrays · tRNA · Chaperones

## Introduction

Living organisms respond to stressful environmental conditions by redirecting protein synthesis to alleviate cell damage. One of the most widely studied stressful environmental conditions is elevated temperature. The cellular response to elevated temperature is termed the heat-shock response. The heat-shock response in Escherichia coli was first described by the Neidhardt and Yura groups in 1978 [26, 46]. These research groups first observed that 20 proteins were very responsive to heat and later determined that the synthesis of these proteins was controlled at the transcription level [13, 23]. Later, these and other researchers identified numerous heatshock proteins by examining protein levels on twodimensional electrophoresis gels [20, 46] and RNA levels via hybridization with genomic libraries [4, 5, 29]. These methods provided the foundation for quantifying the heat-shock response and these identification methods have been extended by the complete sequencing of E. coli [2] and the advent of DNA microarrays [13, 31].

Heat-shock proteins are highly conserved across species. Heat-shock proteins monitor and respond to the level of protein folding in the cell [19]. Many heat-shock proteins are chaperones that promote protein folding, while other heat-shock proteins are proteases, which degrade unfolded or damaged proteins [1, 13]. Interestingly, many other stresses can also elicit the heat-shock response, such as ethanol, viral infections, and recombinant protein production [11, 13, 14, 17, 23, 24, 27, 34, 42, 43]. These and other stress response studies indicate that these stresses do not elicit all the heat-shock response proteins or genes, which suggests overlapping, but distinct regulatory pathways [37]. Additionally, there are a number of heat inducible genes that are controlled by other factors, whose functions and regulations are less well understood [38]. Some studies indicate that the folded state of the recombinant protein elicits heat-shock-related protease activity [27], while other studies indicate that the recombinant protein synthesis rate may play an important role [6, 10, 18, 43]. Mutational studies of the heat-shock proteins indicate that many heat-shock proteins/genes (*dnaK*, *gapA*, *grpE*, *hscA*, *hslJ*, *mopAB*, and *rpoDH*) are essential for normal cellular functions at normal growth temperatures as well as numerous regulatory pathways [9, 13, 16].

DNA microarrays have been used to investigate transcriptome profiles of recombinant E. coli fermentations [3, 15, 27, 34]. These studies identified genes that were sensitive to recombinant protein production. Many of the identified genes overlapped with the classical heatshock and stringent responses [27, 34]. It was also observed that recombinant protein production significantly decreased the transcriptome levels of transcription, translation, and energy synthesis related genes, which would impede recombinant protein production [15]. Additionally, recombinant protein production at elevated temperatures (42°C) has been observed to have higher productivities than normal (37°C) culture temperatures [18, 21, 30, 45]. Consequently, the objective of the present work is to gain a better understanding of the heat-shock response in recombinant cultures and how this response might impact recombinant protein production. To accomplish this objective, the transcriptome response of recombinant cultures subjected to a heatshock and a dual heat-shock recombinant protein induction were analyzed. The transcriptome levels were determined using Affymetrix® E. coli Antisense DNA microarrays, such that the entire genome was evaluated. These two transcriptome responses were also compared to recombinant cultures at normal growth temperature that were not overexpressing the recombinant protein and a set of literature recombinant culture data that were chemically induced to overexpress the recombinant protein [15]. Additionally, the heat-shock response of the recombinant cultures was compared to the literature report of the heat-shock response in wild-type cultures [31]. The results of the global transcriptome analysis demonstrated that recombinant cultures respond differently to a heat-shock stress than wild-type cultures, where the transcriptome response of the recombinant cultures is further modified by production of a recombinant protein.

#### **Materials and methods**

## Culture conditions

The *E. coli* MG1655 [pPROEx-CAT] cells were used for all studies. The pPROEx-CAT plasmid contains a

pBR322 origin of replication, a trc promoter, a lacI gene, and the  $\beta$ -lactamase gene for ampicillin resistance (Invitrogen). Isopropyl-1-thio- $\beta$ -D-galactopyranoside (IPTG) induces chloramphenicol acetyltransferase (CAT) expression via the *trc* promoter. One milliliter of frozen cells was used to inoculate 500 ml shake-flasks with 100 ml LB media containing 0.5% glucose (w/v) and 40 mg/l ampicillin. The LB media was prepared as described by Rodriguez and Tait [33]. The cultures were incubated overnight at 37°C at 200g. A 4-L fed-batch fermenter (Phillips Petroleum), with a 50% working volume, was inoculated with the overnight culture. Air and/or oxygen were sparged continuously into the fermenter at approximately 1,800 ml/min. Sterile antifoam (0.1% v/v) was added to the fermenter. The dissolved oxygen level (DO) was controlled to 60% of saturation by adjusting the agitation speed and air/oxygen flow rate. Sterile 5 M NaOH was used to control pH on-line to 7.0  $\pm$  0.2. The batch media contained LB with 5 g/l glucose. Once the initial glucose was consumed, a feed media was used to maintain the glucose concentration at approximately 1 g/l. The feed media contained 500 g/l glucose and 70 g/l MgSO<sub>4</sub>·7H<sub>2</sub>O. All fermentations were conducted in duplicate or triplicate. CAT expression was induced by 5 mM IPTG, which has been shown to maximize CAT expression [18]. The heat-shock and recombinant protein production phases were synchronized to the cell density of 11.5 OD, which is referred to as sample time 0. For the heat-shocked cultures, the temperature was increased from 37-50°C over 8 min beginning at sample time 0. The temperature and duration used in this study are the same conditions used to evaluate the heat-shock in wild-type cultures [5, 31]. The temperature was then decreased from 50-37°C over 4 min. Richmond et al. [31] observed that the transcriptome levels in heat-shocked wild-type samples taken 5, 10, 15, and 20 min after initiation of the heat-shock (50°C) were not different; therefore only one time point (15 min) was analyzed for the recombinant cultures. For the dual heat-shocked recombinant protein production cultures, 5 mM IPTG was added 8 min after sample time 0. In the Richmond et al. [31] study, IPTG was added to wild-type cells cultured in LB medium without glucose. They observed only nine significantly regulated genes, which included the lac operon genes. In contrast, IPTGadditions to wild-type cultures in the presence of glucose resulted no significant transcriptome changes in the lac operon, due to catabolite repression [15]. The unstressed recombinant cultures were conducted similarly, except without the heat-shock or IPTG-addition. The glucose concentration was measured using a glucose meter (One Touch Profile). The optical density was measured with a Beckman DU<sup>®</sup> 640 spectrophotometer at 595 nm.

Sample preparation

The specific CAT activity, cell harvest, total RNA isolation, cDNA synthesis, fragmentation, labeling, and array hybridization methods were conducted as described previously [15]. The wash and stain procedures were carried out by the fluidics station using the Pro-kGE-fluidics script (Affymetrix, Inc.). Microarray suit 5.0 (Affymetrix, Inc.) was used to process the data. Signal data was prepared and normalized by Affymetrix Data Mining Tool (DMT 3.0), which evaluated the significance of the signal intensity. GeneSpring<sup>®</sup> was used to analyze the data. An ANOVA test using a Benjamini and Hochberg false discovery rate correction was used for the multiple comparisons ( $P \leq 0.001$ ) with Tukey Post-Hoc tests.

#### Data analysis

Transcriptome levels were determined for (1) recombinant cultures not over-expressing the recombinant protein at 37°C (unstressed), (2) recombinant cultured only heat-shocked (heat-shocked), and (3) recombinant cultures both heat-shocked and chemically induced to overexpress the recombinant protein (dual stressed). The unstressed samples were obtained from the exponential phase of the fermentations at the synchronization cell density (~11.5 OD) and 1-hour after the synchronization cell density ( $\sim$ 14 OD). These two time points had significant transcriptome differences no (*t*-test,  $P \leq 0.001$ ), so these data were combined to delineate the unstressed transcriptome levels more precisely. The heat-shocked culture samples were obtained 15 min after the beginning of the heat-shock (15 min after the synchronization cell density with a sample OD  $\sim$  10). The dual stressed culture samples were obtained 15 min after the beginning of the heat-shock (15 min after the synchronization cell density with a sample OD  $\sim$  10). For clarity, the conditions in this study will be referred to as the unstressed, heat-shocked, and dual stressed cultures. For some comparisons, literature data for wildtype cultures and induced recombinant cultures were analyzed. The fermentation conditions for the wild type and induced recombinant cultures were similar, including growth rates, and cell densities at sampling times. The media used (LB with glucose) and fed-batch conditions were the same. When referenced in the text these cultures are referred to as the wild-type and induced cultures, respectively.

Each sample condition was obtained from at least two separate fermentations (two biological replicates). RNA from each biological replicate was purified and processed independently. Prior to hybridization, where only two biological replicates existed, one of the processed samples was divided (two technical replicates), resulting in three separate hybridized chips. The heatshocked and dual stressed culture samples all consisted of three technical replicates from two biological duplicates. For the unstressed culture samples, triplicate samples were obtained for the 11.5 OD and duplicates for the 14 OD conditions. There were no statistical differences between the 11.5 and 14 OD unstressed samples  $(P \leq 0.001)$ . Thus, the unstressed culture transcriptome profile consisted of six technical replicates from five biological replicates and four independent fermentations. The variances between biological replicates and between technical replicates were similar ( $r^2 > 0.90$ ), thus transcriptome levels were weighted equally. For the wild-type culture literature data, duplicate fermentations with triplicate samples were used with an OD  $\sim 11.3$ . For the literature recombinant induced culture data, duplicate fermentations with triplicate samples were used with an OD  $\sim$  13.5. All raw data intensities for the 12 DNA microarrays, individually, may be obtained from the University of Wisconsin E. coli Genome Project via the "A Systematic Annotation Package for Community Analysis of Genomes" as a guest (https:// asap.ahabs.wisc.edu/annotation/php/logon.php) [12]. The total intensity on the DNA microarray was normalized and set to a constant value. Since, ribosome genes contribute greatly to the total intensity of the DNA microarray, several other factors were examined to assure acceptable normalization. For example, the average intensity signals for the intergenic (IG) regions were evaluated and found to be consistent across the conditions examined.

## **Results and discussion**

The response of recombinant E. coli MG1655 [pPROEx-CAT] to elevated culture temperatures and recombinant protein production was investigated. Fed-batch cultures were synchronized with respect to cell densities at the initiation of the various stresses. The growth curves for the three culture conditions (unstressed, heat-shocked, and dual stressed) and the temperature profile for the heat-shocked cultures are shown in Fig. 1, where the unstressed cultures were maintained at 37°C. The glucose concentrations for these fermentations are also shown in Fig. 1. The final cell densities for the unstressed fermentations reached 16.4 OD (Fig. 1). In comparison, the heat-shocked and dual stressed fermentations had final cell densities of 12.2 and 11.2 OD (Fig. 1), respectively. The specific CAT activities for the dual stressed cultures reached 260 U CAT/mg total protein, as shown in Fig. 1, which is lower than similarly IPTG-only induced cultures, where the final specific CAT activity was 430 U CAT/mg total protein [15]. The specific CAT activity for the dual stressed cultures; however, was continuing to increase, but the extent was not determined, as the fermentation was stopped 4hours postinduction. As expected, the cell densities for all the heat-shocked cultures decreased immediately following the heat-shock; however, eventually growth returned. Since, it was unknown at the time of these studies, to what extent the recombinant culture transcriptomes would be changed by a heat-shock, a rather severe heat-shock (50°C) was used. Also, the media selected was similar to the media used for the transcripome analysis of wild-type cultures, although the wild-type



600

500

400

300

200

100

0

12

10

8

Specific CAT

Activity (U/mg

**Fermentation Time (h) Fig. 1** Cell growth characteristics for duplicate *E. coli* MG1655 [pPROEx-CAT] cultures. **a** The temperature profile for the heatshock. **b** Cell density and specific CAT activity for the unstressed (*filled diamond, open diamond*); heat-shocked (*filled triangle, open triangle*); and dual stressed (*filled square, open square*) cultures. Specific CAT activities are shown with standard error bars. **c** Glucose concentrations for the unstressed (*filled diamond, open diamond*); heat-shocked (*filled triangle, open triangle*); and dual stressed (*filled square, open square*) cultures

6

cultures were not fed glucose, as only low cell density shakes were used [31]. Additionally, the selected heatshock was the same temperature and duration used for the published transcriptome analysis of wild-type cultures subjected to heat-shock [31]; however, more severe than typically used in industry for thermo-induction [22]. Therefore, it was not unexpected that the recombinant protein productivities of this study were lower for the dual stressed cultures compared to other induced cultures [15].

In order to provide a comprehensive examination of the affects of heat-shock and recombinant protein production burdens, literature transcriptome data for recombinant cultures induced with IPTG were included [15]. These literature recombinant cultures were grown in the same medium as the cultures in this study with identical growth rates until the point of the stress-additions. Also, the amount of IPTG used was the same, where recombinant protein induction was examined. The literature transcriptome data; however, were obtained 1-hour postinduction, instead of 7-minute postinduction. We examined the appropriateness of this comparison, which will be discussed in the next paragraph, after the statistical criteria have been established. The literature transcriptome data will be referred to as the induced cultures for clarity.

An ANOVA analysis with a Benjamini and Hochberg false discovery rate correction ( $P \le 0.001$ ) was used for the multiple comparisons. Since, one objective of this work was to determine how the combined stress of a heat-shock and recombinant protein induction interacted, it was critical to compare the dual stressed culture responses to both individual stresses. To streamline the presentation of significantly different genes, the literature induced culture data are included in the ANOVAanalysis. The number and identity of the genes declared to have significance differences between the unstressed, heat-shocked, and dual stressed cultures did not change significantly if only the three culture conditions were examined by the ANOVA analysis. Therefore, the number and identity of the genes significantly different between conditions will be presented and described for the four cultures: unstressed, heat-shocked, dual stressed, and induced. Tukey Post-Hoc tests were used to identify genes that were statistically different between pairs of conditions. It was determined that 2,700 of the 4,404 annotated genes on the Affymetrix DNA microarray were significantly different across the four culture conditions. Table 1 lists the number of genes that have different expression levels between the condition pairs, as well as the number of genes with similar expression levels between the pairs. Induced recombinant protein production resulted in the greatest number of differences (2,011 genes) relative to the unstressed cultures; however, both the heat-shocked and dual stressed cultures also had over one thousand genes identified as different.

Since, the sample time postinduction for the dual stressed cultures and literature induced cultures were different, we investigated the transcriptome response of these two conditions to determine if it was reasonable and/or meaningful to compare the transcriptome levels, as a means to determine the affect of heat-shock on recombinant protein expression. In other words, were the dual stressed cultures responding to the IPTG and recombinant protein expression sufficiently to make any conclusion regarding the effects of recombinant protein induction? To address this issue the *lac* operon response was examined. The *lac* operon in recombinant cultures, possessing a lacI-containing plasmid, is very sensitive to IPTG, due to the high level of expression of the *lacI* gene encoded by the plasmid. Thus, the transcriptome response for the lacAYZ and lacI genes were examined across the four cultures, specifically focusing on the dual stressed and induced cultures. The transcriptome levels of the lac operon genes for the dual stressed and induced cultures were determined to not be different. In contrast, the unstressed cultures had significantly different expression levels for these four genes. A summary of the pairwise comparison for the *lac* operon genes is shown in

0

0

2

4

**Table 1** The number of genes with different expression levels (in bold) between the culture pairs, as well as the number of genes with similar expression levels (in italics) between the pairs

Cultures	Unstressed	Heat-Shocked	Dual Heat-Shocked/Induced	Induced
Unstressed	Х	1335	1881	2011
Heat-Shocked	1365	Х	457	890
Dual Heat-Shocked/Induced	819	2243	Х	211
Induced	689	1810	2489	Х

The ANOVA analysis identified a total of 2,700 genes with significant regulation ( $P \le 0.001$ ) across the four cultures. "X" indicates selfcomparison

**Table 2** The number of *lac* operon genes (*lacAYZ* and *lacI*) with different expression levels (in bold) between the culture pairs, as well as the number of genes with similar expression levels (in italics) between the pairs ( $P \le 0.001$ )

Cultures	Unstressed	Heat-Shocked	Dual Heat-Shocked/Induced	Induced
Unstressed	Х	3	4	4
Heat-Shocked	1 (lacZ)	Х	2	4
Dual Heat-Shocked/Induced	0	2 (lacZ, lacI)	Х	0
Induced	0	0	4	Х

"X" indicates self-comparison

Table 2. Although, not a perfect comparison, the dual stressed cultures at the transcriptome level were responding to the presence of the IPTG at levels comparable to the induced cultures 1-hour postinduction. Thus, it is likely that some of the transcriptome differences between the heat-shocked and dual stressed cultures are due to the effects of recombinant protein induction above the heat-shock stress, and likewise differences in transcriptome between the dual stressed and induced cultures is due to the heat-shock. However, due to the time differences between the samples, only conservative inferences will be presented.

#### Classical heat-shock response

The heat-shock response in wild-type E. coli has been extensively studied and is characterized by the up-regulation of 35 proteins and/or genes due to elevated culture temperatures [13, 31]. For this study these genes will be referred to as the classical heat-shock response genes. Since, no previous reports have examined the heat-shock response in recombinant E. coli using DNA microarrays, it was unclear to what extent recombinant cells would elicit a heat-shock response due to a heatshock as assessed by DNA microarrays. Thus, the first analysis of the regulated genes in this study was to determine to what extent the classical heat-shock response genes had been regulated by the three stresses in the recombinant cultures. It was determined that 34 of the 35 classical heat-shock response genes were regulated by one of the three stresses. Thirty-one genes were regulated in the heat-shocked cultures. Also, 31 genes were regulated in the dual stressed cultures; however, not the same 31 genes. The elevated culture temperature was definitely a common factor that regulated these genes in recombinant cultures; however, some of these genes were not regulated in the expected direction (up-regulated). To better highlight the behavior of these atypical genes, the normalized intensity for all 35 classical heat-shock response genes is shown in Fig. 2 for the four cultures [unstressed, heat-shocked (HS), dual stressed (Dual), and induced] and for a fifth culture, a wild-type fermentation (wild) under similar conditions as the unstressed cultures [15]. The line color of a gene's profile remains the same across the five conditions (for example, gapA is red) and indicates the relative expression level of this gene in the unstressed culture. Red indicates high expression in the unstressed,



Fig. 2 Transcriptome profiles for the classical heat-shock response genes for unstressed wild-type *E. coli* cultures and recombinant *E. coli* cultures. The recombinant cultures shown are uninduced (*unstressed*), heat-shocked (*HS*), dual stressed (*Dual*), and induced cultures, in addition to the wild-type (*Wild*) cultures. Standard error bars are shown

blue indicates medium expression, and green indicates low expression. Due to the high number of heat-shock response genes with similar profiles, only the outlier genes or genes with atypical profiles are labeled individually in Fig. 2. As can be readily observed, most of the classical heat-shock response genes were significantly up-regulated in both heat-shocked culture conditions. Table 3 lists all of the classical heat-shock response genes with fold change due to the three stresses relative to the unstressed culture. Interestingly, four of the classical heat-shock response genes (gapA, htgA, and htrBC) were not significantly regulated by the heat-shock, and a few of the classical heat-shock response genes (hscA, lvsU and rfaD) were down-regulated due to the heat-shock for the recombinant cultures. The gapA gene was most likely not significantly regulated due to the elevated temperature, since the expression level of gapA in the recombinant cultures was significantly higher than that observed for the wildtype cultures shown on the right-side of Fig. 2. The genes gapA, hscA, and rfaD also encode essential genes [9], which may play a part in the atypical behavior observed in the already stressed recombinant cultures

due to the metabolic burden of plasmid maintenance. The additive response of the heat-shock response genes for the dual stressed cultures relative to the heatshocked and induced cultures may play an important role in the improved productivities observed in thermoinducible systems. Since, many of the heat-shock response genes are chaperones, the higher chaperone transcriptome levels for the dual stressed cultures likely improves protein folding. For example, the classical heat-shock genes dnaJK, htpG, and mopAB (GroEL/ GroES) all encode chaperones. These chaperone genes were either unaffected or down-regulated due to the chemically induction of the soluble recombinant protein CAT; however, for the dual stressed cultures, these same chaperone genes were all up-regulated. Unlike CAT, it has been reported that expression of insoluble proteins at normal culture temperatures elicits increased heat-shock response transcriptome levels, and increased chaperone levels are known to improve recombinant protein productivities [25, 40, 44]. Taken together, the results of this study support the correlation between improved recombinant productivities at elevated temperature due to higher chaperone levels.

