Transcriptional stress response of Escherichia coli bacteria to heat shock with micro array technique

Ahmad Nizamedien Barzingi

Department of Chemistry/ Faculty of Education/ University of Garmian/ barzingia@yahoo.de

Abstract

In this study, DNA microarray (DNA Chip) technique has been used in tecqnical chemistry depratment from univeristy of Hannover-Germany. This technique was used to study the gene expression of 96 genes of *E*. coli under two different temperature conditions at (37 °C and 50 °C) for 10 min. Thus, the heat shock response of *E*. coli was observed, that most of genes were up regulated, except only 12 genes were down regulated.

Key words: DNA-microarray. Gene Expression, heat shock response of *E*. coli

رد فعل توتري النسخي من البكتيريا القولونية إلى الصدمة الحرارية مع تقنية (DNA microarray)

احمد نظام الدين برزنجى

قسم الكيمياء/فاكلتى التربية/ جامعة كرميان

الخلاصة

اجريت هذه الدراسة فى احدى مختبرات الكيمياء التكنيكية/ جامعة هانوفر الالمانية باستخدام تقنية حديثة وهي ال (DNA microarray) لدراسة التعبير الجيني (Expression Gene) لبكتريا الاشريشية القولونية (*E.coli*) في درجات حرارة مختلفة, وقد اجريت الدراسه على 96 جين في درجتين حراريتين مختلفتين (37 و 50 مْ) لمدة 10 دقائق , لوحظ نتيجة لصدمة الحرارية, استجابة اغلب الجينات للتنظيم الى الاعلى, ما عدا 12 جينا كانت استجابهم للتنظيم الى الاسفل.

كلمات مفتاحية: التعبير الجيني , أثر الصدمة الحرارية لبكتريا الاشريشية القولونية. DNA-microarray

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Introduction

DNA-Chip(DNA microarray)

DNA chip or DNA microarray are fixed array of single-stranded DNA on the Planner surfaces (surfaces usually derivatives glass, rare silicon surfaces) for the detection of RNA or DNA molecules. Have the spots with immobilized DNA has a diameter about 200 µm so we also speak of micro array [1]. The term array is derived from the English by arrangement, in German, but the array is based on the information technology as a "chip" refers to [2]. To detect DNA fragments with DNA chip, DNA is labeled with a dye-Fluorescent then with the chip is brought into contact. The immobilized DNA on the chip are complementary to the DNA samples, is such a bond between the two complementary. The chip is read out, after a washing process, the unbound DNA molecules are removed. The use of fluorescent-labelled DNA is the detection of binding events with a laser scanner (GMS428) simultaneously the quantification of the signal is obtained.

The application of DNA chip is most gene expression studies. For theses such studies, the RNA from the sample to be examined (eg, cells or tissue) obtained, transcribed into cDNA by reverse transcription and with two different fluorescent dyes selected. The sample under test are displayed on the DNA chip is brought into contact, all hybridized simultaneously on the DNA chip located genetic samples with a capture molecules. This demonstrates the significant advantage of this technology over other methods for studying gene expression.

A DNA chip experiment thus consists of five main steps:

1. the gene probe can be selected and printed on the chip.
2. of cells / tissue, the isolated mRNA.
3. The mRNA is transcribed into cDNA and fluorescently labelled reverse.
4. The fluorescently labelled cDNA is hybridized overnight to the chip.
5. The next day, the chip is washed with washing buffer, scanned, and performed the data analysis.

Materials and Methods

I have the use of materials and a precise description of the performance of the experiment in the script "To assess the amount of stress (heat shock) performed on *E. coli* cells, in technical chemistry department from university of Hannover-Germany. To illustrate, however in Fig.2.1 the main steps are shown. The experiment was carried out with total RNA, the work with the RNA requires certain measures because RNAse requires more stable than any DNAse and cofactors for its activity. That's why it was in the course of this experiment always wore gloves and used sterile plastic goods and pure buffer.