Table 3 The classical heat-shock response gene expression levels for the heat-shocked (*HS*), dual stressed and induced cultures relative to the unstressed cultures

Gene	bname	HS	Dual	Induced	Description
clpA	b0882	3.9		-2.2	ATP-binding component of serine protease
clpB	b2592	20.5	6.7		Heat-shock protein
clpP	b0437	5.4	2.7	-3.6	ATP-dependent proteolytic subunit of clpA-clpP serine protease, heat-shock protein F21.5
clpX	b0438	3.8	2.0	-3.1	ATP-dependent specificity component of clpP serine protease, chaperone
dnaJ	b0015	7.6	3.7		Chaperone with DnaK; heat-shock protein
dnaK	b0014	8.4	3.8	-2.2	Chaperone Hsp70; DNA biosynthesis; autoregulated heat-shock proteins
ftsJ	b3179	4.0	2.2	-3.6	Cell division protein
gapA	b1779		-3.4	-7.6	Glyceraldehyde-3-phosphate dehydrogenase A
grpE	b2614	17.2	5.3		Phage lambda replication; host DNA synthesis; heat-shock protein; protein repair
hflB	b3178	5.2	2.0	-2.4	Degrades $\sigma^{32}$ , integral membrane peptidase, cell division protein
hscA	b2526	-2.9	-3.4	1.9	Heat-shock protein, chaperone, member of Hsp70 protein family
hslJ	b1379	5.1	3.0		Heat-shock protein hslJ
hslU	b3931	5.7	2.8		Heat-shock protein hslVU, ATPase subunit, homologous to chaperones
hslV	b3932	4.0	2.2	-2.9	Heat-shock protein hslV2U, proteasome-related peptidase subunit
htgA	b0012		3.1	6.6	Positive regulator for $\sigma^{32}$ heat-shock promoters
htpG	b0473	19.0	5.2		Chaperone Hsp90, heat-shock protein C62.5
htpX	b1829	39.4	22.4		Heat-shock protein, integral membrane protein
htrA	b0161	8.6	5.3		Periplasmic serine protease Do; heat-shock protein HtrA
htrB	b1054				Heat-shock protein
htrC	b3989		4.2	4.2	Heat-shock protein HtrC
htrE	b0139	2.6	4.1	5.2	Probable outer membrane porin protein involved in fimbrial assembly
ibpA	b3687	38.1	21.2		Heat-shock protein
ibpB	b3686	54.6	30.6		Heat-shock protein
ldhA	b1380	16.5	3.4		Fermentative D-lactate dehydrogenase, NAD-dependent
lon	b0439	11.4	5.9	•	DNA-binding, ATP-dependent protease La; heat-shock K-protein
lysU	b4129	-3.3	-3.7	-3.0	Lysine tRNA synthetase, inducible; heat-shock protein
mopA	b4143	14.3	7.0	-2.3	GroEL, chaperone Hsp60, peptide-dependent ATPase, heat-shock protein
торВ	b4142	7.8	4.6	-3.6	GroES, 10 kDa chaperone binds to Hsp60 in pres. Mg-A1P, suppressing its A1Pase activity
pspA	b1304	3.5	2.0	-2.6	Phage shock protein, inner membrane protein
rfaD	b3619	-5.5	-3.4	-2.5	ADP-L-glycero-D-mannoheptose-6-epimerase
rpoD	63067	4.4		-2.0	RNA polymerase, $\sigma_{\rm F}^{0}$ factor; regulation of proteins induced at high temperatures
rpoE	025/3	3.8	2.2	-2.7	KINA polymerase, $\sigma^-$ factor; heat-shock and oxidative stress
rpoH	b3461	5.0	2.2	-2.1	RNA polymerase, $\sigma^{-2}$ factor; regulation of proteins induced at high temperatures
yrfH	b3400	11.9	3.8		ort, hypothetical protein ( <i>hslR</i> )
yrf1	b3401	1.2	2.1		ori, nypotnetical protein (hslO)

Blanks indicate the fold change was not significant ( $P \ge 0.001$ )

Heat-sensitive genes observed in wild-type cultures

In the heat-shock response work described by Richmond et al. [31] for wild-type cultures, only 20 of the 35 classical heat-shock response genes were reported to have been up-regulated by the heat-shock. Richmond et al. [31] applied a 5-fold criterion to identify regulated genes in additional to statistical significance. If a 5-fold criterion were applied to the recombinant culture data of the current study, 19 of the heat-shock response genes would have been classified as up-regulated by the heatshock. Thus, the recombinant and wild-type cultures responded to a heat-shock in a very consistent manner with respect to the classical heat-shock response genes. Richmond et al. [31] also identified 97 additional genes (5-fold regulated) that were heat-sensitive in the wildtype cultures. A comparison of these Richmond identified genes with the significantly regulated genes (including less than 5-fold regulated genes) observed for the high-cell density recombinant E. coli, an overlap of 73 genes was observed, of which 65 genes were also regulated similarly in one of the heat-shocked cultures of this study. Thus, it appears that the recombinant cells elicit a heat-shock response that is similar to the wildtype cultures, although not identical. All of the Richmond identified heat-sensitive genes are listed in Table 4 with fold-changes for the heat-shocked, dual stressed, and induced cultures relative to the unstressed cultures.

## Heat-sensitive genes in recombinant cultures

Further analysis of the heat-sensitive genes for the heatshocked and dual stressed cultures identified numerous genes that were highly responsive to the elevated temperature in the recombinant cultures that were not observed in the wild-type cultures. Specifically, of the 1,335 genes observed to be significantly regulated in the heatshocked cultures (Table 1), 1,093 genes had at least a 2-fold transcriptome level change, 260 genes had at least a 5-fold change, and 75 genes had at least a 10-fold change. Fifty-six of the 10-fold genes are not classified as classical heat-shock genes or identified by Richmond et al. [31]. Not unexpectedly, seven of these 56 genes were amino acid-tRNA genes, which are known to be heat-shock sensitive. Unexpectedly, 32 of the 56 10-fold genes were hypothetical or putative genes. This very high fraction of hypothetical/putative genes for these highly regulated genes indicates that recombinant cultures utilize many genes not normally utilized to combat a heatshock in wild-type cultures.

Of the 1,881 genes observed to be significantly regulated in the dual stressed cultures (Table 1), 1,633 genes had at least a 2-fold transcriptome level change, 521 genes had at least a 5-fold change, and 170 genes had at least a 10-fold change. One hundred and sixty of the 10fold genes have not been classified as classical heatshock genes or identified by Richmond et al. [31]. Unexpectedly, 113 of the 160 10-fold genes were hypothetical or putative genes. Since, it is generally accepted that 87% of the *E. coli* genome has been assigned function to some degree of confidence [31, 32], the high percentage of hypothetical/putative genes observed to be regulated in the recombinant cultures under the dual stressed conditions would seem to indicate that many more genes are needed to cope with the dual stress than either stress alone. This observation is also supported by the greater growth inhibition observed for the dual stressed cultures compared to the single stress cultures.

#### Metabolism genes

Based on the dramatic change in cell growth rate due to heat-shock (Fig. 1), it was expected that numerous metabolic pathways would be affected at the transcriptome level. Roughly, 25% of the genes in any one pathway were affected by the elevated temperature, indicating a global response [5, 8, 39]. The metabolic genes most uniformly affected by the heat-shock were energy and protein synthesis related. Oxidative phosphorylation genes responsible to energy synthesis were significantly down-regulated in the heat-shocked cultures. The oxidative phosphorylation genes include ATP synthase, cytochrome, fumarate reductase, NADH dehydrogenase, and polyphosphate kinase genes (40 genes total). The ATP synthase genes (*atpABDEFGH*) were significantly down-regulated approximately 3-fold. The ATP synthase genes were also down-regulated in the dual stressed and induced cultures at approximately the same level. The cytochrome genes (*appBC*, *cydA*, and cvoABCDE) were also down-regulated in the heatshocked cultures; however, the down-regulation observed for the dual stressed and induced cultures for these genes was slightly lower than the heat-shocked cultures. The NADH dehydrogenase genes (nuoBCEFGHIJKLM) were all significantly down-regulated in the heat-shocked cultures and in nearly all of the nuo genes were down-regulated in the dual stressed cultures, whereas for the induced cultures, only half of these genes were significantly regulated. Interestingly, overall, the induced cultures had only 18 of the 40 oxidative phosphorylation genes down-regulated compared to 28 and 24 genes down-regulated for the heat-shocked dual stressed cultures, respectively. The response of the oxidative phosphorylation genes indicates an impaired energy synthesis system under heat-shock, which could in part account for the observed reduced growth rates.

Protein synthesis relies on aminoacylated-tRNAs and ribosomes for translation. The aminoacyl-tRNA biosynthesis (synthetases) genes were significantly down-regulated on average 2.6-fold in the heat-shocked cultures (13 of the 25 genes), whereas 17 of the 25 genes were significantly down-regulated ( $\sim$ 1.9-fold) and five genes were up-regulated ( $\sim$ 1.8-fold) in the dual stressed cultures. The fold changes for the individual tRNA synthatase genes are listed in Table 5. For the induced cultures, 17 aminoacyl-tRNA synthetase genes were

Table 4 The heat-sensitive genes identified by Richmond et al. [31] including the classical heat-shock genes

abd.b417*4.14.2Biodegradative arginine dearboxylae Off. hypothetical protein Off. hypo	Gene	bname	HSP	Down in Richmond	HS	Dual	Induced	Description
biss         Orf. Spothetical protein           0302         5302           0302         5302           0302         5302           0302         5302           0302         5302           0302         5302           0302         5302           0302         5302           0303         07. Typothetical protein           044         bills         59           0503         17. T2.         Lysine decarboxylase           044         bills         57           045         1887         *         2.2           0503         7         Heatshock protein           0464         bills         *         2.2           0514         0437         *         3.8         2.0           0514         5.4         2.7         -3.6         ATP-dependent proteolytic submit of clpA-clpP           echt         bills         *         3.8         2.0         -3.1         ATP-dependent proteolytic submit of clpA-clpP           echt         bills         5.0         Restore protein         Corport protein constase protein           cdb         bills         5.0         -3.1         ATP-dependent proteolyti	adiA	b4117		*		4.1	4.2	Biodegradative arginine decarboxylase
b1903       Orf. hypothetical protein         b1409       b140       40.1       24.4       2.7         cadd       b413       3.7       4.1       Transport of Tysine         cadd       b4132       3.7       4.1       Transport of Tysine         carb       b0032       3.7       4.1       Transport of Tysine         carb       b0033       Carbamoly-phosphate synthesis, glutamine (small) subunit         carb       b0032       2.7       -1.6         carb       b0033       2.2       Positive regulator of CheA protein activity         c/ph       b1887       *       2.2       Positive regulator of Serie protease         c/ph       b1837       *       2.2       -3.4       ATP-binding component of depl serine protease, chaperone         c/ph       b1338       *       3.8       2.0       -3.1       ATP-dependent proteolytic subanit of clpA-clpP         serine       South active regulator       Cheap containsize       Containsize       Containsize         c/ph       b1338       *       3.8       2.0       -3.1       ATP-thomas protein regulator         c/ph       b1338       *       3.7       -2.2       Chapeneter proteon       Chapeneter proteon	b1593	b1593						Orf, hypothetical protein
b202       b202       Orf. hypothetical protein         b4440       b4140       40.1       24.2       Orf. hypothetical protein         cadd       b4131       5.9       11.7       17.2       Dynobiola protein         cadd       b4132       7       Transport of hysic       Carbamos/ phophate synthese large submit         card       b0038       *       4.4       4.1       Garbamos/ phophate synthese large submit         chell       b1887       *       2.2       Positive regulator of CheA protin activity         chell       b1887       *       2.2       Positive regulator of CheA protin activity         chell       b1887       *       2.2       Positive regulator of CheA protin activity         chell       b1887       *       2.7       -3.6       ATP-dependent proteclytic submit of clpA-clpP         chell       b1837       *       3.8       2.0       -3.1       ATP-dependent specificity component of clpA-clpP         chell       b1836       *       3.8       2.0       -3.1       ATP-dependent specificity component of clpA-clpP         chell       b1363       *       Corper boncotasis protein       Corper boncotasis protein       Corper boncotasis protein         cord       b1874       <	<i>b1903</i>	b1903						Orf, hypothetical protein
b4140 = b4140 = 40.1 24.4 2.7 Orf. hypothetical protein       cadb b413 = 5.9 11.7 12.2 Lypic decarboxylas 1       Transport of lysine       Carbamoyl-phosphate synthetase, glutamine (small) subunit       Carbamoyl-phosphate synthetase large subanit       Carbamoyl-phosphate synthese large subanit       Carbamoyl-phosphate synthetase specific f	<i>b3022</i>	b3022						Orf, hypothetical protein
$      cadd = b413 \\ cadd = b4032 \\ cadd = b4033 \\ cadd = b4132 \\ cadd = b4142 \\ cad$	<i>b4140</i>	b4140			40.1	24.4	2.7	Orf, hypothetical protein
cadBb41323.74.1Transport of lysine Carbamoy-phosphate synthesize large subunit Carbamoy-phosphate synthesize large subunit Carbamoy-phosphate synthesize large subunit Carbamoy-phosphate synthesize large subunit 	cadA	b4131			5.9	11.7	17.2	Lysine decarboxylase 1
$ \begin{array}{c} card & b0023 \\ cbcd \\ b1888 \\ cbcd \\ b1887 \\ cbcd \\ b1888 \\ cbcd \\ cbcd \\ b1888 \\ cbcd \\ cbcd \\ b1888 \\ cbcd \\ cbcd \\ cbcd \\ b1888 \\ cbcd \\ $	cadB	b4132				3.7	4.1	Transport of lysine
$ \begin{array}{c} carbon bound bou$	car A	b0032						Carbamoyl-phosphate synthetase, glutamine (small) subunit
chedb1888*4.44.1Sensory transducer knass between chemo- signal receptors and CheB and CheP positive regulator of CheA protein activity cpl b0852*2.2Positive regulator of CheA protein activity cpl b0437*2.2Positive regulator of CheA protein is protease them botto by the submit of cplAn-clpPchyb0437*3.82.0-3.1CheB and CheB and CheP Positive transducer proteoly the submit of cplAn-clpPchyb0437*3.82.0-3.1ATP-dependent specificity component of cplP serine protease, chaperone Cytosine deminance (cy component of cplP serine proteins Cold shock protein Transport of o-alanine, n-serine, and glycine Transport of o-alanine, n-serine, and glycine Transport of o-alanine, n-serine, and glycine Transport of proteinsendb2045*2.53.7DNA-specific endonuclease I analys (liament capping protein; Transport of protein Transport of protein Transport of protein Transport of protein that shock proteinfibb2045*2.53.6Flagglar bosynthesis, flament capping protein; that shock protein moteinfifb1924*1.23.1Chaptrone HspO(b), host	carB	b0033						Carbamoyl-phosphate synthase large subunit
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	che A	b1888		*		4.4	4.1	Sensory transducer kinase between chemo- signal receptors
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	ah a W	L1007		*		2.2		and Cheb and Cher
	che W	b0882	*		3.0	1.0		ATP-binding component of serine protease
$ \begin{array}{c} cpp \\ cpp $	clpA clnR	b0882 b2592	*		20.5	6.7		Heat-shock protein
$ \begin{array}{cccc} correct cor$	clpD clnP	b0437	*		5 4	27	-36	ATP-dependent proteolytic subunit of clpA-clpP
$ \begin{array}{cccc} cdA & b033 & * & 3.8 & 2.0 & -3.1 & ATP-dependent specificity component of clpP serine protease, chaperone Cytosine doraminase Cytosine doraminase Cytosine parmease Cytosine particle part particle particle part particle particle particle partic$	cipi	00107			5.1	2.7	5.0	serine protease, heat-shock protein F21.5
codd $bol36$ *Cytosine deaminaseCytosine primaseCytosine primase $cordbol36*Cytosine primaseCytosine primasecordbl3165.0Mg2+ transport, system IcreBbl3864.43.4Catabolic regulation response regulatorcurdbl8744.4Copper homestasis proteincurdbl8744.4Copper homestasis proteincurdbl8744.4Copper homestasis proteinduadbol15*7.63.7bul16*3.8-3.1duadbol15*7.6duadbol245*2.5duadbol245*2.5duadbol35*duadbol35*7.6duadbl37*bla5duadbl37*bla5duadbl37*2.5duadbl37*bla5duadbl37*bla5duadbl37*bla5duadbl37*bla5duadbl37*bla5duadbl37*bla5duadbl37*bla5duadbl37*bla5duadbl37*bla5duadbl37*bla5duadbl37*bl37duadbl37<$	clnX	b0438	*		3.8	2.0	-3.1	ATP-dependent specificity component of clpP serine protease, chaperone
codBb0336*Cytosine permeasecordb33165.0Mg2+transport, system IcreBb43984.43.4Catabolic regulation response regulatorcordb3816Cold shock proteincurCb18744.4Copper homeostasis proteincurCb18744.4Copper homeostasis proteincurCb18744.4Copper homeostasis proteincurCb2035*7.7curLb2045*2.2chakb0014*8.4b2045*2.53.7curLb2045*2.5curLb2045*2.5curLb2045*2.5curLb2045*2.5curLb2045*2.5curLb216*3.5curLFragelar bosynthesis, hook proteinfizbb3179*4.02.2curLcurLcurLCurLfizbb3179*4.02.2curLcurLcurLcurLcurLfizbb2507*1.5-4.4guaAb2507*1.5-4.4fixbb41732.32.3-3.6fixbb41732.32.3-3.6fixbb41732.32.3-3.6fixbb41732.3-2.6fixbb41732.2-2.8fixbb41735.72.8	codA	b0337		*				Cytosine deaminase
$ \begin{array}{ccc} cords & b3816 \\ cords & b3806 \\ crelb & b4398 \\ cords & b3806 \\ cords & cords & cords & b3806 \\ cords & cords & cords & b3806 \\ cords & cords & cords & cords & b3806 \\ cords & cord$	codB	b0336		*				Cytosine permease
creBb43984.43.4Catabolic regulation response regulator Cold shock proteincutCb18744.4Copper homeostasis protein Transport of D-alanine, D-serine, and glycinecyrAb23131.9dmalb0015*6.5dmalb0015*7.6dmalb0015*2.5dmalb015*2.5dmalb015*2.5dmalb016*2.5endb2779*-3.1endb2925*-3.5endb2925*-3.5flbb12242.53.6flgbb12242.53.6flgbb1224*2.6flbb12242.7Fractose-bisphosphate aldolase, class IIflgbb1217*4.02.2glydb2517*-3.6flgbb1224*2.5glydb2517*-3.6glydb2517*-3.6glydb2517*-3.6glydb2517*-3.6glydb2518*-3.6glydb2517*-3.6glydb2507*-3.6guadb2508*glydb2517*glydb251*glydb3931*flydb41723.11.5d-4Host flactor I for bacteriophage Q beta replication, a growth-related	corA	b3816			5.0			Mg2+ transport, system I
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	creB	b4398			4.4	3.4		Catabolic regulation response regulator
curCb18744.4Copper homeostasis proteincvpAb23131.9cvpAb23131.9dvadb0015*dvadb0014*b2015*2.5dvadb0014*8.43.8endAb2945*endAb2945*endAb2945*endAb2945*endAb2945*endAb2945*endAb2945*endAb2945*endAb2945*endAb2945*endAb2945*endAb2945*fibb1924*2.53.7DNA-specific endonuclease Ifibb1924*enablesfilament assemblyfifabb179*dyadb2507*gyadb2508gyadb2508hffabb4172dyad2.3enablesfilambda replication; host DNA synthesis;hffabb4172dyadb331hffabb4331hffabb3931hffabb3931hffabb3931hffabb3931hffabb3931hffabb3931hffabb3931hffabb3931hffabb3931hffabb3931hffabb3931hffabb3931hffab <td>cspD</td> <td>b0880</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>Cold shock protein</td>	cspD	b0880						Cold shock protein
$ \begin{array}{c} cp/A & b2313 \\ cp/A & b228 \\ dnaK & b0015 & * & 7.6 & 3.7 & -2.2 \\ dnaK & b0015 & * & 7.6 & 3.7 & -2.2 \\ dnaK & b0014 & * & 8.4 & 3.8 & -3.1 \\ chaperone & with DnaK; heat-shock protein \\ heat-shock proteins \\ heat-shock proteins \\ heat-shock proteins \\ heat-shock proteins \\ heat-shock protein heat \\ heat-shock protein heat \\ heat-shock protein \\ heat-shock$	cutC	b1874			4.4			Copper homeostasis protein
$\begin{array}{ccccc} bd208 \\ bdad b0015 * & 7.6 & 3.7 & -2.2 \\ bdad b014 * & 8.4 & 3.8 & -3.1 \\ chaperone klsp70; DNA biosynthesis; autoregulated \\ heat-shock proteins \\ bd2945 * & 2.5 & 3.7 \\ bd2925 * & -3.5 & -2.7 \\ bd292 * & 2.5 & 3.6 \\ bd2925 * & -3.5 & -2.7 \\ bd292 * & -3.6 \\ bd292 * & 2.5 & 3.6 \\ bd292 * & 2.3 & -3.6 \\ cd10 + bd297 \\ bd292 * & 2.3 & -3.6 \\ cd10 + bd297 \\ bd2931 * & 5.7 & 2.8 \\ bd292 * & 4.0 & 2.2 & -2.9 \\ bd292 * & 4.0 & 2.2 & -2.9 \\ bd292 * & 4.0 & 2.2 & -2.9 \\ bd2931 * & 5.7 & 2.8 \\ bd2931 * & 5.7 & 2.8 \\ bd2932 * & 4.0 & 2.2 & -2.9 \\ bd2931 * & 2.1 \\ bd2931 * & 19.0 & 5.2 \\ chaperone blave box brotein \\ bd20 + bd293 * & 39.4 \\ 2.1 \\ bd20 + bd293 * & 39.4 \\ 2.2 \\ bd20 + bd297 \\ bd21 & -2.9 \\ bd21 & -2.9 \\ bd22 + & 2.1 \\ bd21 & -2.9 \\ bd21 & -2.9 \\ bd22 + & 2.1 \\ bd21 & -2.9 \\ bd21 & -2.9 \\ bd21 & -2.9 \\ bd21 & -2.9 \\ bd22 + & -2.9 \\ bd21 & -2.9 \\ bd21 & -2.9 \\ bd21 & -2.9 \\ bd22 + & -2.9 \\ bd21 & -2.9 \\ bd21 & -2.9 \\ bd22 + & -2.9 \\ bd21 & -2.9 \\ bd22 + & -2.9 \\ bd21 & -2.9 \\ bd21 & -2.9 \\ bd21 & -2.9 \\ bd22 + & -2.9 \\ bd22 + & -2.9 \\ bd21 & -2.9 \\ bd21 & -2.9 \\ bd22 + & -2.9 \\ bd22 + & -2.9 \\ bd22 + & -2.9 \\ bd21 & -2.9 \\ bd21 & -2.9 \\ bd22 + & -2.9 \\ bd21 & -2.9 \\ bd22 + & -2.9 \\$	cvpA	b2313					1.9	Membrane protein required for colicin V production
dnak dnak boll *7.63.7-2.2Chaperone Hsp70; DNA biosynthesis; autoregulated heat-shock proteinsend bollb2945*2.53.7DNA-specific endonuclease I 	cycA	b4208						Transport of D-alanine, D-serine, and glycine
datakbould*8.43.8 $-3.1$ Chaperone Hsp/0; DNA biosynthesis; autoregulated heat-shock proteinsendb2945*2.53.7DNA-specific endonuclease Ienob2779* $-3.1$ $-6.6$ $-4.0$ Enolaseflabb1924*2.53.7Fructose-bisphosphate aldolase, class IIflgEb1076*3.54.0Flagellar biosynthesis; filament capping protein; enables filament assemblyflsJb3179*4.02.2 $-3.6$ Cell division protein genables filament assemblyflsJb2517*-Same Pictation; bost DNA synthesis; heat-shock protein repair gmath b2507guadb2508.IMP dehydrogenase lambda cII repressorhfqb41723.11.5 $-4.4$ hsIUb3931*5.72.8htqbb3932*4.02.2enables filament assemblyfor bacteriophage Q beta replication, a growth-related protein homologous to chaperoneshsIUb3931*5.72.8htqbb3932*3.9.42.2htqbb15313.2.Chaperone Hsp00, heat-shock protein protein hyab b3686htqbb2618*3.9.42.4htqbb15313.2.Heat-shock protein hyab b1530htqbb15313.2.Multiple antibiotic resistance protein; repressor filmar opticin petidase subunit hyab b1530htqdb	dnaJ	b0015	*		7.6	3.7	-2.2	Chaperone with DnaK; heat-shock protein
end4b2945*2.53.7DNA-specific codouclease Ienob2779*-3.1-6.6-4.0Enolaseflabb2925*-3.5-2.7Fractose-bisphosphate aldolase, class IIflgEb1076*3.54.0Flagellar biosynthesis, hook proteinflibb1924*2.53.6Flagellar biosynthesis, filament capping protein; enables filament assemblyfisJb3179*4.02.2-3.6Cell division protein strein; protein repairguaAb2501*Scine hydroxymethyltransferaseguaAb2507GMP synthetase (glutamine-hydrolyzing)guaAb2508IMP dehydrogenasehffXb41723.11.5hslUb3931*5.72.8heat-shock protein hslVU, ATPase subunit, homologous to chaperonehomologous to chaperoneshffXb41723.11.5-4.4host factor I for bacteriophage Q beta replication, a growth-related protein homologous to chaperone hslVU, attactor I for bacteriophage Q beta replication, a growth-related protein homologous to chaperoneshslVb3932*4.02.2-2.9Heat-shock protein hydrogenase-119.05.2Chaperone Hsp90, heat-shock protein homologous to chaperone factor in for the strein hydrogenasehffXb473*19.05.2Chaperone Hsp90, heat-shock protein petidas subunithtpGb0473*19.05.2htpat hish5.	апаК	60014	^		8.4	3.8	-3.1	Chaperone Hsp/0; DNA biosynthesis; autoregulated
end $b2779$ * $-3.1$ $-6.6$ $-4.0$ Enclosefba $b2925$ * $-3.1$ $-6.6$ $-4.0$ Enclosefba $b2925$ * $-3.5$ $-2.7$ Fructose-bisphosphate aldolase, class IIfibb $b1924$ * $2.5$ $3.6$ Flagellar biosynthesis, hook proteinfibl $b1924$ * $2.5$ $3.6$ Flagellar biosynthesis, hook proteinfisl $b179$ * $4.0$ $2.2$ $-3.6$ Cell division proteinglyA $b2551$ *Cell division proteingerine hydroxymethyltransferasegurA $b2507$ GMP synthesiac (glutamine-hydrolyzing)guaA $b2507$ IMP dehydrogenaseguaB $b2508$ IMP dehydrogenasehffq $b4172$ $3.1$ $1.5$ $-4.4$ Host factor I for bacteriophage Q beta replication, a a growth-related proteinhslU $b3931$ * $5.7$ $2.8$ haff $b473$ * $19.0$ $5.2$ hpg $b0473$ * $19.0$ $5.2$ hpab bof73* $2.1$ Hydrogenase-large subunithpd $b0387$ * $38.1$ $21.2$ hpd $b164$ $-2.9$ $-3.8$ $-2.9$ hpd $b164$ $-2.9$ $-3.8$ $-2.9$ hpd $b164$ $-2.9$ $-3.8$ $-2.9$ hpd $b387$ * $38.1$ $21.2$ hpd $b173$ $-2.9$ $-3.8$ $-2.9$ hpd $b173$ <td>and 1</td> <td>h2045</td> <td></td> <td>*</td> <td></td> <td>2.5</td> <td>27</td> <td>neat-snock proteins</td>	and 1	h2045		*		2.5	27	neat-snock proteins
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	enuA	02943 b2770		*	2.1	2.5	5.7 4.0	Englase
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	eno fha	b2925		*	-5.1	-0.0	-4.0 -2.7	Eriotase Fructose-bisphosphate aldolase, class II
JackJackJackFigellar biosynthesis; filament capping protein; enables filament capsemblyfisJb5179*4.02.2-3.6Cell division protein serine hydroxymethyltransferaseglyAb2551**Serine hydroxymethyltransferasegrpEb2614*17.25.3Phage lambda replication; host DNA synthesis; heat-shock protein; protein repair (BMP synthetase (glutamine-hydrolyzing)guaAb2507GMP synthetase (glutamine-hydrolyzing)guaAb2508IMP dehydrogenasehffXb41723.11.5hslUb3931*5.7b3932*4.02.2-2.9Heat-shock protein in IsIVU, ATPase subunit, homologous to chaperoneshslVb3932*19.0hslZ-2.9Heat-shock protein in IsIVU, proteasome-related peptidase subunithpgb0473*19.0hyaBb0973*2.1hydBb3686*54.6holl b4.15.9-1.9hutFb0439*11.4hyaBb016*-2.9-3.8-2.9Lipoamide chydrogenase (NADH); component of 2-oxodehydrogenase and pruvate complexe; transcriptional activator of defense systems marAh15313.2Multiple antibiotic resistance protein; repressor of mar operonmiAb15313.2Multiple antibiotic resistance protein atmasferase and pruvate complexes; transcriptional activator of defense systems marAh1531<	flo E	b1076		*		35	4.0	Flagellar biosynthesis, hook protein
firsdb3179*4.02.2-3.6Cell division protein $flyA$ b2551**Cell division protein $grpE$ b2614*17.25.3Phage lambda replication; host DNA synthesis; heat-shock protein; protein repair $guaA$ b2507GMP synthesis (glutamine-hydrolyzing) $guaB$ b2508IMP dehydrogenase $hfq$ b41732.32.3-3.6 $GTP$ -binding subunit of protease specific for phage lambda cll repressorbeta replication, had a growth-related protein $hslU$ b3931*5.72.8 $hslV$ b3932*4.02.2 $hslV$ b3932*4.02.2 $hslV$ b3932*1.05.2 $harsen karsen ka$	fliD	b1924		*		2.5	3.6	Flagellar biosynthesis; filament capping protein:
ftsJb3179*4.02.2 $-3.6$ Cell division protein Serine hydroxymethyltransferase grpt b2614gtyAb2551*17.25.3Phage lambda replication; host DNA synthesis; heat-shock protein; protein repair GMP synthetase (glutamine-hydrolyzing) IMP dehydrogenaseguaAb2507GTP-binding subunit of protease specific for phage lambda cll repressorhfqb41723.11.5-4.4hslUb3931*5.72.8hslVb3932*4.02.2-2.9htpGb0473*19.05.2htpAb1829*3.42.1htpBb3686*34.6htpBb3687*38.1htpBb3687*38.1htpAb15313.2-1.4marAb15313.2Groelene stance; transcriptional activator of dense system and pyruate complexe; transcriptional activator of dense sys	Juz	0172.				2.0	210	enables filament assembly
glyAb2551*Serine hydroxymethyltransferasegrpEb2614*17.25.3Phage lambda replication; host DNA synthesis; heat-shock protein; protein repairguaAb2507GMP synthetase (glutamine-hydrolyzing)guaBb2508IMP dehydrogenasehftXb41732.32.3-3.6hfqb41723.11.5-4.4hsUb3931*5.72.8hfqb41723.11.5-4.4hsIVb3932*4.02.2hgGb0473*19.05.2htpGb0473*19.05.2htpBb3686*3.42.4htpBb3687*38.12.1htpAb15313.2-1.4Heat-shock proteinhtpAb0116*-2.9-3.8-2.9htpAb15313.2Multiple antibiotic resistance; transcriptional activator of defense systemsmarAb15313.2Multiple antibiotic resistance; transcriptional activator of defense systemsmarAb1531 <t< td=""><td>ftsJ</td><td>b3179</td><td>*</td><td></td><td>4.0</td><td>2.2</td><td>-3.6</td><td>Cell division protein</td></t<>	ftsJ	b3179	*		4.0	2.2	-3.6	Cell division protein
grpEb2614*17.25.3Phage lambda replication; host DNA synthesis; heat-shock protein; protein repair (GMP synthetase (glutamine-hydrolyzing)) IMP dehydrogenase $guaA$ b2508IMP dehydrogenase $hfIX$ b41732.32.3-3.6 $hfq$ b41723.11.5-4.4 $hslU$ b3931*5.72.8 $hslU$ b3932*4.02.2-2.9 $hslV$ b3932*1.05.2 $hslV$ b3932*1.05.2 $hslV$ b3932*1.05.2 $hslV$ b3932*1.05.2 $hslV$ b3932*1.05.2 $hslV$ b3933*1.1.41.2 $hslV$ b3937*2.1Heat-shock protein hslVU, proteasome-related peptidase subunit $hyaB$ b0973*2.1 $hyaB$ b0973*2.1 $hyaB$ b3687*38.1 $lipA$ b016*-2.9 $-3.8$ -2.9-3.8-2.9 $lipA$ b0116*-2.9 $narA$ b15313.2Multiple antibiotic resistance; transcriptional activator of defense systems marA $marA$ b15302.8Multiple antibiotic resistance; protein of glycine cleavage complex and pyruvate complexes; t-protein of glycine cleavage complex and pyruvate complexes; t-protein of glycine cleavage complex and pyruvate complexes; transcriptional activator of defense systems and pyruvate complexes; transc	glyA	b2551		*				Serine hydroxymethyltransferase