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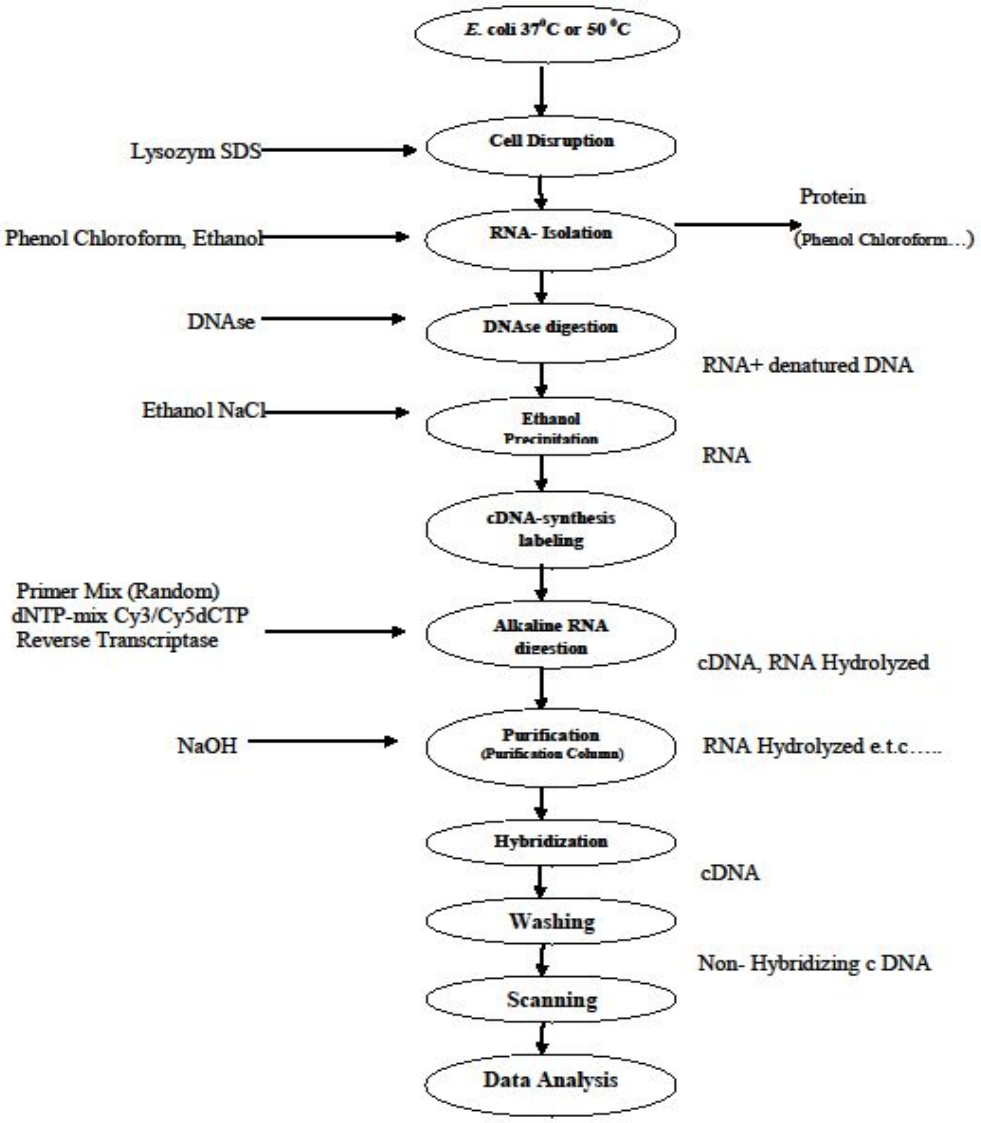

Figure 2.1: Implementation of the DNA micro array experiment.

Preparation of *E. coli* culture and RNA extraction

1- Prepared two *E. coli* cultures, one at 50 °C for 10 min and chased the other remains at 37 °C, both are with multi centrifuge at 4000rpm for 5-8 min and the pellet was placed in freezer.

2- RNA isolated from two *E. coli* cultures with Phenol/chloroform method

3- The concentration and purity of RNA Measured with Micro volume spectrophotometer

Labelling and cDNA synthesis

Direct labeling with the Super Script III or total script OLS

PCR-Purification-Kit

PCR purification kit *(QIAquick® PCR Purification Kit*, Qiagen) is used to design the unbound Cy3, Cy5.

Drying of the sample in the Speed vac

The sample is dried to remove the residual water molecules and does not hybridize helix cDNA.

Washing

The Slides to be washed in three Buffers (Sodium chloride + Sodium citrate) each for 5 min, The hybridized chip in the array scanner Axon 4000B read simultaneously at 532 nm (Cy3) and 635 nm (Cy5)

Data Analysis

The saved TIFF files are loaded into the PC program "ImaGene". Over the spots a raster is then placed, indicating the program area are in which the spots and the background area. In the ImaGene a computer program is integrated, which converts the spots and the background in numerical values.

All genes are significantly regulated with a ratio <-2 and > 2, this result for example are illustrated as a histogram in Figure 3.1. It shows all 96 genes. The Y-axis represents the ratio

value and the x-axis genes can be read, how many genes are accordingly regulated. In this gene regulation almost genes are up regulated and about very few genes (Approx. 12 genes) are down regulated

Result

Table 3.1 shows the results of the DNA chip: the cDNA produced from the mRNA of E. coli was labelled: the cDNA from the heat-treated was labelled with the Fluor chrome (Cy3) strain and the cDNA from the untreated was labelled with (Cy5) Fluor chrome strain. The two have different Fluor chrome excitation and emission wavelengths. Because the genes which shown in the table are fixed on the chip, the labelled cDNA can hybridize with their complementariness and the intensity of fluorescence is measured, the Intensity ratio is a measure of the expression of E. coli under the two culture conditions (50 ° C and 37 ° C).

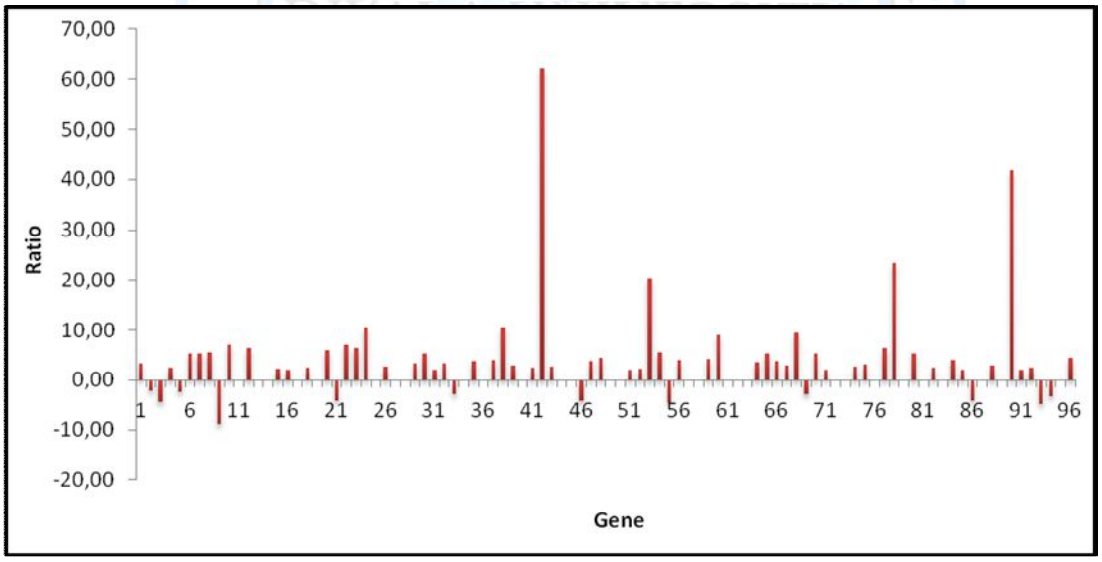

Figure 3.1 Expression profiles of E. coli under thermal stress

A ratio of 0.5 or corresponds to a two-fold under-expression or over-expression of the gene under stressful conditions (heat treatment). To obtain meaningful results, the values were

represented <1 as negative reciprocals. Only absolute values greater than 2 are considered to be representative and discussed in the following:

Table 3.1 Composition of the examined genes and their expression ratio

Nr.	ID Gene	Ratio
1	Chaperone Hsp70; DNA biosynthesis; autoregulated heat shock proteins; dnaK	3,27
2	ATP-binding component of sn-glycerol 3-phosphate transport system; ugpC	-2
3	50S ribosomal subunit protein L10; rplJ	-4,38
4	PTS system, N-acetylglucosamine-specific enzyme IIABC; nagE	2,42
5	probable third cytochrome oxidase, subunit I; appC	-2,24
6	nitrate/nitrite response regulator (sensor NarQ); narP	5,22
7	fermentative D-lactate dehydrogenase, NAD-dependent; ldhA	5,21
8	host factor I for bacteriophage Q beta replication, a growth-related protein; hfq	5,48
9	orf, hypothetical protein; ybgF	-8,9
10	NADH dehydrogenase I chain F; nuoF	6,98
11	tagatose-bisphosphate aldolase 1; gatY	
12	cytochrome o ubiquinol oxidase subunit II; cyoA	6,51
13	putative ligase; yjfG	
14	superoxide dismutase, iron; sodB	
15	50S ribosomal subunit protein L4, regulates expression of S10 operon; rplD	2,31
16	N-acetylglucosamine metabolism; nagD	2,14
17	putative phosphatase; yrfG	
18	GroES, 10 Kd chaperone binds to Hsp60 in pres. Mg-ATP, suppressing its ATPase activity mopB	2,41
19	heat shock protein; ibpB	
20	phage lambda replication; host DNA synthesis; heat shock protein; protein repair; grpE	5,88