heat-shock protein;	grpE	b2614	*		17.2	5.3		Phage lambda replication; host DNA synthesis;
guad guad b2507b2507 IMP dehydrogenase IMP dehydrogenase Imbda clir repressorGMP synthetase (glutamine-hydrolyzing) IMP dehydrogenase lambda clir repressorhfq hgb41723.11.5-4.4Host factor I for bacteriophage Q beta replication, a growth-related proteinhslU b3931b3931*5.72.8Heat-shock protein hslVU, ATPase subunit, homologous to chaperoneshslV b3932*4.02.2-2.9Heat-shock protein hslVU, proteasome-related peptidase subunithtpG b0473b0473 s*19.05.2Chaperone Hsp00, heat-shock protein C 62.5htpX b1829b1829 s*38.121.2Heat-shock proteinhtpB b0473b3686 s*5.9-1.9DNA-binding, ATP-dependent protease La; heat-shock K-protein putative phage integraselon b0439*11.45.9-1.9DNA-binding, ATP-dependent protease La; heat-shock K-protein and pyruvate complexes; L-protein of glycine cleavage complex and pyruvate complexes; L-protein of glycine cleavage complex and pyruvate complexes; L-protein of glycine cleavage complex and pyruvate complexes; L-protein of and peronmarA marA b15313.2Multiple antibiotic resistance; transcriptional activator of defense systems Multiple antibiotic resistance; protein of marker and pyruvate complexes; L-protein of glycine cleavage complex and pyruvate complexes; L-protein of slycencleavage complex and pyruvate complexes; L-protein of slycencleavage complex and pyruvate complexes; L-protein of glycencleavage complex and pyruvate complexes; L-protein of glycencleavage complex <b< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>heat-shock protein; protein repair</td></b<>								heat-shock protein; protein repair
guadb2508IMP dehydrogenase $hfIX$ b41732.32.3-3.6GTP-binding subunit of protease specific for phage lambda cII repressor $hfq$ b41723.11.5-4.4Host factor I for bacteriophage Q beta replication, a growth-related protein $hsIU$ b3931*5.72.8Heat-shock protein hsIVU, ATPase subunit, homologous to chaperones $hsIV$ b3932*4.02.2-2.9Heat-shock protein hsIVU, proteasome-related peptidase subunit $htpG$ b0473*19.05.2Chaperone Hsp90, heat-shock protein C 62.5 $htpK$ b1829*39.422.4Heat-shock protein, integral membrane protein $hyaB$ b0973*2.1Hydrogenaselarge subunit $hpB$ b3686*36.6Heat-shock protein $htpB$ b3686*31.6Heat-shock protein $htpB$ b0439*11.45.9-1.9 $DNA-binding, ATP-dependent protease La; heat-shock K-proteinPutative phage integraselonb0116*-2.9-3.8-2.9marAb15302.8Multiple antibiotic resistance; transcriptional activator of defense systemsmarAb15302.8Multiple antibiotic resistance protein; repressor of mar operonmiAAb1413*14.37.0-2.3marAb15302.8GroEL, chaperone Hsp60, petide-dependent ATPase, heat-shock proteinmarAb15302.8Formamidopy$	guaA	b2507						GMP synthetase (glutamine-hydrolyzing)
hftxb41/32.32.3-3.6G1P-binding subunit of protease specific for phage lambda CII repressorhfqb41723.11.5-4.4Host factor I for bacteriophage Q beta replication, a growth-related proteinhslUb3931*5.72.8Heat-shock protein hslVU, ATPase subunit, homologous to chaperoneshslVb3932*4.02.2-2.9Heat-shock protein hslVU, proteasome-related peptidase subunithtpGb0473*19.05.2Chaperone Hsp90, heat-shock protein C 62.5htpXb1829*39.422.4Heat-shock proteinhyabb0973*2.1Hydrogenase-1 large subunithpdb3687*38.121.2Heat-shock proteinibpBb3686*54.630.6Heat-shock proteinlonb0439*11.45.9-1.9DNA-binding, ATP-dependent protease La; heat-shock K-proteinlpdAb0116*-2.9-3.8-2.9Lipoamide dehydrogenase (NADH); component of 2-oxodehydrogenase and pyruvate complexes; t-protein of glycine cleavage complexmarAb15313.2Multiple antibiotic resistance; transcriptional activator of defense systems marA b15302.8Multiple antibiotic resistance protein; repressor of mar operonmiaAb41712.1-4.7Delta (2)-isopentenylpyrophosphate tRNA-adenosine transferase and pyruvate complexes; to haperone Hsp60, peptide-dependent ATPase, heat-shock protein ir sATPase activitymorbb4143*1	guaB	b2508			• •	• •	2.6	IMP dehydrogenase
hfqb41723.11.5 $-4.4$ Host factor I for bacteriophage Q beta replication, a growth-related proteinhslUb3931*5.72.8Heat-shock protein hslVU, ATPase subunit, homologous to chaperoneshslVb3932*4.02.2 $-2.9$ Heat-shock protein hslVU, proteasome-related peptidase subunithtpGb0473*19.05.2Chaperone Hsp90, heat-shock protein C 62.5htpXb1829*39.422.4Heat-shock proteinhtpAb3687*38.121.2Heat-shock proteinibpAb3687*38.121.2Heat-shock proteinintFb02810.6Heat-shock proteinPutative phage integraselonb0439*11.45.9 $-1.9$ DNA-binding, ATP-dependent protease La; heat-shock K-proteinmarAb15313.2Multiple antibiotic resistance; transcriptional activator of defense systemsmarAb15302.8Multiple antibiotic resistance protein; repressor of mar operonmiaAb4143*14.37.0 $-2.3$ GroEL, chaperone Hsp60, peptide-dependent ATPase, heat-shock proteinmutMb36354.12.92.2Formanidopyrimidine DNA glycosylasemarAb1318.66.9NitratemutMb36354.12.92.2Formanidopyrimidine DNA glycosylasemarAb284*-5.1 $-3.3$ $-2.2$ NADH dehydrogenase I chain C, D	hflX	b4173			2.3	2.3	-3.6	GIP-binding subunit of protease specific for phage
hyq $b41/2$ $5.1$ $1.3$ $-4.4$ Host relator relator pacteriophage Q deta replication, a growth-related protein hslVU, ATPase subunit, homologous to chaperones $hslV$ $b3932$ * $5.7$ $2.8$ Heat-shock protein hslVU, ATPase subunit, homologous to chaperones $hslV$ $b3932$ * $4.0$ $2.2$ $-2.9$ Heat-shock protein hslVU, proteasome-related peridase subunit $htpG$ $b0473$ * $19.0$ $5.2$ Chaperone Hsp90, heat-shock protein C 62.5 $htpX$ $b1829$ * $39.4$ $22.4$ Heat-shock protein, integral membrane protein $hyaB$ $b0973$ * $2.1$ Hydrogenase-1 large subunit $ibpA$ $b3687$ * $38.1$ $21.2$ Heat-shock protein $ibpB$ $b3686$ * $54.6$ $30.6$ Heat-shock protein $miF$ $b0281$ $00.6$ $-2.9$ $-3.8$ $-2.9$ Lipoamide dehydrogenase (NADH); component of 2-oxodehydrogenase $marA$ $b1531$ $3.2$ Multiple antibiotic resistance; transcriptional activator of defense systems $marA$ $b1531$ $3.2$ Multiple antibiotic resistance protein; repressor of mar operon $miaA$ $b1413$ * $14.3$ $7.0$ $-2.3$ GroEL, chaperone Hsp60, peride-dependent ATPase, heat-shock protein $mopB$ $b4142$ * $7.8$ $4.6$ $-3.6$ GroEL, chaperone binds to Hsp60 in pres. Mg-ATP, suppressing its ATPase activity $mutM$ $b3635$ $4.1$ $2.9$ $2.2$ Formanidopyrimidine DNA glycosyl	1.6.	h4172			2 1	15	4.4	lambda cili repressor
hslUb3931*5.72.8Heat-shock protein hslVU, ATPase subunit, homologous to chaperoneshslVb3932*4.02.2-2.9Heat-shock protein hslVU, proteasome-related peptidase subunithtpGb0473*19.05.2Chaperone Hsp90, heat-shock protein C 62.5htpXb1829*39.422.4Heat-shock proteinhyaBb0973*2.1Hydrogenase-1 large subunitibpAb3686*54.630.6Heat-shock proteinimFb0281Putative phage integraseDNA-binding, ATP-dependent protease La; heat-shock K-proteinlpdAb0116*-2.9-1.9DNA-binding, ATP-dependent protease La; heat-shock K-proteinmarAb15313.2Multiple antibiotic resistance; transcriptional activator of defense systemsmarAb15313.2Multiple antibiotic resistance; transcriptional activator of defense systemsmiaAb4143*14.37.0-2.3mopBb4142*7.84.6-3.6mutMb36354.12.92.2Formamidopyrimidine DNA glycosylasemutMb36354.12.92.2Formamidopyrimidine DNA glycosylasemucCb2286*-5.1-3.3-2.2NADH dehydrogenase I chain C, D	njq	04172			5.1	1.5	-4.4	a growth related protein
halob3912.3Ale Statehalob391barrowbarrowhalob392*barrowhalobarrow<	hell	h3031	*		57	28		A growth-related protein Heat-shock protein hslVUL ATPase subunit
hslVb3932*4.02.2-2.9Heat-shock protein hslVU, proteasome-related peptidase subunithtpGb0473*19.05.2Chaperone Hsp90, heat-shock protein C 62.5htpXb1829*39.422.4Heat-shock protein, integral membrane proteinhyaBb0973*2.1Hydrogenase-1 large subunitibpAb3687*38.121.2Heat-shock proteinibpBb3686*54.630.6Heat-shock proteinintFb0281PUtative phage integrasePUtative phage integraselonb0439*11.45.9-1.9lpdAb15313.2Multiple antibiotic resistance; transcriptional activator of defense systemsmarAb15302.8Multiple antibiotic resistance protein; repressor of mar operonmiaAb41712.1-4.7Delta (2)-isopenten Hsp60, peptide-dependent ATPase, heat-shock proteinmopBb4142*7.84.6-3.6morBb4142*7.84.12.92.2mutMb36354.12.92.2Formamidopyrimidine DNA glycosylasenurCb2286*-5.1-3.3-2.2NADH dehydrogenase I chain C, D	nsiO	03731			5.7	2.0		homologous to chaperones
harrybib 20harryharryperidasehtpGb0473*19.05.2Chaperone Hsp90, heat-shock protein C 62.5htpXb1829*39.422.4Heat-shock protein, integral membrane proteinhyaBb0973*2.1Hydrogenase-1 large subunitibpAb3687*38.121.2Heat-shock proteinibpBb3686*54.630.6Heat-shock proteinintFb02812.9-3.8-2.9DNA-binding, ATP-dependent protease La; heat-shock K-proteinlpdAb0116*-2.9-3.8-2.9Lipoamide dehydrogenase (NADH); component of 2-oxodehydrogenase and pyruvate complexes; L-protein of glycine cleavage complexmarAb15313.2Multiple antibiotic resistance; transcriptional activator of defense systems Multiple antibiotic resistance protein; repressor of mar operonmiaAb41712.1-4.7Delta (2)-isopentenylpyrophosphate tRNA-adenosine transferasemopAb4143*14.37.0-2.3GroEL, chaperone Hsp60, peptide-dependent ATPase, heat-shock protein its ATPase activitymutMb36354.12.92.2Formamidopyrimidine DNA glycosylasenarPb21938.66.9NitratenuoCb2286*-5.1-3.3-2.2NADH dehydrogenase I chain C, D	hslV	b3932	*		4.0	2.2	-2.9	Heat-shock protein hslVU, proteasome-related
htpGb0473*19.05.2Chaperone Hsp90, heat-shock protein C 62.5htpXb1829*39.422.4Heat-shock protein, integral membrane proteinhyaBb0973*2.1Hydrogenase-1 large subunitibpAb3687*38.121.2bbpBb3686*54.630.6Heat-shock proteininiFb0281Putative phage integraselonb0439*11.45.9-1.9lpAAb15313.2Multiple antibiotic resistance; transcriptional activator of defense systemsmarAb15302.8Multiple antibiotic resistance; protein, irrepressor of mar operonmiaAb41712.1-4.7Delta (2)-isopentenylpyrophosphate tRNA-adenosine transferasemopBb4142*7.84.6-3.6mutMb36354.12.92.2Formamidopyrimidine DNA glycosylasemurCb2286*-5.1-3.3-2.2NADH dehydrogenase I chain C, D								peptidase subunit
htpXb1829*39.422.4Heat-shock protein, integral membrane proteinhyaBb0973*2.1Hydrogenase-1 large subunitibpAb3687*38.121.2Heat-shock proteinibpBb3686*54.630.6Heat-shock proteinintFb0281Putative phage integrasePutative phage integraselonb0439*11.45.9-1.9lpdAb0116*-2.9-3.8-2.9lipbBb15313.2Multiple antibiotic resistance; transcriptional activator of defense systemsmarAb15313.2Multiple antibiotic resistance protein; repressor of mar operonmiaAb41712.1-4.7Delta (2)-isopentenylpyrophosphate tRNA-adenosine transferasemopAb4143*14.37.0-2.3GroEL, chaperone Hsp60, peptide-dependent ATPase, heat-shock proteinmutMb36354.12.92.2Formamidopyrimidine DNA glycosylasenarPb21938.66.9NitratenuoCb2286*-5.1-3.3-2.2NADH dehydrogenase I chain C, D	htpG	b0473	*		19.0	5.2		Chaperone Hsp90, heat-shock protein C 62.5
hyaBb0973*2.1Hydrogenase-1 large subunit $ibpA$ $b3687$ * $38.1$ $21.2$ Heat-shock protein $ibpB$ $b3686$ * $54.6$ $30.6$ Heat-shock protein $intF$ $b0281$ Putative phage integrasePutative phage integrase $lon$ $b0439$ * $11.4$ $5.9$ $-1.9$ $lpdA$ $b0116$ * $-2.9$ $-3.8$ $-2.9$ $marA$ $b1531$ $3.2$ Multiple antibiotic resistance; transcriptional activator of defense systems $marA$ $b1530$ $2.8$ Multiple antibiotic resistance protein; repressor of mar operon $miaA$ $b4143$ * $14.3$ $7.0$ $-2.3$ $mopA$ $b4142$ * $7.8$ $4.6$ $-3.6$ $mopB$ $b4142$ * $7.8$ $4.6$ $-3.6$ $marP$ $b2193$ $8.6$ $6.9$ Nitrate $muoC$ $b2286$ * $-5.1$ $-3.3$ $-2.2$ NADH dehydrogenase I chain C, D	htpX	b1829	*		39.4	22.4		Heat-shock protein, integral membrane protein
ibpA $b3687$ * $38.1$ $21.2$ Heat-shock protein $ibpB$ $b3686$ * $54.6$ $30.6$ Heat-shock protein $intF$ $b0281$ $11.4$ $5.9$ $-1.9$ DNA-binding, ATP-dependent protease La; heat-shock K-protein $lon$ $b0439$ * $11.4$ $5.9$ $-1.9$ DNA-binding, ATP-dependent protease La; heat-shock K-protein $lpdA$ $b0116$ * $-2.9$ $-3.8$ $-2.9$ Lipoamide dehydrogenase (NADH); component of 2-oxodehydrogenase and pyruvate complexes; 1-protein of glycine cleavage complex $marA$ $b1531$ $3.2$ Multiple antibiotic resistance; transcriptional activator of defense systems Multiple antibiotic resistance protein; repressor of mar operon $miaA$ $b4171$ $2.1$ $-4.7$ Delta (2)-isopentenylpyrophosphate tRNA-adenosine transferase GroEL, chaperone Hsp60, peptide-dependent ATPase, heat-shock protein GroES, 10 kDa chaperone binds to Hsp60 in pres. Mg-ATP, suppressing its ATPase activity $mutM$ $b3635$ $4.1$ $2.9$ $2.2$ Formamidopyrimidine DNA glycosylase Nitrate $mucC$ $b2286$ * $-5.1$ $-3.3$ $-2.2$ NADH dehydrogenase I chain C, D	hyaB	b0973		*			2.1	Hydrogenase-1 large subunit
<i>ibpB</i> b3686*54.630.6Heat-shock protein Putative phage integrase <i>lon</i> b0439*11.45.9-1.9DNA-binding, ATP-dependent protease La; heat-shock K-protein <i>lpdA</i> b0116*-2.9-3.8-2.9Lipoamide dehydrogenase (NADH); component of 2-oxodehydrogenase and pyruvate complexes; 1-protein of glycine cleavage complex <i>marA</i> b15313.2Multiple antibiotic resistance; transcriptional activator of defense systems Multiple antibiotic resistance protein; repressor of mar operon <i>miaA</i> b41712.1-4.7Delta (2)-isopentenylpyrophosphate tRNA-adenosine transferase GroEL, chaperone Hsp60, peptide-dependent ATPase, heat-shock protein gits ATPase activity <i>mutM</i> b36354.12.92.2Formamidopyrimidine DNA glycosylase <i>narP</i> b21938.66.9Nitrate <i>nuoC</i> b2286*-5.1-3.3-2.2NADH dehydrogenase I chain C, D	ibpA	b3687	*		38.1	21.2		Heat-shock protein
intFb0281Putative phage integraselonb0439*11.45.9-1.9lpdAb0116*-2.9-3.8-2.9Lipoamide dehydrogenase (NADH); component of 2-oxodehydrogenase and pyruvate complexes; 1-protein of glycine cleavage complexmarAb15313.2Multiple antibiotic resistance; transcriptional activator of defense systems Multiple antibiotic resistance protein; repressor of mar operonmarAb15302.8Multiple antibiotic resistance protein; repressor of mar operonmiaAb41712.1-4.7Delta (2)-isopentenylpyrophosphate tRNA-adenosine transferasemopAb4143*7.0-2.3GroEL, chaperone Hsp60, peptide-dependent ATPase, heat-shock protein groES, 10 kDa chaperone binds to Hsp60 in pres. Mg-ATP, suppressing its ATPase activitymutMb36354.12.92.2murVb36354.12.92.2murVb2286*-5.1-3.3-2.2NADH dehydrogenase I chain C, D	ibpB	b3686	*		54.6	30.6		Heat-shock protein
lon $b0439$ * $11.4$ $5.9$ $-1.9$ DNA-binding, AIP-dependent protease La; heat-shock K-protein $lpdA$ $b0116$ * $-2.9$ $-3.8$ $-2.9$ DNA-binding, AIP-dependent protease La; heat-shock K-protein $marA$ $b1531$ $3.2$ Lipoamide dehydrogenase (NADH); component of 2-oxodehydrogenase and pyruvate complexes; 1-protein of glycine cleavage complex $marA$ $b1530$ $3.2$ Multiple antibiotic resistance; transcriptional activator of defense systems Multiple antibiotic resistance protein; repressor of mar operon Delta (2)-isopentenylpyrophosphate tRNA-adenosine transferase GroEL, chaperone Hsp60, peptide-dependent ATPase, heat-shock protein GroES, 10 kDa chaperone binds to Hsp60 in pres. Mg-ATP, suppressing its ATPase activity $mutM$ $b3635$ $4.1$ $2.9$ $2.2$ Formamidopyrimidine DNA glycosylase Nitrate $mucC$ $b2286$ * $-5.1$ $-3.3$ $-2.2$ NADH dehydrogenase I chain C, D	intF	b0281					1.0	Putative phage integrase
IpdAb0116*-2.9-3.8-2.9Lipoamide dehydrogenase (NADH); component of 2-oxodehydrogenase and pyruvate complexes; 1-protein of glycine cleavage complexmarAb15313.2Multiple antibiotic resistance; transcriptional activator of defense systems Multiple antibiotic resistance protein; repressor of mar operonmarAb15302.8Multiple antibiotic resistance; transcriptional activator of defense systems Multiple antibiotic resistance protein; repressor of mar operonmiaAb41712.1-4.7Delta (2)-isopentenylpyrophosphate tRNA-adenosine transferase GroEL, chaperone Hsp60, peptide-dependent ATPase, heat-shock protein its ATPase activitymutMb36354.12.92.2Formamidopyrimidine DNA glycosylase NitratemuoCb2286*-5.1-3.3-2.2NADH dehydrogenase I chain C, D	lon	b0439	×		11.4	5.9	-1.9	DNA-binding, ATP-dependent protease La; heat-shock K-protein
marAb15313.2Multiple antibiotic resistance; transcriptional activator of defense systemsmarRb15302.8Multiple antibiotic resistance; transcriptional activator of defense systemsmiaAb41712.1-4.7Delta (2)-isopentenylpyrophosphate tRNA-adenosine transferasemopAb4143*14.37.0-2.3GroEL, chaperone Hsp60, peptide-dependent ATPase, heat-shock proteinmopBb4142*7.84.6-3.6GroES, 10 kDa chaperone binds to Hsp60 in pres. Mg-ATP, suppressing its ATPase activitymutMb36354.12.92.2Formamidopyrimidine DNA glycosylasenuoCb2286*-5.1-3.3-2.2NADH dehydrogenase I chain C, D	lpdA	60116		*	-2.9	-3.8	-2.9	Lipoamide dehydrogenase (NADH); component of 2-oxodehydrogenase
mark5.2Multiple antibiotic resistance, transcriptional activator of defense systemsmarkb15302.8Multiple antibiotic resistance, transcriptional activator of defense systemsmiaAb41712.1-4.7Delta (2)-isopentenylpyrophosphate tRNA-adenosine transferasemopAb4143*14.37.0-2.3mopBb4142*7.84.6-3.6GroEL, chaperone Hsp60, peptide-dependent ATPase, heat-shock proteinmutMb36354.12.92.2Formamidopyrimidine DNA glycosylasenarPb21938.66.9NitratenuoCb2286*-5.1-3.3-2.2NADH dehydrogenase I chain C, D	mand	b1521			2.2			And pyruvate complexes, L-protein of glycine cleavage complex
mark $01350$ $2.6$ mark $12.6$	mar P	b1530			3.2 2.8			Multiple antibiotic resistance protein: repressor of mar operor
mopAb4143*14.37.0-2.3GroEL, chaperone Hsp60, peptide-dependent ATPase, heat-shock protein groEL, chaperone Hsp60, peptide-dependent ATPase, heat-shock protein its ATPase activitymutMb36354.12.92.2Formamidopyrimidine DNA glycosylase NitratenuoCb21938.66.9NitratenuoCb2286*-5.1-3.3-2.2NADH dehydrogenase I chain C, D	mia A	h4171			2.0		_4 7	Delta (2)-isopentenylnyronhosnhate t $\mathbf{RNA}$ -adenosine transferase
mopB $b4142$ *7.84.6 $-3.6$ GroEL, chapterine rapport, population reprint and product appendent reprint	mon A	h4143	*		14.3	7.0	_2 3	GroEL chaperone Hsp60 pentide-dependent ATPase heat-shock protein
mutMb36354.12.92.2Formamidopyrimidine DNA glycosylase $narP$ b21938.66.9Nitrate $nuoC$ b2286* $-5.1$ $-3.3$ $-2.2$ NADH dehydrogenase I chain C, D	monB	b4142	*		7.8	4.6	-3.6	GroES. 10 kDa chaperone binds to Hsp60 in pres. Mg-ATP. suppressing
mutMb36354.12.92.2Formamidopyrimidine DNA glycosylase $narP$ b21938.66.9Nitrate $nuoC$ b2286* $-5.1$ $-3.3$ $-2.2$ NADH dehydrogenase I chain C, D							2.0	its ATPase activity
nar P         b2193         8.6         6.9         Nitrate $nuoC$ b2286         * $-5.1$ $-3.3$ $-2.2$ NADH dehydrogenase I chain C, D	mutM	b3635			4.1	2.9	2.2	Formamidopyrimidine DNA glycosylase
<i>nuoC</i> b2286 * $-5.1$ $-3.3$ $-2.2$ NADH dehydrogenase I chain C, D	nar P	b2193			8.6	6.9		Nitrate
	nuoC	b2286		*	-5.1	-3.3	-2.2	NADH dehydrogenase I chain C, D