21	periplasmic protein involved in the tonb-independent uptake of group A colicins; tolB	-4
22	outer membrane porin protein; locus of qsr prophage; nmpC	7,09
23	membrane-bound ATP synthase, F1 sector, alpha-subunit; atpA	6,48
24	cytochrome d terminal oxidase, polypeptide subunit I; cydA	10,56
25	putative transport ATPase; yhiD	
26	16S pseudouridylate 516 synthase; rsuA	2,77
27	50S ribosomal subunit protein L3; rplC	
28	mechanosensitive channel; mscL	
29	IS186 hypothetical protein; yi81_1	3,28
30	GroEL, chaperone Hsp60, peptide-dependent ATPase, heat shock protein; mopA	5,3
31	heat shock protein; ibpA	2,09
32	cell division protein; ftsJ	3,26
33	2-oxoglutarate dehydrogenase (dihydrolipoyltranssuccinase E2 component); sucB	-2,77
34	methylglyoxal synthase; mgsA	
35	galactitol-specific enzyme IIA of phosphotransferase system; gatA	3,86
36	orf, hypothetical protein; b1824	
37	putative transport system permease protein; yhfT	4,06
38	30S ribosomal subunit protein S16; rpsP	10,47
39	50S ribosomal subunit protein L2; rplB	2,88
40	heat shock protein, chaperone, member of Hsp70 protein family; hscA	
41	orf, hypothetical protein; yhfY	2,43
42	delta(2)-isopentenylpyrophosphate tRNA-adenosine transferase; miaA	62,14
43	heat shock protein, integral membrane protein; htpX	2,71
44	44. chaperone with DnaK; heat shock protein; dnaJ	
45	2-oxoglutarate dehydrogenase (decarboxylase component); sucA	
46	internal control	-4,1
47	fumarate reductase, anaerobic, membrane anchor polypeptide; frdD	3,7

48	membrane-bound ATP synthase, F1 sector, delta-subunit; atpH	4,47
49	orf, hypothetical protein; yhaL	
50	30S ribosomal subunit protein S7, initiates assembly; rpsG	
51	D-ribulose-5-phosphate 3-epimerase; rpe	2,03
52	coproporphyrinogen III oxidase; hemF	2,28
53	putative EC 2.1 enzymes; ycjX	20,38
54	multiple antibiotic resistance; transcriptional activator of defense systems; marA	5,49
55	heat shock protein hslVU, proteasome-related peptidase subunit; hslV	-4,55
56	DNA biosynthesis; DNA primase; dnaG	3,99
57	phosphoenolpyruvate carboxykinase; pckA	
58	PTS enzyme IIAB, mannose-specific; manX	
59	fumarate reductase, anaerobic, membrane anchor polypeptide; frdC	4,31
60	membrane-bound ATP synthase, F1 sector, gamma-subunit; atpG	9,1
61	orf, hypothetical protein; yfjA	
62	50S ribosomal subunit protein L30; rpmD	
63	bifunctional pyrimidine deaminase/reductase in pathway of riboflavin synthesis; ribD	
64	GTP-binding export factor binds to signal sequence, GTP and RNA; ffh	3,61
65	orf, hypothetical protein; yccV	5,29
66	DNA-binding, ATP-dependent protease La; heat shock K-protein; lon	3,76
67	heat shock protein hslVU, ATPase subunit, homologous to chaperones; hslU	3
68	heat shock protein; clpB	9,39
69	NADH dehydrogenase I chain M; nuoM	-2,76
70	isocitrate dehydrogenase, specific for NADP+; icdA	5,4
71	formate dehydrogenase-O, major subunit; fdoG	2,01
72	membrane-bound ATP synthase, F1 sector, beta-subunit; atpD	
73	putative amino acid/amine transport protein; yean	

74	50S ribosomal subunit protein L23; rplW	2,65
75	UDP-D-galactose:(glucosyl)lipopolysaccharide-1,6- D-galactosyltransferase; rfaB	3,19
76	sodium-calcium/proton antiporter; chaA	
77	putative ATP-binding component of a transport system; ybjZ	6,39
78	chaperone Hsp70; DNA biosynthesis; autoregulated heat shock proteins; dnaK	23,42
79	heat shock protein hslJ; hslJ	
80	orf, hypothetical protein; b1541	5,36
81	NADH dehydrogenase I chain L; nuoL	
82	serine hydroxymethyltransferase; glyA	2,53
83	cytochrome o ubiquinol oxidase subunit III; cyoC	
84	pyruvate dehydrogenase (decarboxylase component); aceE	4,1
85	putative oxidoreductase; ydfI	2,06
86	50S ribosomal subunit protein L18; rplR	-4,1
87	NADH dehydrogenase I chain J; nuoJ	
88	orf, hypothetical protein; b3000	3,02
89	putative ATP-binding protein in pho regulon; ybeZ	
90	Arabidopsis Control Oligonucleotide	41,79
91	DNA polymerase III, chi subunit; holC	2
92	arginine 3rd transport system permease protein; artQ	2,54
93	NADH dehydrogenase I chain H; nuoH	-4,77
94	putative tagatose 6-phosphate kinase 1; gatZ	-3,3
95	cytochrome o ubiquinol oxidase subunit I; cyoB	
96	membrane-bound ATP synthase, F1 sector, alpha-subunit; atpA	4,37

Table 3.2 Summary of some over expressing genes of E. coli by heat shock

Genname	Proteinname	Funktion
dnaK	chaperone Hsp70; DNA biosynthesis; autoregulated heat shock proteins; dnaK	Plays an essential role in the initiation of phage lambda DNA replication, where it acts in an ATP-dependent fashion with the dnaJ protein to release lambda O and P proteins from the preprimosomal complex. DnaK is also involved in chromosomal DNA replication, possibly through an analogous interaction with the dnaA protein. Also participates actively in the response to hyperosmotic shock [4]
nagE	PTS system, N-acetylglucosamine-specific enzyme IIABC; nagE	The phosphoenolpyruvate-dependent sugar phosphotransferase system (sugar PTS), a major carbohydrate active -transport system, catalyzes the phosphorylation of incoming sugar substrates concomitantly with their translocation across the cell membrane. This system is involved in N-acetylglucosamine transport. [6]
narP	nitrate/nitrite response regulator (sensor NarQ); narP	This protein activates the expression of the nitrate reductase (narGHJI) and formate dehydrogenase-N (fdnGHI) operons and represses the transcription of the fumarate reductase (frdABCD) operon in response to a nitrate/nitrite induction signal transmitted by either the narX or narQ proteins.[7]
nuoF	NADH dehydrogenase I chain F; nuoF	NDH-1 shuttles electrons from NADH, via FMN and iron-sulfur (Fe-S) centers, to quinones in the respiratory chain. The immediate electron acceptor for the enzyme in this species is believed to be ubiquinone. Couples the redox reaction to proton translocation (for every two electrons transferred, four hydrogen ions are translocated across the cytoplasmic membrane), and thus conserves the redox energy in a proton gradient.
cyoA	cytochrome o ubiquinol oxidase subunit II; cyoA	Cytochrome o terminal oxidase complex is the component of the aerobic respiratory chain of E.coli that predominates when cells are grown at high aeration.[14]
rplD	50S ribosomal subunit protein L4, regulates expression of S10 operon; rplD	One of the primary rRNA binding proteins, this protein initially binds near the 5'-end of the 23S rRNA. It is important during the early stages of 50S assembly. It makes multiple contacts with different domains of the 23S rRNA in the assembled 50S subunit and ribosome. [15]