	pflB phoB	b0903 b0399		*	13.2	$-10.4 \\ 6.4$		Formate acetyltransferase 1 Positive response regulator for pho regulon, sensor is PhoR (or CreC)
prdC b3498 3.6   prdE b3297 *   purE b3237 *   purE b323 *   purE b324 *   purE b325 *   purE b336 *	phoR	b0400			33.4	13.0		Positive and negative sensor protein for pho regulon
piob2297*PhosphortnasactylasepurCb237SatCaR synthesisepurKb0523SatCaR synthesisepurKb0524SatCaR synthesisepurKb0525SatCaR synthesisepurKb0526SatCaR synthesisepurKb2500Phosphortboxylaminoimdizole carboxylase =purKb2500Phosphortboxylaminoimdizole carboxylase =purKb2500Phosphortboxylaminoimdizole carboxylase =purKb2500Phosphortboxylaminoimdizole carboxylase =purKb2510SattCaR synthesisepurKb2527SattCaR synthesisepurDb062SattCaR synthesisepurDb1662SattCaR synthesisepurDb2464SattCaR synthesisepurDb2464SattCaR synthesisepurDb2677SattCaR synthesisepurDb2677SattCaR synthesisepurDb2677SattCaR synthesisestarCaR synthesisePhosphortboxylaminoimdizole carboxylasepurDb2678SattCaR synthesisestarCaR synthesisePhosphortboxylaminoimdizole carboxylasepurDb2679SattCaR synthesisestarCaR synthesisePhosphortboxylaminoimdizole carboxylasepurDb2687SattCaR synthesisestarCaR synthesisePhosphortboxylaminoimdizole carboxylasestarCaR synthesisePhosphortboxylaminoimdizole carboxylasestarCaR synthesisePhosphortboxylaminoimdizole carboxylasestarCaR synthesisePhosphortboxylam	prlC	b3498			3.6			Oligopeptidase A
purCb113 purCAdeinylosuccinate bysic synthetisse - SAICAR synthetissepurEb05233.1Phosphotiobsylaminoimidazole-succincearboxnaic synthetisse - SAICAR synthetissepurKb05233.1Phosphotiobsylaminoimidazole-succincearboxnaic synthetisse - SAICAR synthetissepurKb05233.1Phosphotiobsylayininoimidazole-succincearboxnaic erboyhoriboxylayininoimidazole carboxnaicpurKb166*-3.4Phyraute kinase [former] F). Incrose simulated Dilydro-orotalepurKb1676*-2.6Aspartate carboxnoyltransferase, catalytic subunit pyrB b244*purKb1676*-2.6Aspartate carboxnoyltransferase, catalytic subunit pyrB obtoxicpurLb4246*15.011.05.0purLb4247*2.83.3Aspartate carboxnoyltransferase, regulatory subunit induced at high temperatures 	pta	b2297		*				Phosphotransacetylase
$\mu \mu E$ b2476Phosphoribosylaminoimidazole succincerosmaide $\mu \mu E$ b05233.1Phosphoribosylaminoimidazole carboxylae - $\mu \mu K$ b0500All carboxylae, catalytic subunit $\mu \mu K$ b0500All carboxylae, catalytic subunit $\mu \mu K$ b1676*-3.4 $\mu \mu K$ b1676*-3.4 $\mu \mu K$ b1676*-3.4 $\mu \mu K$ b1676*-3.4 $\mu \mu K$ b1676*-2.5 $\mu \mu K$ b1676*-2.6 $\mu \mu K$ b1676*-2.7 $\mu \mu K$ b1676*-2.8 $\mu \mu K$ b1676*-2.8 $\mu \mu K$ b1676*-2.7 $\mu K$ b1677*-2.7 $\mu K$ b1676*-2.7 $\mu \mu K$ b1676*-2.7 $\mu \mu K$ b1676*-2.7 $\mu \mu K$ b1679-1.3 $\mu \mu K$ b1679-2.1 $\mu \mu K$ b1679-1.3 $\mu \mu K$ b1679-3.4 $\mu \mu K$ b1679-3.4 $\mu \mu K$ b1679-3.4 $\mu \mu K$ b1679-3.4 $\mu \mu \mu $	purB	b1131						Adenylosuccinate lyase
purkb05233.1Phosphorbosylaminoimidazole carbosylas = AIR carbosylas, catably: subunit Phosphorbosylaminoimidazole carbosylas = AIR carbosylas, catably: subunit Phosphorbosylayicaminoimidazole carbosylas = AIR carbosylas, catably: subunit Phosphorbosylgycinamide formyltransferase 1 Phosphorbosylgycinamide formyltransferase 1 Phosphorbosylawice carbosylas = A 2507prifb1246 **2.8 1.03.3 Apartale carbonyltransferase, regulatory subunit pyrif b1246 with the phosphorbosylawice phosphore sigma-f. factor, peathowck and oxidative stress sigma-f. factor, peathowck and oxidative stress sigma-f. factor peag protein (emory.ubpf)profb2573 with the phosphorbosylawice construction of proteins induced at high temperatures b2573-profb2476 with the phosphorbosylawice construction of upper for and colkin K (emory.ubpf)profb2480 with b315-with b315 with b3154.0 with b315-with b315 with b315with b315 with b315with b315 with b315with b315 with b315with b315 with b315with b315 with b315with b315 with b315 <td>purC</td> <td>b2476</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>Phosphoribosylaminoimidazole-succinocarboxamide synthetase = SAICAR synthetase</td>	purC	b2476						Phosphoribosylaminoimidazole-succinocarboxamide synthetase = SAICAR synthetase
purk         b522         Phosphoribosylaminidic carboxylase = $prr         b1676         *         -3.4         Phosphoribosylagy carboxylase, CO_Chising subunit           prr         b1676         *         -3.4         Pyruste kinase I (formery IP, furctose stimulated           prr         b1622         *         2.5         Aspartate carbamoyltransferase, regulatory subunit           prr         b1642         *         2.8         3.3         Aspartate carbamoyltransferase, regulatory subunit           prr         b2424         *         2.8         3.3         Aspartate carbamoyltransferase, regulatory subunit           prL         b244         *         2.8         Probable science transports         Receive transports           prL         b244         *         2.9         -4.7         Receive transports           prob         b3067         *         4.4         -2.0         Probable science transports           prde         b114         *         1.6         1.8         Probable formate acceptransports           sgr         b4300         5         6.4         Probable formate acceptroptional regulator           ideE         b114         *         1.6         Trehabase -P hydrolase           $	purE	b0523					3.1	Phosphoribosylaminoimidazole carboxylase = AIR carboxylase, catalytic subunit
pp/K         bi2500         Phosphoribos/glycinamide formytransferase 1           pp/K         bi366         *         -3.4           pp/K         bi062         *         2.5           pp/K         bi087         Supproversite         Dihydro-orotase           pp/L         bi244         *         2.8         3.3           pp/L         bi244         *         2.8         Napartate carbanoytransferase, regulatory subunit           pp/L         bi244         *         2.8         Photobib formate acety functional regulator           pp/L         bi243         *         -2.7         RNA polymerase, signac Elacty heat-shock and oxidative stress           side         bi370         *         -2.7         Photobib formate acety functional regulator           side         bi373         *         1.6         Photobib formate acety functional regulator           side         bi374         8         2.9         Photobib formate acety functional regulator           side         bi38 <td< td=""><td>purK</td><td>b0522</td><td></td><td></td><td></td><td></td><td></td><td>Phosphoribosylaminoimidazole carboxylase = AIR carboxylase, CO(2)-fixing subunit</td></td<>	purK	b0522						Phosphoribosylaminoimidazole carboxylase = AIR carboxylase, CO(2)-fixing subunit
pyrB         b4245         *         -3.4         Pyruak kinase I (formerly P), furctose simulated           pyrC         b0945         *         2.6         2.5         Aspartate carbamoyltransferase, catalytic subunit           pyrD         b04246         *         1.7         1.8         Dihydro-orotase           pyrL         b4246         *         2.8         3.3         Aspartate carbamoyltransferase, regulatory subunit           pyrL         b4246         *         1.5.0         10.50         pyrB1 optical teddy for the stress induced at thigh temperatures           rpoE         b5067         *         4.4         -0         RNA polymerase, sigma.F factor, beat-shock and oxidative stress induced at thigh temperatures           rpoE         b5273         *         3.8         -2.7         RNA polymerase, sigma.F factor, beat-shock and oxidative stress induced at thigh temperatures           scale         b5270         11.3         4.8         2.9         Probable scrine transporter           sdaC         b2374         3.8         1.5         0.4         Probable scrine transporter           scale         b3141         3.8         1.5         0.4         Probable scrine transporter           scale         b3249         mareloporino syntrase carbinor or phage T6 and colicin K <td>purN</td> <td>b2500</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>Phosphoribosylglycinamide formyltransferase 1</td>	purN	b2500						Phosphoribosylglycinamide formyltransferase 1
pyrCblo2*2.62.5Asparatue carbomolytransferase, calabie subunit $pyrC$ blo2*1.71.8Dihydro-orotaseDihydro-orotase $pyrJ$ bd244*2.83.3Asparate carbomolytransferase, regulatory subunit $pyrJ$ bd244*1.05.0 $pyrH$ $pyrL$ bd276*4.4-2.0RNA polymerase, sigma - factor, negative regulatory protein $pyrL$ bd277*3.8-2.7RNA polymerase, sigma - factor, negative regulatory protein $succ$ bd277611.34.82.9Probable serine transporter $succ$ bd2305.56.4Putative DEOR-type transcriptional regulator $tdE$ bd314*1.61.8Probable serine transporter $succ$ bd2398.311.5Trehalses of Phydrolaseputative activator of uhp T transcription $tax$ bd669 $-3.4$ Valuecoside channel; receptor of page proteinKason uhpB $uprd$ bd2095.9Orf, hypothetical protein $uprd$ bd2095.9Value stransport $uprd$ bd3154.04.6Orf, hypothetical protein $uprd$ bd3692.1-3.3Orf, hypothetical protein $uprd$ bd5892.1-3.3Orf, hypothetical protein $uprd$ bd692.38.3Putative transcriptional regulator LVSR-type $uprd$ bd5892.1-3.3Orf, hypothetical protein $uprd$ bd589	pykF	b1676		*		-3.4		Pyruvate kinase I (formerly F), fructose stimulated
pr/C         b042         *         1.7         1.8         Dihydro-crotate         pr/d/progenase           pr/L         b424         *         2.8         3.3         Aspartate carbamoyltransferase, regulatory subunit           pr/L         b4246         *         1.0         5.0         pyll bydro-crotate         bydro-crotate         bydro-crotate           pr/L         b4246         *         2.8         3.3         Aspartate carbamoyltransferase, regulatory subunit           pr/L         b4246         *         1.0         5.0         Probable Serine transporter           signal         b4300         .5.5         6.4         Putative DEOR-type transferase 3         DNA topoisomerase type I, onega protein           tize         b430         .5.5         6.4         Putative DEOR-type transferase 3         DNA topoisomerase type I, onega protein           tize         b44         1.6         1.8         Probable formate acetyltransferase 3         DNA topoisomerase type I, onega protein           tize         b441         2.3         Nucleoside channel; receptor of phage T6 and colicin K           weak         b2497         *         11.3         11.7           ya/D         b0209         5.9         Putative fanseritation suftesis protein <tr< td=""><td>pyrB</td><td>b4245</td><td></td><td>*</td><td></td><td>2.6</td><td>2.5</td><td>Aspartate carbamoyltransferase, catalytic subunit</td></tr<>	pyrB	b4245		*		2.6	2.5	Aspartate carbamoyltransferase, catalytic subunit
$p_{PT}$ b945         *         Dihydro-orotate dehydrogenase $p_{PT}$ b424         *         2.8         3.3         Asparate carbanoyltransferase, regulatory subunit $p_{PL}$ b4246         *         15.0         11.0         5.0 $p_{PT}$ $p_{PL}$ b4267         *         3.8         -2.7         RNA polymerase, signa-E factor, negative regulatory protein $read$ b2572         2.9         -4.2         Signa-E factor, negative regulatory protein $read$ b1300         5.5         6.4         Putative DEOR-type transcriptional regulator $redE$ b3114         *         1.6         1.8         Probable scine transporter $redE$ b4301         .         .6         Putative DEOR-type transcription are gulator $redE$ b411         .         8.3         11.5         Trehalses 6-P hydrolase $redE$ b429         .         11.3         11.7         Urceil rhophydrohoyllransferase $redE$ b4301         .         .         0.6         phydrohydrohyllransferase $redE$ b4304         .         .         .         11.3	pyrC	b1062		*		1.7	1.8	Dihydro-orotase
pr/L         b424         *         2.8         3.3         Aspartate carbamoyltransferase, regulatory submit           pr/L         b426         *         1.0         5.0         pyBl optical         byBl optical         byBl optical           pr/D         b426         *         1.0         5.0         pyBl optical         byBl optical         byBl optical           pr/D         b426         *         3.8         -2.7         RNA polymerase, sigma (70) factor, regulation of proteins induced at high temperatures           stack         b2573         *         3.8         -2.7         RNA polymerase, sigma (70) factor, regulator protein           stack         b430         -5.5         6.4         Putative DEOR-type transferase a         b114           stack         b430         1.5         Tenhase 6-P hydrolase         b208         consistence type (1 omega protein for an colicin K           tree         b4299         8.3         1.5         Tenhase 6-P hydrolase         Uracil phosphoribosyltransferase           up/d         b2497         *         11.3         11.7         Uracil phosphoribosyltransferase           ya/d         b0210         -3.4         Cracil phosphoribosyltransferase         Putative thoin synthesis protein           ya/d         b031	pyrD	b0945		*				Dihydro-orotate dehydrogenase
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	pyrI	b4244		*		2.8	3.3	Aspartate carbamoyltransferase, regulatory subunit
by borns         *         4.4         -2.0         RNA polymerase, sigma (70) factor; regulation of proteins induced at high temperatures           induced at high temperatures         sigmat E factor, regulation of proteins           start         b 2573         *         3.8         -2.7         RNA polymerase, sigmat E factor, regulatory protein           start         b 2572         2.9         -4.2         Sigmat E factor, regulatory protein           start         b 2572         2.9         -4.2         Sigmat E factor, regulatory protein           start         b 2574         3.8         DNA topoisonerase type I, omega protein           start         b 2174         3.8         DNA topoisonerase type I, omega protein           start         b 2439         8.3         11.5         Trechalse 6-P hydrolase           start         b 2449         Uracil transport         Orf, hypothetical protein         Putative boin synthesis protein           start         b 2497         *         11.3         11.7         Uracil transport         Orf, hypothetical protein           start         b 2497         *         11.3         11.7         Uracil transport         Orf, hypothetical protein           start         b 2498         Uracil transport         Orf, hypothetical protein         Pu	pvrL	b4246		*	15.0	11.0	5.0	pyrBI operon leader peptide
ppE         b2373         *         3.8         -2.7         RNA polymerase. sigma E factor, nearby regulatory protein           stacc         b2372         2.9         -4.2         Sigma E factor, nearby regulatory protein           stacc         b130         11.3         4.8         2.9         Probable serine transporter           gcR         b4300         11.3         4.8         2.9         Probable formate acetyltransferase 3           tork         b4239         8.3         11.5         Trchalase 6-P hydrolase         0.0000 nonear regulator, positive activator of uhpT transcription (sensor, uhpB)           trx         b0411         2.3         Nucleoside channel, receptor of phage T6 and colicin K           upA         b3669         -3.4         Resonsore regulator, positive activator of uhpT transcription (sensor, uhpB)           upA         b2498         Uracil phosphoribosyltransferase           urad         b2497         *         11.3         11.7           yalb         b0315         4.0         4.6         Orf, hypothetical protein           yald         b0315         4.0         4.6         Putative facella resistance protein           ybk         b0659         2.1         -3.3         orf, hypothetical protein           ybk         b0	rpoD	b3067	*		4.4		-2.0	RNA polymerase, sigma (70) factor; regulation of proteins induced at high temperatures
$r_{add}$ b2372       2.9       -4.2       Sigma E factor, negative regulatory protein $vadc$ b2376       11.3       4.8       2.9       Probable serie transporter $vadc$ b314       *       1.6       1.8       Probable serie transporter $vadc$ b114       *       1.6       1.8       Probable serie transporter $vadc$ b233       8.3       11.5       Trehalase 6-P hydrolase $varc$ b2497       *       11.3       Vaccoside channet, receptor of phage T6 and colicin K $vard$ b2497       *       11.3       Uraci lposphoribosyltransferase $vard$ b2497       *       11.3       Uraci lposphoribosyltransferase $vard$ b2497       *       11.3       Uraci lposphoribosyltransferase $vard$ b0315       4.0       4.6       Putative transcriptional regulator LYSR-type $vard$ b0316       2.6       Putative transcriptional regulator LYSR-type $vard$ b0316       2.6       Putative transcriptional regulator LYSR-type $vard$ b0491       Putative meal resistance protein $vbbM$ b0492       4.1       Putative trans	rdoE	b2573	*		3.8		-2.7	RNA polymerase, sigma-E factor: heat-shock and oxidative stress
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	rse A	b2572			2.9		-4.2	Sigma-E factor, negative regulatory protein
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	sdaC	b2796			11.3	48	2.9	Probable serine transporter
NetherProbable formate actylinans/product probable formate actylinans/product product pr	sacR	b4300			11.5	5.5	6.4	Putative DEOR-type transcriptional regulator
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	tdeF	b3114		*		1.6	1.8	Probable formate acetultransferase 3
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	ton A	b1274			28	1.0	1.0	DNA topoisomerose type L omega protein
Inc.bitsb	topA traC	b1274			5.8	0 2	11.5	Trabalage 6 D hydrologe
hx $b2491$ $2.5$ Functional contained conta	trec	04239 b0411				8.5	11.5	Nucleaside channels recenter of phase T6 and calicin K
upp $b.3669$ $-3.4$ Response regulator, positive activator of unp1 transcription (sensor, uhpB) Uracil phosphoribosyltransferase $upp$ $b2498$ $Uracil phosphoribosyltransferaseuradb2497*11.311.7yafDb02095.9Orf, hypothetical proteinyafAb03154.04.6Orf, hypothetical proteinyahAb03154.04.6Orf, hypothetical proteinyahBb03162.6Putative transcriptional regulator LYSR-typeyabMb0491Putative transcriptional proteinybbNb04924.1Putative transcriptionybbXb06592.1-3.3ybdZb06602.3Putative transcriptionycePb106052.626.8ycePb106052.626.8ycfFb11123.5Orf, hypothetical proteinycfFb1322*Orf, hypothetical proteinycfFb132*-2.1yedUb1967-2.2-1.4yhgHb3435.22.44yhgHb3135.22.7yhgHb3135.22.7yhgHb3435.22.7yhgHb3435.22.7yhgHb3435.24.9yhgHb3435.24.9yhgHb3411.36.0$	ISX	00411			2.4		2.3	Nucleoside channel; receptor of phage 16 and concin K
upp         b2498         Uracil phosphorhosyltransferase           yafD         b0209         5.9         Orf, hypothetical protein           yafE         b0210         Orf, hypothetical protein           yafB         b0315         4.0         4.6           yafB         b0315         4.0         4.6           yafB         b0316         2.6         Putative transcriptional regulator LYSR-type           yabM         b0491         Putative transcriptional regulator LYSR-type           ybbN         b0492         4.1         Putative transcriptional regulator LYSR-type           ybbV         b0659         2.1         -3.3         orf, hypothetical protein           ybZ         b0660         2.3         Putative thoredoxin-like protein           yceV         b0966         9.0         4.4         -3.1           yceP         b1060         52.6         26.8         Orf, hypothetical protein           yceV         b1060         52.6         26.8         Orf, hypothetical protein           yceV         b1060         52.6         26.8         Orf, hypothetical protein           yceV         b1060         52.6         26.8         Orf, hypothetical protein           ycfC         b132<	uhpA	03669			-3.4			(sensor, uhpB)
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	upp	b2498						Uracil phosphoribosyltransferase
yaffb02095.9Orf, hypothetical proteinyaffb0316Putative bioin synthesis proteinyahAb03154.04.6yahBb0362.6yahBb03748.2ybbNb0491Putative fragellin structural proteinybbNb04924.1ybbZb06692.1ybcZb06602.3ybcZb06602.3yccVb09669.0yccVb09669.0yccVb09669.0ycfFb112ycfFb112ycfFb1322ycfFb132ycfFb1322ycaffb112ycfFb132ycfFb132ycfFb132ycfFb132ycfFb132ycfFb132ycfFb132ycfFb132ycaffb116ycaffb117ycaffb178ycaffb178ycaffb178ycaffb172ycaffb133ycaffb172ycaffb172ycaffb172ycaffb172ycaffb172ycaffb172ycaffb172ycaffb172ycaffb172ycaffb172ycaffb173ycaffb172ycaffb172ycaffb173ycaffb173ycaff <t< td=""><td>uraA</td><td>b2497</td><td></td><td>*</td><td></td><td>11.3</td><td>11.7</td><td>Uracil transport</td></t<>	uraA	b2497		*		11.3	11.7	Uracil transport
yafAb03154.04.6Putative bioin synthesis proteinyahAb03154.04.6Orf, hypothetical proteinyahBb03162.6Putative transcriptional regulator LYSR-typeyaiUb03748.2Putative metal resistance proteinybbMb0491Putative metal resistance proteinybbNb04924.1Putative metal resistance proteinybrZb06602.3Putative ATP-binding component of a transport systemycVb09669.04.4-3.1ycRb1132*Orf, hypothetical proteinycRb1132*Orf, hypothetical proteinycFb1328.03.3Orf, hypothetical proteinycFb1328.03.3Orf, hypothetical proteinycFb1328.03.3Orf, hypothetical proteinycfFb1328.03.3Orf, hypothetical proteinycfFb1328.03.51.9yedUb1967-2.1-8.7Orf, hypothetical proteinyhgHb3435.22.4Orf, hypothetical proteinyhgEb34022.53.73.3yhgEb34022.53.73.3yhgEb3512*-3.9-4.9yhgEb368511.06.64.6yidEb368511.06.64.6yidEb368511.06.6yidEb36812.5-4.0yidEb3681	yafD	60209			5.9			Orf, hypothetical protein
yah B         b0315         4.0         4.6         Orf, hypothetical protein           yah B         b0316         2.6         Putative transcriptional regulator LYSR-type           yau U         b0374         8.2         Putative meal resistance protein           ybbN         b0491         Putative diagellin structural protein           ybeY         b0659         2.1         -3.3           ybfZ         b0660         2.3         Putative thioredoxin-like protein           ybfZ         b0879         23.5         8.3           yceV         b0966         9.0         4.4         -3.1           ycfC         b1132         *         Orf, hypothetical protein           ycfC         b1132         *         Orf, hypothetical protein           ycfK         b1112         3.5         Orf, hypothetical protein           ycfK         b1182         *         -2.2         -2.2           ycfK         b1782         *         -2.1         -8.7           yffD         b2613         3.5         1.9         Putative transport protein           yffD         b343         5.2         2.4         Orf, hypothetical protein           yffH         b3413         -3.7	yafE	b0210						Putative biotin synthesis protein
yahbb03162.6Putative transcriptional regulator LYSR-typeyaiUb03748.2Putative tinggellin structural proteinybbMb0491Putative tincedoxin-like proteinybbYb06592.1-3.3ybZb06602.3Putative ATP-binding protein in pho regulonybjZb08669.04.4ycCVb09669.04.4ycCVb096652.626.8ycfRb11123.5Orf, hypothetical proteinycfKb13228.03.3ycfKb13228.03.3ycfKb132*Orf, hypothetical proteinycfKb132 $*$ -2.2ycfKb132 $*$ Orf, hypothetical proteinycfKb132 $*$ -2.1ycfKb132 $*$ -2.2ycfKb132 $*$ -2.1ycfKb132 $*$ -2.2ycfKb1330rf, hypothetical proteinyfJDb26133.51.9yhubhb32936.01.6yhucb33435.22.4yhufHb34022.53.7yhufHb3413Orf, hypothetical proteinyildEb368511.06.6yildEb368511.06.6yildEb368511.06.6yildEb08712.74.1yildEb0872*2.7yildEb0572*2.7yildE	yahA	b0315			4.0	4.6		Orf, hypothetical protein
yaiUb03748.2Putative flagellin structural proteinybbMb0491Putative metal resistance proteinybbNb04924.1Putative thioredoxin-like proteinyheZb06592.1-3.3ybfZb06602.3Putative ATP-binding component of a transport systemybfZb087923.58.3yccVb09669.04.4yccPb106052.626.8ycfCb1132*ycfKb11123.5ycfKb11123.5ycfKb11228.0ycalb1867-2.2ycalb1967ycalb1967ycalb160ycalb167ybalb167ycalb167ybalb167ybalb167ybalb167ybalb167ybalb167ybalb167ybalb167ybalb167ybalb167<	yahB	b0316					2.6	Putative transcriptional regulator LYSR-type
ybbMb0491Putative metal resistance proteinybbNb04924.1Putative thioredoxin-like proteinybeYb06592.1 $-3.3$ orf, hypothetical proteinybzZb06602.3Putative ATP-binding protein in pho regulonybjZb087923.58.3Putative ATP-binding component of a transport systemyccVb09669.04.4 $-3.1$ Orf, hypothetical proteinyccVb09669.04.4 $-3.1$ Orf, hypothetical proteinyccVb106052.626.8Orf, hypothetical proteinycfCb1132*Orf, hypothetical proteinycfFb13228.03.3Orf, hypothetical proteinycfFb13228.03.3Orf, hypothetical proteinycfLb1867 $-2.1$ $-8.7$ Orf, hypothetical proteinycfLb187 $-2.2$ $-2.4$ $-7.1$ yfDb2613 $3.5$ $1.9$ Putative transport proteinyfbHb3402 $2.5$ $3.7$ $3.3$ yhgEb3402 $2.5$ $3.7$ $3.3$ yhatti1 $13.7$ $8.2$ $1.9$ yf8H1 $1.6$ $4.4$ $2.9$ yf8Hb3685 $11.0$ $6.6$ $4.6$ yidEb3685 $11.0$ $6.6$ $4.6$ yidEb3685 $11.0$ $6.6$ $4.6$ yidEb3280 $-1.6$ Orf, hypothetical proteinyidEb3280 $-1.6$ Orf, hypoth	yaiU	b0374					8.2	Putative flagellin structural protein
ybbNb04924.1Putative thioredoxin-like proteinybeZb06592.1 $-3.3$ orf, hypothetical proteinybZb087923.58.3Putative ATP-binding protein in pho regulonybZb09669.04.4 $-3.1$ Orf, hypothetical proteinycePb106052.626.8Orf, hypothetical proteinycfCb1132*Orf, hypothetical proteinycfRb11123.5Orf, hypothetical proteinycfRb11228.03.3Orf, hypothetical proteinyeaFb1782* $-2.2$ $-2.2$ ycfRb11333.51.9yeaFb1782* $-2.1$ $-8.7$ Off, hypothetical proteinypothetical proteinyeaFb18633.51.9ylfDb26133.59.7yhdNb32936.01.6 $-1.4$ off, hypothetical proteinorf, hypothetical proteinyhgEb34022.53.73.3yhuEb341300.42.9yi81113.78.21.9yi8214.02.42.9yi84b4115*3.3yi64b5521.06.6yi64b0572*2.7yi64b0572*2.7yi64b0572*2.7yi74b3401*7.2yi74b3401*7.2yi74b3401*<	ybbM	b0491						Putative metal resistance protein
ybeYb06592.1-3.3orf, hypothetical protein $ybZ$ b06602.3Putative ATP-binding component of a transport system $ycZV$ b09669.04.4-3.1Orf, hypothetical protein $yceV$ b106052.626.8Orf, hypothetical protein $ycfC$ b1132*Orf, hypothetical protein $ycfR$ b1228.03.3Orf, hypothetical protein $ycfR$ b1322*-2.2-1.4 $ycfR$ b1322*-2.2-1.4 $ycfR$ b1782*-2.2-1.4 $ycfR$ b13236.01.6-1.4 $yfD$ b26133.51.9Putative transport protein $yhdL$ b33435.22.4Orf, hypothetical protein $yhgE$ b34022.53.73.3 $yhgH$ b3413Orf, hypothetical protein $yhgE$ 11.3.78.2 $yhdL$ b368511.06.6 $yhdE$ b368511.06.6 $yhdE$ b368511.06.6 $yhdE$ b368511.06.6 $yhdE$ b368511.06.6 $yhdE$ b368511.0 $yhdE$ b368511.0 $yhdE$ b3280-1.6 $yhdE$ b0572*2.7 $yhdE$ b0572*2.7 $yhdE$ b05812.5 $yhdE$ b05820-1.6 $yhdE$ b3280-1.6 $y$	vbbN	b0492			4.1			Putative thioredoxin-like protein
ybeZb06602.3Putative ATP-binding protein in pho regulonybjZb087923.58.3Putative ATP-binding component of a transport systemyccVb09669.04.4 $-3.1$ Orf, hypothetical proteinyccPb106052.626.8Orf, hypothetical proteinycfRb11123.5Orf, hypothetical proteinycfRb11228.03.3Orf, hypothetical proteinycafFb13228.03.3Orf, hypothetical proteinyeafFb1782* $-2.2$ $-2.2$ ycafFb1782* $-2.1$ ycafFb13236.01.6 $-1.4$ ybarb26133.51.9Putative transport proteinyhalb32936.01.6 $-1.4$ ybarb34022.53.73.3yhalb34130rf, hypothetical proteinyhalb34130rf, hypothetical proteinyik1113.78.2yik214.02.4yik214.02.4yik213.6yik4b368511.0b368511.06.6yik4b0872*yik4b3812.5yik4b08812.5yik4b08812.5yik4b08812.5yik4b08812.5yik4b08812.5yik4b08812.5yik4b0340*<	vbe Y	b0659			2.1		-3.3	orf, hypothetical protein
ybjZb087923.58.3Putative ATP-binding component of a transport system $yccV$ b09669.04.4 $-3.1$ Orf, hypothetical protein $yceP$ b106052.626.8Orf, hypothetical protein $ycfC$ b1132*Orf, hypothetical protein $ycfK$ b11123.5Orf, hypothetical protein $ycfK$ b1123.5Orf, hypothetical protein $ycfK$ b1782* $-2.2$ $2.3.5$ 8.03.3 $ycaF$ b1782* $ycaF$ b1782* $ycaF$ b1732* $ycaF$ b1732* $ycaF$ b1782* $ycaF$ b2133.5 $ycaF$ b1923 $b100$ $c.6$ -1.4 $ycaF$ b343 $ycaF$ b343 $ycaF$ b3413 $ycaF$ b3512 $ycaF$ -3.9 $yi8F$ b3685 $1.0$ 6.6 $4.6$ Putative transport protein $yi8E$ b3685 $1.0$ 6.6 <td>vbeZ</td> <td>b0660</td> <td></td> <td></td> <td>2.3</td> <td></td> <td></td> <td>Putative ATP-binding protein in pho regulon</td>	vbeZ	b0660			2.3			Putative ATP-binding protein in pho regulon
yccVb09669.04.4 $-3.1$ Orf, hypothetical proteinycePb106052.626.8Orf, hypothetical proteinycfCb1132*Orf, hypothetical proteinycfRb13228.03.3Orf, hypothetical proteinyceFb13228.03.3Orf, hypothetical proteinycefFb13228.03.3Orf, hypothetical proteinyeaFb1782* $-2.2$ $-2.2$ $-1.4$ yffDb26133.51.9Putative transport proteinyhdNb32936.01.6 $-1.4$ Orf, hypothetical proteinyhdLb33435.22.4Orf, hypothetical proteinyhgHb3413Orf, hypothetical proteinOrf, hypothetical proteinyik1113.78.21.9yik214.02.42.9yik31113.78.21.9yik41536511.06.64.6yik21b368511.06.64.6yik42b368511.06.64.6yik43b3233.33.6yik44b3812.74.1yik45b368511.06.6yik46b3572*2.7yik47b3832.52.7yik48b3280 $-1.6$ yik48b3280 $-1.6$ yik48b3280 $-1.6$ yik48b3280 $-1.6$ yik49b3280 $-1$	vbiZ	b0879			23.5	8.3		Putative ATP-binding component of a transport system
ycePb106052.626.8Orf, hypothetical proteinycfRb11123.5Orf, hypothetical proteinycfRb11123.5Orf, hypothetical proteinyceFb13228.03.3Orf, hypothetical proteinyeaFb1782* $-2.2$ $-2.2$ $-1.4$ yedFb167 $-2.1$ $-8.7$ Orf, hypothetical proteinyedUb1967 $-2.1$ $-8.7$ Orf, hypothetical proteinyhIDb26133.51.9Putative transport proteinyhaKb34335.22.4Orf, hypothetical proteinyhgEb34022.53.73.3yhgEb34022.53.73.3yhaKb3512* $-3.9$ $-4.9$ yikI113.78.21.9yikI113.78.21.9yikIEb368511.06.64.6yikI4.11.8Putative transport proteinyikIb368511.06.64.6yikIb36851.06.64.6yikIb36832.5 $-4.0$ Orf, hypothetical proteinyikIb38312.5 $-4.0$ Orf, hypothetical proteinyikIb3280 $-1.6$ Orf, hypothetical proteinyrdBb3280 $-1.6$ Orf, hypothetical proteinyrdBb3280 $-1.6$ Orf, hypothetical proteinyrdBb3401* $7.2$ 2.1yrdFb340	vccV	b0966			9.0	4.4	-3.1	Orf, hypothetical protein
ycfCb132*Off, hypothetical proteinycfRb11123.5Orf, hypothetical proteinycfRb11123.5Orf, hypothetical proteinycfFb13228.03.3Orf, hypothetical proteinyeaFb1782*-2.2-2.2yeaFb1782*-2.2yeaFb167-2.1-8.7yeaFb26133.51.9yhaNb32936.01.6yhaLb33435.22.4yhgEb34022.53.7yhgHb3413Orf, hypothetical proteinyhaHb3512*-3.9yi81113.78.2yi4Eb368511.06.6yi6Eb368511.06.6yi6Eb368511.06.6yi6Eb368511.06.6yi6Eb368511.06.6yi6Eb368511.06.6yi6Eb368511.06.6yi6Eb0572*2.7yi7Bb3280-1.6Orf, hypothetical proteinyi7Bb3280-1.6Orf, hypothetical proteinyi7Bb3401*7.22.1yi7Eb42091.92.42.8Orf, hypothetical proteinyitative protein	vceP	b1060			52.6	26.8		Orf, hypothetical protein
ycfRb11123.5Orf, hypothetical proteinycjFb13228.03.3Orf, hypothetical proteinyeaFb1782 $*$ $-2.2$ $-2.2$ $-1.4$ Orf, hypothetical proteinyedUb1967 $-2.1$ $-8.7$ Orf, hypothetical proteinyfjDb26133.51.9Putative transport proteinyhaNb32936.01.6 $-1.4$ Orf, hypothetical proteinyheLb33435.22.4Orf, hypothetical proteinyhgHb34130Orf, hypothetical proteinyhiEb3512 $*$ $-3.9$ $-4.9$ yi81113.78.21.9yi8214.02.42.9yidEb368511.06.6yidEb368511.0yidEb0572 $*$ 2.7yidEb0572 $*$ 2.7yidBb3280 $-1.6$ Orf, hypothetical proteinyifBb3280 $-1.6$ Orf, hypothetical proteinyifBb3280 $-1.6$ Orf, hypothetical proteinyifBb3280 $-1.6$ Orf, hypothetical proteinyifBb3280 $-1.6$ Orf, hypothetical proteinyifBb3401 $*$ $7.2$ 2.1Off, hypothetical proteinYrf, hypothetical protein	vcfC	b1132		*	02.0	2010		Orf hypothetical protein
$y_{efF}$ $b132$ $b16$	vcfR	b1112			3 5			Orf, hypothetical protein
yearb172*-2.2-2.2-1.4Orf, hypothetical proteinyedUb1967-2.1-8.7Orf, hypothetical proteinyfDb26133.51.9Putative transport proteinyhdNb32936.01.6-1.4Orf, hypothetical proteinyhgLb33435.22.4Orf, hypothetical proteinyhgEb34022.53.73.3Putative transportyhgHb3413Orf, hypothetical proteinOrf, hypothetical proteinyhiEb3512*-3.9-4.9Orf, hypothetical proteinyi81113.78.21.9IS186 hypothetical proteinyi8214.02.42.9IS186 and IS421 hypothetical proteinyidEb368511.06.64.6Putative transport proteinyidEb4115*3.33.6Putative transportyidEb0572*2.74.14.6Putative resistance proteinyl/Ab08812.5-4.0Orf, hypothetical proteinyrdBb3280-1.6Orf, hypothetical proteinyrfGb33991.9Putative phosphataseyrfIb3401*7.22.1Orf, hypothetical protein	vciF	b1322			8.0	33		Orf hypothetical protein
year $0102$ $-2.2$ $-1.4$ Orr, hypothetical proteinyear $0162$ $-2.1$ $-8.7$ Orr, hypothetical proteinyfjD $b2613$ $3.5$ $1.9$ Putative transport proteinyhall $b3293$ $6.0$ $1.6$ $-1.4$ Orr, hypothetical proteinyhgE $b3402$ $2.5$ $3.7$ $3.3$ Putative transportyhgH $b3413$ $5.2$ $2.4$ Orr, hypothetical proteinyhgE $b3512$ * $-3.9$ $-4.9$ yhiE $b3512$ * $-3.9$ $-4.9$ yi811 $13.7$ $8.2$ $1.9$ yi821 $4.0$ $2.4$ $2.9$ yi841 $1.0$ $6.6$ $4.6$ yidE $b3685$ $11.0$ $6.6$ 4.0 $2.4$ $2.9$ yi64 $b4115$ * $3.3$ $3.6$ Putative transportyi64 $b0572$ * $2.7$ $4.1$ $4.6$ Putative transportyick $b0572$ * $2.7$ $4.1$ $4.6$ Putative transportyick $b3280$ $-1.6$ $0rf$ , hypothetical proteinyrdB $b3280$ $-1.6$ $0rf$ , hypothetical proteinyrfG $b3399$ $1.9$ yrfI $b3401$ * $7.2$ $2.1$ $2.1$ $0rf$ , hypothetical proteinyrfE $b4209$ $1.9$ $2.4$ $2.8$ $0rf$ , hypothetical protein <td>veaF</td> <td>b1782</td> <td></td> <td>*</td> <td>_2 2</td> <td>_2 2</td> <td>_14</td> <td>Orf, hypothetical protein</td>	veaF	b1782		*	_2 2	_2 2	_14	Orf, hypothetical protein
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	vedU	b1967			-2.2	-2.2	-1.4	Orf hypothetical protein
yhdN $b3293$ $6.0$ $1.6$ $-1.4$ Orf, hypothetical protein $yheL$ $b3343$ $5.2$ $2.4$ Orf, hypothetical protein $yhgE$ $b3402$ $2.5$ $3.7$ $3.3$ Putative transport $yhgH$ $b3413$ Orf, hypothetical proteinOrf, hypothetical protein $yhiE$ $b3512$ * $-3.9$ $-4.9$ Orf, hypothetical protein $yi81$ 1 $13.7$ $8.2$ $1.9$ IS186 hypothetical protein $yi82$ 1 $4.0$ $2.4$ $2.9$ IS186 and IS421 hypothetical protein $yidE$ $b3685$ $11.0$ $6.6$ $4.6$ Putative transport protein $yidE$ $b4115$ * $3.3$ $3.6$ Putative transport $yieH$ $b4141$ $4.1$ $1.8$ Putative transport $ylcB$ $b0572$ * $2.7$ $4.1$ $4.6$ $ylgA$ $b0881$ $2.5$ $-4.0$ Orf, hypothetical protein $yrdB$ $b3280$ $-1.6$ Orf, hypothetical protein $yrfG$ $b3399$ $1.9$ Putative phosphatase $yrff$ $b3401$ * $7.2$ $2.1$ $VrfE$ $b4209$ $1.9$ $2.4$ $2.8$ $Orf, hypothetical protein$	vfiD	b2613			3 5	-2.1	-0.7	Putative transport protein
yhatb3293 $0.0$ $1.0$ $-1.4$ Off, hypothetical proteinyheLb3343 $5.2$ $2.4$ Orf, hypothetical proteinyhgEb3402 $2.5$ $3.7$ $3.3$ Putative transportyhgHb3413Orf, hypothetical proteinOrf, hypothetical proteinyhiEb3512* $-3.9$ $-4.9$ Orf, hypothetical proteinyi81113.7 $8.2$ $1.9$ IS186 hypothetical proteinyi821 $4.0$ $2.4$ $2.9$ IS186 and IS421 hypothetical proteinyidEb3685 $11.0$ $6.6$ $4.6$ Putative transport proteinyidEb4115* $3.3$ $3.6$ Putative transportyidBb0572* $2.7$ $4.1$ $4.6$ yidBb0572* $2.7$ $4.1$ $4.6$ yidBb3280 $-1.6$ Orf, hypothetical proteinyrdBb3280 $-1.6$ Orf, hypothetical proteinyrfGb3399 $1.9$ Putative phosphataseyrfIb3401* $7.2$ $2.1$ Orf, hypothetical protein $0.7$ , hypothetical protein	$y_{JJD}$	b2013			5.5	1.7	1.4	Orf hypothetical protain
yheL $5.2$ $2.4$ Off, hypothetical proteinyhgE $5343$ $2.5$ $3.7$ $3.3$ Putative transportyhgH $5343$ $2.5$ $3.7$ $3.3$ Putative transportyhgH $5312$ * $-3.9$ $-4.9$ Orf, hypothetical proteinyi811 $13.7$ $8.2$ $1.9$ IS186 hypothetical proteinyi821 $4.0$ $2.4$ $2.9$ IS186 and IS421 hypothetical proteinyidE $b3685$ $11.0$ $6.6$ $4.6$ Putative transport proteinyidE $b4115$ * $3.3$ $3.6$ Putative transport proteinyidE $b4141$ $4.1$ $1.8$ Putative transportyieH $b4141$ $4.1$ $1.8$ Putative transportyieB $b0572$ * $2.7$ $4.1$ $4.6$ yifA $b0881$ $2.5$ $-4.0$ Orf, hypothetical proteinyrdB $b3280$ $-1.6$ Orf, hypothetical proteinyrfG $b3399$ $1.9$ Putative phosphataseyrfI $b3401$ * $7.2$ $2.1$ Orf, hypothetical protein $7.2$ $2.1$ Orf, hypothetical protein	ynan l.at	b3293			0.0 5.2	1.0	-1.4	Orf, hypothetical protein
yhgE $b3402$ $2.5$ $3.7$ $3.3$ Putative transport Orf, hypothetical protein $yhgH$ $b3413$ Orf, hypothetical protein $yhiE$ $b3512$ * $-3.9$ $-4.9$ $yi8I$ 1 $13.7$ $8.2$ $1.9$ $yi82$ 1 $4.0$ $2.4$ $2.9$ $yidE$ $b3685$ $11.0$ $6.6$ $4.6$ $yidE$ $b4115$ * $3.3$ $yieH$ $b4141$ $4.1$ $1.8$ $yieH$ $b4141$ $4.1$ $1.8$ $yieB$ $b0572$ * $2.7$ $4.1$ $4.6$ Putative transport $yieB$ $b0572$ * $2.7$ $4.1$ $4.6$ Putative resistance protein $yidB$ $b3280$ $-1.6$ Orf, hypothetical protein $yrfG$ $b3399$ $1.9$ Putative phosphatase $yrff$ $b3401$ * $7.2$ $2.1$ $yrfE$ $b4209$ $1.9$ $2.4$ $2.8$ $Orf, hypothetical protein$	yneL	03343			5.2	2.4	2.2	Dri, hypothetical protein
yngH $63413$ $63413$ Orr, hypothetical protein $yhE$ $63512$ * $-3.9$ $-4.9$ Orf, hypothetical protein $yi81$ 1 $13.7$ $8.2$ $1.9$ IS186 hypothetical protein $yi82$ 1 $4.0$ $2.4$ $2.9$ IS186 and IS421 hypothetical protein $yidE$ $b3685$ $11.0$ $6.6$ $4.6$ Putative transport protein $yjdE$ $b4115$ * $3.3$ $3.6$ Putative transport protein $yjeH$ $b4141$ $4.1$ $1.8$ Putative transport $ylcB$ $b0572$ * $2.7$ $4.1$ $4.6$ $yrdB$ $b3280$ $-1.6$ Orf, hypothetical protein $yrfG$ $b3399$ $1.9$ Putative phosphatase $yrfI$ $b3401$ * $7.2$ $2.1$ $yrfE$ $b4209$ $1.9$ $2.4$ $2.8$ $Orf, hypothetical protein$	yngE	b3402			2.5	3.7	3.3	Putative transport
yhE $63512$ * $-3.9$ $-4.9$ Orf, hypothetical proteinyi81113.78.21.9IS186 hypothetical proteinyi8214.02.42.9IS186 and IS421 hypothetical proteinyidEb368511.06.64.6Putative transport proteinyjdEb4115*3.33.6Putative amino acidyjeHb41414.11.8Putative transportylcBb0572*2.74.14.6ydB5280-1.6Orf, hypothetical proteinyrfGb33991.9Putative phosphataseyrfIb3401*7.22.1VfEb42091.92.42.8Orf, hypothetical protein	yngH	63413				• •	4.0	Ori, hypothetical protein
yi81113.78.21.9IS186 hypothetical protein $yi82$ 14.02.42.9IS186 and IS421 hypothetical protein $yidE$ b368511.06.64.6Putative transport protein $yjdE$ b4115*3.33.6Putative amino acid $yjeH$ b41414.11.8Putative transport $ylcB$ b0572*2.74.14.6 $yljA$ b08812.5-4.0Orf, hypothetical protein $yrdB$ b3280-1.6Orf, hypothetical protein $yrfG$ b33991.9Putative phosphatase $yrfI$ b3401*7.22.1Orf, hypothetical protein $ytfE$ b42091.92.42.8Orf, hypothetical protein	yhiE	63512		*	10 5	-3.9	-4.9	Orf, hypothetical protein
yi8214.02.42.9IS186 and IS421 hypothetical protein $yidE$ $b3685$ 11.06.64.6Putative transport protein $yjdE$ $b4115$ *3.33.6Putative transport protein $yjeH$ $b4141$ 4.11.8Putative transport $ylcB$ $b0572$ *2.74.14.6 $yljA$ $b0881$ 2.5-4.0Orf, hypothetical protein $yrdB$ $b3280$ -1.6Orf, hypothetical protein $yrfG$ $b3399$ 1.9Putative phosphatase $yrff$ $b3401$ *7.22.1 $ytfE$ $b4209$ 1.92.42.8 $ytfE$ $b4209$ 1.92.42.8	yi81	1			13.7	8.2	1.9	IS186 hypothetical protein
yidE $b3685$ $11.0$ $6.6$ $4.6$ Putative transport proteinyjdE $b4115$ * $3.3$ $3.6$ Putative transportyjeH $b4141$ $4.1$ $1.8$ Putative transportylcB $b0572$ * $2.7$ $4.1$ $4.6$ Putative resistance proteinyljA $b0881$ $2.5$ $-4.0$ Orf, hypothetical proteinyrdB $b3280$ $-1.6$ Orf, hypothetical proteinyrfG $b3399$ $1.9$ Putative phosphataseyrfI $b3401$ * $7.2$ $2.1$ Orf, hypothetical proteinytfE $b4209$ $1.9$ $2.4$ $2.8$ Orf, hypothetical protein	yi82	1			4.0	2.4	2.9	IS186 and IS421 hypothetical protein
yjdEb4115*3.33.6Putative amino acid $yjeH$ b41414.11.8Putative transport $ylcB$ b0572*2.74.14.6Putative resistance protein $yljA$ b08812.5-4.0Orf, hypothetical protein $yrdB$ b3280-1.6Orf, hypothetical protein $yrfG$ b33991.9Putative phosphatase $yrfI$ b3401*7.22.1 $ytfE$ b42091.92.42.8	yidE	b3685			11.0	6.6	4.6	Putative transport protein
yjeHb41414.11.8Putative transport $ylcB$ b0572*2.74.14.6Putative resistance protein $yljA$ b08812.5-4.0Orf, hypothetical protein $yrdB$ b3280-1.6Orf, hypothetical protein $yrfG$ b33991.9Putative phosphatase $yrfI$ b3401*7.22.1 $yrfE$ b42091.92.42.8	yjdE	b4115		*		3.3	3.6	Putative amino acid
ylcB $b0572$ * $2.7$ $4.1$ $4.6$ Putative resistance protein $yljA$ $b0881$ $2.5$ $-4.0$ Orf, hypothetical protein $yrdB$ $b3280$ $-1.6$ Orf, hypothetical protein $yrfG$ $b3399$ $1.9$ Putative phosphatase $yrfI$ $b3401$ * $7.2$ $2.1$ $vfE$ $b4209$ $1.9$ $2.4$ $2.8$ $Orf, hypothetical protein$	yjeH	b4141			4.1		1.8	Putative transport
yljAb08812.5-4.0Orf, hypothetical protein $yrdB$ b3280-1.6Orf, hypothetical protein $yrfG$ b33991.9Putative phosphatase $yrfI$ b3401*7.22.1 $ytfE$ b42091.92.42.8	ylcB	b0572		*	2.7	4.1	4.6	Putative resistance protein
yrdBb3280 $-1.6$ Orf, hypothetical protein $yrfG$ b33991.9Putative phosphatase $yrfI$ b3401*7.22.1 $ytfE$ b42091.92.42.8	yljA	b0881			2.5		-4.0	Orf, hypothetical protein
yrfGb33991.9Putative phosphataseyrfIb3401* $7.2$ $2.1$ Orf, hypothetical proteinytfEb42091.9 $2.4$ $2.8$ Orf, hypothetical protein	vrdB	b3280			-1.6			Orf, hypothetical protein
yrfIb3401*7.22.1Orf, hypothetical proteinytfEb42091.92.42.8Orf, hypothetical protein	vrfG	b3399			1.9			Putative phosphatase
ytfE b4209 1.9 2.4 2.8 Orf, hypothetical protein	vrfI	b3401	*		7.2	2.1		Orf, hypothetical protein
	ytfE	b4209			1.9	2.4	2.8	Orf, hypothetical protein

The gene expression levels relative to the unstressed cultures are shown for the heat-shocked (*HS*), dual stressed, and induced cultures. *Asterisks* in blanks indicate the fold change was not significant ( $P \ge 0.001$ )

significantly down-regulated (~1.8-fold) and four genes were up-regulated (~2.1-fold). In contrast, most of the amino acid-tRNA genes (51 of 78 genes) were significantly up-regulated in the heat-shocked cultures with only seven genes significantly up-regulated in the dual stressed cultures. Whereas, 36 of the amino acid-tRNA genes were significantly down-regulated in the induced cultures. Over half of the ribosome genes (39 of 73) were also significantly down-regulated on average over 3-fold in the heat-shocked and dual stressed cultures. This mixed protein translation transcriptome response indicates that heat-shock and recombinant protein induction have many common negative effects on the translation apparatus, except for the amino acid-tRNA genes.

In addition to the oxidative phosphorylation genes used to synthesis cell energy, central carbon metabolism plays an important role, namely glycolysis and the tricarboxylic acid (TCA) cycle. Only 13 of the 42 genes in glycolysis were significantly regulated in the heatshocked cultures. The aceEF, eno, galM, lpdA, pfkA, pgi, pgk, and yibO genes were down-regulated between 1.8- and 7.4-fold. The aldH, bglB, and glvCG genes in glycolysis were up-regulated between 2.1- and 3.8-fold in the heat-shocked and dual stressed cultures. These up-regulated genes catalyze reactions not part of the main glucose to acetyl-CoA pathway, but side reactions, for example acetate synthesis (*aldH*). For the TCA cycle, only eight (icdA, fumB, gltA, lpdA, pckA, and sucAB) of the 27 genes were significantly regulated; however, all of these affected genes were down-regulated between 2- and 5-fold. In comparison, a cold temperature shift for wildtype E. coli cultured in shake flasks was observed to limitedly affect metabolic genes, where TCA cycle genes were reported to be up-regulated [7]. Since, only wildtype cultures were examined in the cold temperature shift study, it is difficult to extrapolate the reported behaviors to recombinant cultures. The response of the central carbon metabolism genes indicates impaired carbon utilization, which could in part account for the observed reduced growth rates during and after a heat-shock.

#### Dual stress sensitive genes

Many gene families were regulated by the heat-shock and recombinant protein induction with varying responses in the dual stressed cultures. Basically, the overall responses of the genes to the dual stress divided in to four categories: (1) heat-shocked-like response, (2) induced-like response, (3) in between the heat-shocked and induced responses, and (4) a greater than either heat-shocked or induced response. In order to evaluate the dual stress transcriptome response, the initial screening was limited to the genes that were greater than 5-fold regulated, and excluded the classical heat-shock genes and Richmond identified heat-sensitive genes. This included 260 genes for the heat-shocked and 521 genes for the dual stressed cultures. The union of these two pools was 563 genes. Pair wise comparisons between the unstressed and the three stressed cultures for the 563 genes identified 433, 457, and 426 genes that were significantly regulated ( $P \leq 0.001$ ) for the heat-shocked, dual stressed, and induced cultures, respectively. This procedure identified genes with less than 5-fold regulation for the other stresses and also more stringently selected genes with statistically significant changes. Thus, for the dual stressed cultures, the original 521-gene list was reduced to 456 genes.

 Table 5
 The tRNA synthetase gene expression levels for the heat-shocked (HS), dual stressed and induced cultures relative to the control cultures

Gene	bname	HS	Dual	Induced	Description
alaS	b2697	-1.9	-2.6	-2.8	Alanyl-tRNA synthetase
argS	b1876		3.0	3.3	Arginine tRNA synthetase
asnS	b0930				Asparagine Trna synthetase
aspS	b1866	-2.3	-2.9	-2.5	Aspartate tRNA synthetase
cysS	b0526		1.9	1.9	Cysteine tRNA synthetase
fmt	b3288	-3.8	-3.8	-2.8	10-Formyltetrahydrofolate: L-methionyl-tRNA(fMet) N-formyltransferase
glnS	b0680		-1.4		Glutamine tRNA synthetase
gltX	b2400		-1.3	-1.5	Glutamate tRNA synthetase, catalytic subunit
glyQ	b3560	-3.1	-2.6	-2.0	Glycine tRNA synthetase, alpha subunit
glyS	b3559	-2.7	-2.2	-2.0	Glycine tRNA synthetase, beta subunit
hisS	b2514	-2.6	-3.5	-2.7	Histidine tRNA synthetase
ileS	b0026		-1.9	-4.3	Isoleucine tRNA synthetase
leuS	b0642	-1.9	-2.4	-2.3	Leucine tRNA synthetase
lysS	b2890		-1.5	-1.3	Lysine tRNA synthetase, constitutive; suppressor of ColE1 mutation in primer RNA
lysU	b4129	-3.3	-3.7	-3.0	Lysine tRNA synthetase, inducible; heat-shock protein
metG	b2114			-1.1	Methionine tRNA synthetase
pheS	b1714	-3.3	-3.5	-1.8	Phenylalanine tRNA synthetase, alpha-subunit
pheT	b1713	-1.7	-3.7	-3.8	Phenylalanine tRNA synthetase, beta-subunit
proS	b0194		1.2		Proline tRNA synthetase
serS	b0893	-2.3	-2.9	-2.2	Serine tRNA synthetase; also charges selenocystein tRNA with serine
thrS	b1719	-3.8	-5.3	-4.1	Threonine tRNA synthetase

Blanks indicate the fold change was not significant ( $P \ge 0.001$ )

The objective of the analysis of the dual stressed transcriptome was to determine if a global response existed that was different for the dual stressed cultures that was not solely due to the individual stresses, thus a holistic approach was used. The classification of the genes into the four categories was not absolute. For example, the ybfH gene was up-regulated 36.6-fold, upregulated 31.6-fold, and not significantly regulated for the heat-shocked, dual stressed, and induced cultures, respectively, so this gene was classified as heat-shockedlike, where it might also be considered an "in between" response. The *cfa* gene is an example of the greater than either individual stress response, as it was down-regulated 5.6-fold, down-regulated 13.6-fold, and downregulated 5.4-fold for the heat-shocked, dual stressed, and induced cultures, respectively. The heat-shockedlike and induced-like responses were the most arbitrary classifications, and also yielded little new information. The higher than either stress provided the most useful information to better understand the dual stressed cultures. All 563 genes with fold changes grouped by category can be found in the electronic supplemental materials (Table S1) via a link at http://www.ces.clemson.edu/bio/people/harcum.htm.

The heat-shocked-like category for the dual stressed cultures was the smallest category with only 14 genes. Only three of these genes had known functions (*cbl*, *gapC*, and *uidC*), where *gapC* and *cbl* were down-regulated and *uidC* was up-regulated for the dual stressed and heat-shocked cultures. The *gapC* gene is a non-functional glyceraldehyde 3-phosphate dehydrogenase gene and *cbl* is a transcriptional regulator for *cys* regulon (cysteine biosynthesis regulator). Interestingly, none of the classical heat-shock gene or the Richmond identified genes had this behavior in the dual stressed cultures.

The induced-like category for the dual stressed cultures contained 55 genes. The most interesting genes in the induced-like group were the ribosome genes rplEFJX, rpmDIJ, and rpsH, which were all down-regulated at least 5-fold for the dual stressed and induced cultures, but only down-regulated approximately 3-fold for the heat-shocked cultures. Thirty-five of these induced-like genes are putative or hypothetical genes. The remaining 12 known-function genes had no commonality. Additionally, one of the classical heat-shock genes (htrC) and eleven of the Richmond identified genes (aidA, cheA flgE, pyrBCI, sgcR, uraA, yidE, yjdE, and ytfE) had characteristics of the induced-like response in the dual stressed cultures.

Most of the genes that were significantly regulated in the dual stressed cultures fell into the "in between" the heat-shocked and induced response category. This group included 225 genes out of 456 significantly regulated genes. This also described the behavior of most of the classical heat-shock genes (29 of the 31 significantly regulated genes), as well as the majority of the heatsensitive identified Richmond genes (53 of the 67 significantly regulated genes). Three cytochrome genes (cyoBCD) were down-regulated, and six flagella/fimbriae genes (flgBCI, fihA, and fliLQ) were up-regulated. Additionally, 134 genes that had "in between" behavior were putative or hypothetical genes. Unfortunately, this high number of uncharacterized genes makes if difficult to develop a theoretical understanding of the in-between dual stress response.

The category of genes that responded more profoundly in the dual stressed cultures than either stress alone contains 163 genes. Additionally, two of the classical heat-shock genes (hscA and lysU) and three Richmond identified genes (hflX, vahA, and veaF) had this behavior. Unlike the other three categories, this category contains numerous gene families indicating a coordinated response. The aceABEF genes were all significantly down-regulated  $\sim$ 9-fold, as was the poxB gene, indicating pyruvate metabolism was affected. Two bgl genes (bglBG) were both up-regulated by the dual stress and *bglF* was up-regulated  $\sim$ 4-fold in the heatshocked cultures, but did not meet the significance criteria for the dual stressed culture. The bgl genes are crytic genes associated with glucosidase metabolism. The *cadC* gene, which is a transcriptional activator, was upregulated, where Richmond et al. [31] observed the *cadA* gene up-regulated due to heat-shock in wild-type cultures. This study also observed *cadA* up-regulation due to heat-shock, and more so in the dual stressed cultures. Two cold shock genes (cspAC) were down-regulated (~8-fold) more by the dual stress; however, cspE was also down-regulated by the dual stress, but at lower levels. Many glutamine biosynthesis genes were downregulated (~9-fold), including glnAHKPO. Two of the molybdopterin genes (moaCD) were classified into this category, while two other molybdopterin biosynthesis genes (moaAB) were classified in to the in-between category. Despite falling into different categories, these four molybdopterin genes were observed to be down-regulated (>5-fold) under all three stressed culture conditions. Two outer membrane protein genes (ompCX) were also down-regulated (>7-fold) by the dual stress more profoundly than either stress alone. Rhs element protein genes, *rhsAB*, were both up-regulated (11-fold) by the dual stress. This is interesting in that RhsA protein overexpression has been observed to reduce cell survival in the stationary phase [41], and the heat-shocked and dual stressed cultures stopped growing immediately following the heat-shock stress. Many of the ribosome genes (*rplKMNRY* and *rpsDKMNPR*) were profoundly down-regulated (> 5-fold) by the dual stress, which may provide insight into the long observed phenomena of decreased ribosome levels in heat-shocked cells [19]. Also, eight ribosome genes were classified and inducedlike and were down-regulated  $\sim$ 6-fold by the dual stress. The RNA polymerase genes (rpoAS) were down-regulated by the dual stress, in contrast to the known heatshock RNA polymerase genes, rpoDEH. Only rpoH was significantly up-regulated in the dual stressed cultures, where *rpoDE* were not significantly regulated. Additionally, the relA gene was more profound down-regulated 7-fold in the dual stressed cultures, where this gene regulates the stringent response. Also, the *slp* gene, which is normally induced after a carbon starvation, was down-regulated 10-fold, indicating glucose was sufficient. The tRNA synthetase genes were nearly uniformly down-regulated ( $\sim$ 2-fold) Overall, there were no genes that had unique responses to the dual stressed cultures; however, for many of the ribosome and metabolic pathway genes the dual stressed cultures were more profoundly affected that the single stressed cultures. More importantly, the dual stressed cultures transcriptome profiles were most often "in between" the transcriptional responses of heat-shocked and induced cultures, which mitigated the negative effects of these stresses in many cases.

#### Recombinant protein production implications

In order to reduce the need for large quantities of IPTG for industrial-scale processes, thermo-inducible system are often used to elicit recombinant protein production [18, 21, 28, 30, 35]. Recombinant protein production has been observed to result in significant transcriptome changes that would seem to be targeted to impede recombinant protein production [15]. It has also been observed that recombinant protein production at elevated culture temperatures can actually improve recombinant protein productivity [18, 21, 30, 36]; however, a viable mechanism that explains these observations has not been developed. Although the heat-shock (50°C) used in this study is greater than would be used in industry for thermo-induction (42°C), examining the differences between the induced and dual stressed cultures could provide insight into the mechanism by which thermo-induction improve recombinant protein production over chemical induction at normal growth temperatures. Thus, an analysis of the transcriptome level differences between the induced and dual stressed cultures was under taken to provide a basis for a preliminary mechanism. Based on the pair wise Tukey Post-Hoc tests, only 211 genes had different expression levels between the induced and dual stressed cultures. A majority of these genes (148 genes) also had different expression levels relative to the unstressed, whereas 63 of the 211 genes were not differentially expressed between the unstressed and induced cultures.

The most prevalent gene family identified by comparing transcriptome levels under the induced and dual stressed cultures were the amino acid-tRNA genes. Thirty-seven of the 211 genes were amino acid-tRNA genes and all had higher expression in the dual stressed cultures compared to the induced cultures. Since, amino acid-tRNA genes play a critical role in protein translation, up-regulation of these genes could provide an explanation for improve recombinant protein productivities, as the necessary tools to counteract the other negative effects that recombinant protein production has on the translation reactions. In *E. coli*, there are 78 identified amino acid-tRNA genes, of which 58 of these genes were observed to be significantly regulated across the four cultures examined. The heat-shocked cultures had the highest amino acid-tRNA transcriptome levels. Both of the heat-shocked and induced cultures significantly regulated the tRNA transcriptome levels; however, in opposite directions, thus when the amino acid-tRNA transcriptome levels for the dual stressed cultures are comparable to the unstressed cultures, and very few amino acid-tRNA genes appear to be regulated, if one only compares the dual stressed to the unstressed cultures. In the electronic supplemental materials, Table S2 (http://www.ces.clemson.edu/bio/people/harcum.htm), all 211 genes with fold change are listed. Also in the electronic supplemental materials, in Table S3 (http://www.ces.clemson.edu/bio/people/harcum.htm), all 78 amino acid-tRNA genes are listed with fold changes relative to the unstressed cultures.

Interestingly, the SYNPBR322 probe, which is a control probe on the Affymetrix DNA microarrays designed to hybridize with the  $\beta$ -lactamase (ampicillin resistance) portion of the pBR322 plasmid, was identified as over 2-fold higher for the dual stressed cultures compared the induced cultures. The transcriptome level for the SYNBR322 probe between the unstressed, heatshocked and dual stressed cultures were not significantly different. The pPROEx-CAT plasmid is a pBR322 derivative, so transcriptome signal attributed to the plasmid was not unexpected. In previous wild-type versus recombinant culture comparisons, the signal from plasmid-related synthetic probes, where a chromosomal gene existed, correlated well with the known copy number of the pPROEx-CAT plasmid, for example the lacI signal. Therefore, recombinant protein productivities may be higher for the dual stressed cultures relative to the chemically induced cultures due, in part, to higher plasmid copy number.

In addition to the amino acid-tRNA genes, numerous other transcription and translation genes were also upregulated by the elevated temperature. These other upregulated genes included *ffh*, *ffs*, *holC*, *miaA*, *nusB*, *rffH*, *rmf*, *rnc*, *rnpB*, *ssrAS*, and *stpA*. These genes generally catalyze transcription or translation reactions. Specifically, *miaA* and *rnpB* are involved in tRNA processing, while *rseAB* encode the  $\sigma^{E}$  factor and its regulator, respectively. While *ffs* and *rmf* encode factors that modulate the ribosome, up-regulation of these genes would result in improved protein synthesis, and therefore high recombinant protein synthesis.

#### Summary

The recombinant cultures responded to a heat-shock in a similar manner as the reported wild-type cultures; however, many more genes, in particular putative and hypothetical genes, were significantly regulated. The dual stressed cultures also had transcriptome responses to the heat-shock similar to the wild-type cultures for the classical heat-shock genes, and many more genes were highly affected. For the recombinant cultures, both the heat-shock and dual stressed cultures had significant down-regulation of energy and protein translation genes. Numerous other metabolic and nonmetabolic gene families were regulated including the fimbriae, flagellar, cold-shock, molybdopterin, amino acid-tRNA, and tRNA synthetase, and ribosome gene families. This study demonstrated that recombinant cultures have an active and responsive heat-shock apparatus for which some transcriptome responses are mitigated by recombinant protein induction and others are exacerbated. The highly sensitive heat-sensitive genes observed for the heat-shocked and dual stressed recombinant cultures may provide insight into the differences observed in the growth characteristics of recombinant and wild-type cultures, once characterized.

The dual stressed cultures were observed to have numerous differences from the induced cultures, specifically higher amino acid-tRNA and chaperone gene transcriptome levels. Also, the plasmid copy number may have been higher. Although ribosome levels were still down-regulated for the dual stressed cultures compared to the unstressed cultures, the up-regulation of the amino acid-tRNA genes relative to the induced cultures, in concert with the higher chaperone gene levels may mitigate some of the metabolic burden associated with recombinant protein production. To confirm that these results are applicable to industrial thermo-induction, 42°C heat-shocked and dual stressed culture transcriptome profiles need to be assessed.

## **Supporting material**

All raw data intensities for the 12 DNA microarrays, individually, may be obtained from the University of Wisconsin *E. coli* Genome Project via the "A Systematic Annotation Package for Community Analysis of Genomes" as a guest https://asap.ahabs.wisc.edu/annotation/php/logon.php) [12].

Acknowledgment This material is based upon work supported by the National Science Foundation under Grant No. 0303782.

#### References

- Baneyx F, Mujacic M (2004) Recombinant protein folding and misfolding in *Escherichia coli*. Nat Biotechnol 22:1399–1408
- Blattner FR, Plunkett G, Bloch CA, Perna NT, Burland V, Riley M, ColladoVides J, Glasner JD, Rode CK, Mayhew GF, Gregor J, Davis NW, Kirkpatrick HA, Goeden MA, Rose DJ, Mau B, Shao Y (1997) The complete genome sequence of *Escherichia coli* K-12. Science 277:1453–1474
- Bonomo J, Gill R (2005) Amino acid content of recombinant proteins influences the metabolic burden response. Biotechnol Bioeng 90:116–126
- Chuang S-E, Blattner FR (1993) Characterization of twenty-six new heat shock genes of *Escherichia coli*. J Bacteriol 175:5242– 5252

- Chuang SE, Daniels DL, Blattner FR (1993) Global regulation of gene-expression in *Escherichia coli*. J Bacteriol 175:2026–2036
- DeLisa MP, Li J, Rao G, Weigand WA, Bentley WE (1999) Monitoring GFP-operon fusion protein expression during high cell density cultivation of *Escherichia coli* using an on-line optical sensor. Biotechnol Bioeng 65:54–64
- Gadgil M, Kapur V, Hu WS (2005) Transcriptional response of Escherichia coli to temprature shift. Biotechnol Prog 21:689– 699
- Gao HC, Wang Y, Liu XD, Yan TF, Wu LY, Alm E, Arkin A, Thompson DK, Zhou JZ (2004) Global transcriptome analysis of the heat shock response of *shewanella oneidensis*. J Bacteriol 186:7796–7803
- Gerdes SY, Scholle MD, Campbell JW, Balazsi G, Ravasz E, Daugherty MD, Somera AL, Kyrpides NC, Anderson I, Gelfand MS, Bhattacharya A, Kapatral V, D'Souza M, Baev MV, Grechkin Y, Mseeh F, Fonstein MY, Overbeek R, Barabasi AL, Oltvai ZN, Osterman AL (2003) Experimental determination and system level analysis of essential genes in *Escherichia coli* MG1655. J Bacteriol 185:5673–5684
- Gill RT, Delisa MP, Valdes JJ, Bentley WE (2001) Genomic analysis of high cell density recombinant *Escherichia coli* fermentation and "cell conditioning" for improved recombinant protein yield. Biotechnol Bioeng 72:85–95
- Gill RT, Valdes JJ, Bentley WE (1999) Reverse transcription-PCR differential display analysis of *Escherichia coli* global gene regulation in response to heat shock. Appl Environ Microbiol 65:5386–5393
- Glasner JD, Liss P, Plunkett G, Darling A, Prasad T, Rusch M, Byrnes A, Gilson M, Biehl B, Blattner FR, Perna NT (2003) ASAP, a systematic annotation package for community analysis of genomes. Nucleic Acids Res 31:147–151
- Gross CA (1996) Function and regulation of the heat shock. In: Neidhardt FC, Curtiss R, Lin ECC, Low KB, Magasanik B, Reanikoff WS, Riley M, Schaechter M, Umbarger HE (eds) *Escherichia coli* and *Salmonella*. ASM Press, Washington DC, pp 1382–1399
- Grossman AD, Taylor WE, Burton ZF, Burgess RR, Gross CA (1985) Stringent response in *Escherichia coli* induces expression of heat shock proteins. J Mol Biol 186:357–365
- Haddadin FT, Harcum SW (2005) Transcriptome profiles for high-cell-density recombinant and wild-type *Escherichia coli*. Biotechnol Bioeng 90:127–153
- Han MJ, Park SJ, Park TJ, Lee SY (2004) Roles and applications of small heat shock proteins in the production of recombinant proteins in *Escherichia coli*. Biotechnol Bioeng 88:426–436
- Harcum SW, Bentley WE (1993) Response dynamics of 26-, 34-, 39-, 54-, and 80-kDa proteases in induced cultures of recombinant *Escherichia coli*. Biotechnol Bioeng 42:675–685
- Harcum SW, Bentley WE (1999) Heat-shock and stringent responses have overlapping protease activity in *Escherichia coli*: Implications for heterologous protein yield. Appl Biochem Biotechnol 80:23–37
- Henry MD, Yancey SD, Kushner SR (1992) Role of the heat shock response in stability of mRNA in *Escherichia coli* K-12. J Bacteriol 174:743–748
- Herendeen SL, VanBogelen RA, Neidhardt FC (1979) Levels of major proteins of *Escherichia coli* during growth at different temperatures. J Bacteriol 139:185–194
- Hoffmann F, Rinas U (2001) Plasmid amplification in *Escherichia coli* after temperature up shift is impaired by induction of recombinant protein synthesis. Biotechnol Lett 23:1819– 1825
- Hoffmann F, Weber J, Rinas U (2002) Metabolic adaptation of *Escherichia coli* during temperature-induced recombinant pro- tein production: 1. Readjustment of metabolic enzyme syn-thesis. Biotechnol Bioeng 80:313–319
- 23. Kanemori M, Mori H, Yura T (1994) Induction of heat shock proteins by abnormal proteins results from stabilization and not increased synthesis of  $\sigma^{32}$  in *Escherichia coli*. J Bacteriol 176:5648–5653

- Kucharczyk K, Laskowska E, Taylor A (1991) Response of Escherichia coli cell membranes to induction of 1 cl857 prophage by heat shock. Mol Microbiol 5:2935–2945
- 25. Lee SC, Olins PO (1992) Effect of overproduction of heat shock chaperones GroESL and DnaK on human procollagenase production in *Escherichia coli*. J Biol Chem 267:2849–2852
- Lemaux PG, Herendeen SL, Bloch P, Neidhardt FC (1978) Transient rates of synthesis of individual polypeptides in *E. coli* following temperature shifts. Cell 13:427–434
- Lesley SA, Graziano J, Cho CY, Knuth MW, Klock HE (2002) Gene expression response to misfolded protein as a screen for soluble recombinant protein. Protein Eng 15:153–160
- Makrides SC (1996) Strategies for achieving high-level expression of genes in *Escherichia coli*. Microbiol Rev 60:512
- 29. Nagai H, Yuzawa H, Yura T (1991) Regulation of the heat shock response in *E. coli:* involvement of positive and negative *cis*-acting elements in translational control of  $\sigma^{32}$  synthesis. Biochimie 73:1473–1479
- Pilon AL, Yost P, Chase TE, Lohnas GL, Bentley WE (1996) High-level expression and efficient recovery of ubiquitin fusion proteins from *Escherichia coli*. Biotechnol Prog 12:331–337
- Richmond CS, Glasner JD, Mau R, Jin H, Blattner FJ (1999) Genome-wide expression profiling in *Escherichia coli* K-12. Nucleic Acids Res 27:3821–3835
- 32. Riley M, Abe T, Arnaud MB, Berlyn MKB, Blattner FR, Chaudhuri RR, Glasner JD, Horiuchi T, Keseler IM, Kosuge T, Mori H, Perna NT, Plunkett G, Rudd KE, Serres MH, Thomas GH, Thomson NR, Wishart D, Wanner BL (2006) Escherichia coli K-12: a cooperatively developed annotation snapshot - 2005. Nucleic Acids Res 34:1–9
- 33. Rodriguez RL, Tait RE (1983) Recombinant DNA techniques: an introduction. Benjamin/Cummings, Menlo Park, CA
- 34. Rohlin L, Oh MK, Liao JC (2002) DNA microarray for microbial biotechnology: gene expression profiles in *Escherichia coli* during protein overexpression. J Chin Inst Chem Eng 33:103–112
- 35. Schmidt M, Babu KR, Khanna N, Marten S, Rinas U (1999) Temperature-induced production of recombinant human insulin in high-cell density cultures of recombinant *Escherichia coli*. J Biotechnol 68:71–83

- 36. Schmidt M, Viaplana E, Hoffmann F, Marten S, Villaverde A, Rinas U (1999) Secretion-dependent proteolysis of heterologous protein by recombinant *Escherichia coli* is connected to an increased activity of the energy-generating dissimilatory pathway. Biotechnol Bioeng 66:61–67
- 37. Srivastava R, Peterson MS, Bentley WE (2001) Stochastic kinetic analysis of the *Escherichia coli* stress circuit using  $\sigma^{32}$ targeted antisense. Biotechnol Bioeng 75:120–129
- Storz G, Regine H-A (2000) Bacterial stress response. ASM Press, Wachington, DC
- Thomas JG, Baneyx F (1996) Influence of a global deregulation of the heat-shock response on the expression of heterologous proteins in *Escherichia coli*. Ann N Y Acad Sci 782:478–485
- Thomas JG, Baneyx F (1996) Protein folding in the cytoplasm of *Escherichia coli*: requirements for the DnaK-DnaJ-GrpE and GroEL-GroES molecular chaperone machines. Mol Microbiol 21:1185–1196
- Vlazny D, Hill C (1995) A stationary-phase-dependent viability block governed byt two different polypeptides from the *rhsa* genetic element of *Escherichia coli* K-12. J Bacteriol 177:2209– 2213
- Wegrzyn A, Wegrzyn G, Taylor K (1996) Disassembly of the coliphage l replication complex due to heat shock induction of the groE operon. Virology 217:594–597
- Wild J, Walter WA, Gross CA, Altman E (1993) Accumulation of secretory protein precursors in *Escherichia coli* induces the heat shock response. J Bacteriol 175:3992–3997
   Xu HM, Zhang GY, Ji XD, Cao L, Shu L, Hua ZC (2005)
- 44. Xu HM, Zhang GY, Ji XD, Cao L, Shu L, Hua ZC (2005) Expression of soluble, biologically active recombinant human endostatin in *Escherichia coli*. Protein Expres Purif 41:252–258
- Yabuta M, Onaimiura S, Ohsuye K (1995) Thermo-inducible expression of a recombinant fusion protein by *Escherichia coli lac* repressor mutants. J Biotechnol 39:67–73
- 46. Yamamori T, Ito K, Nakamura Y, Yura T (1978) Transient regulation of protein synthesis in *Escherichia coli* upon shift-up of growth temperature. J Bacteriol 134:1133–1140