mopB	GroES, 10 Kd chaperone binds to Hsp60 in pres. Mg-ATP, suppressing its ATPase activity; mopB	Binds to Cpn60 in the presence of Mg-ATP and suppresses the ATPase activity of the latter
atpA	membrane-bound ATP synthase, F1 sector, alpha-subunit; atpA	Produces ATP from ADP in the presence of a proton gradient across the membrane. The alpha chain is a regulatory subunit.
cydA	cytochrome d terminal oxidase, polypeptide subunit I; cydA	Cytochrome d terminal oxidase complex is the component of the aerobic respiratory chain of E.coli that predominates when cells are grown at low aeration.
rsuA	16S pseudouridylate 516 synthase; rsuA	Responsible for synthesis of pseudouridine from uracil-516 in 16S ribosomal RNA[12]
mopA	GroEL, chaperone Hsp60, peptide-dependent ATPase, heat shock protein; mopA	Prevents misfolding and promotes the refolding and proper assembly of unfolded polypeptides generated under stress conditions. Essential for the growth of the bacteria and the assembly of several bacteriophages. Also plays a role in coupling between replication of the F plasmid and cell division of the cell.
ibpA	heat shock protein; ibpA	Associates with aggregated proteins, together with ibpB, to stabilize and protect them from irreversible denaturation and extensive proteolysis during heat shock and oxidative stress. Aggregated proteins bound to the ibpAB complex are more efficiently refolded and reactivated by the ATP-dependent chaperone systems clpB and dnaK/dnaJ/grpE. Its activity is ATP-independent[13]
ftsJ	cell division protein; ftsJ	Specifically methylates the uridine in position 2552 of 23S rRNA in the fully assembled 50S ribosomal subunit
yhfT	putative transport system permease protein; yhfT	Integral Membrane Protein
rpsP	30S ribosomal subunit protein S16; rpsP	In addition to being a ribosomal protein, S16 also has a cation-dependent endonuclease activity

miaA	delta(2)-isopentenylpyrophosphate tRNA-adenosine transferase; miaA	Catalyzes the first step in the biosynthesis of 2-methylthio-N6-(delta(2)-isopentenyl)-adenosine (MS[2]I[6]A) adjacent to the anticodon of several tRNA species. [17]
htpX	heat shock protein, integral membrane protein; htpX	Membrane-localized protease able to endoproteolytically degrade overproduced SecY but not YccA, another membrane protein. It seems to cleave SecY at specific cytoplasmic sites. Does not require ATP. Its natural substrate has not been identified. Probably plays a role in the quality control of integral membrane proteins. [18]

Table 3.3 Summary of some down expressing genes of E. coli with heat stress

Genname	Proteinname	Funktion
ugpC	ATP-binding component of sn-glycerol 3-phosphate transport system;	Part of the ABC transporter complex ugpABCE involved in sn-glycerol-3-phosphate import. Responsible for energy coupling to the transport system. Can also transport glycerophosphoryl diesters. Activated by gluconate, inhibited by fumarate and internal phosphate. Internal phosphate may bind to UgpC and reduce its affinity for UgpA and UgpE. [25]
rplJ	50S ribosomal subunit protein L10	Protein L10 is also a translational repressor protein. It controls the translation of the rplJL-rpoBC operon by binding to its mRNA
appC	probable third cytochrome oxidase, subunit I;	Probable cytochrome oxidase subunit
ybgF	orf, hypothetical protein; ybgF	Uncharacterized protein ybgF precursor
tolB	periplasmic protein involved in the tonb-independent uptake of group A colicins; tolB	Involved in the tonB-independent uptake of group A colicins (colicins A, E1, E2, E3 and K). Necessary for the colicins to reach their respective targets after initial binding to the bacteria

sucB	2-oxoglutarate dehydrogenase (dihydrolipoyltranssuccinase E2 component); sucB	The 2-oxoglutarate dehydrogenase complex catalyzes the overall conversion of 2-oxoglutarate to succinyl-CoA and CO(2). It contains multiple copies of three enzymatic components: 2-oxoglutarate dehydrogenase (E1), dihydrolipoamide succinyltransferase (E2) and lipoamide dehydrogenase (E3).
hslV	heat shock protein hslVU, proteasome-related peptidase subunit; hslV	Protease subunit of a proteasome-like degradation complex
rplR	50S ribosomal subunit protein L18; rplR	This is one of the proteins that mediates the attachment of the 5S rRNA subcomplex onto the large ribosomal subunit where it forms part of the central protuberance. Binds stably to 5S rRNA; increases binding abilities of L5 in a cooperative fashion; both proteins together confer 23S rRNA binding. The 5S rRNA and some of its associated proteins might help stabilize positioning of ribosome-bound tRNAs. [26]
gatZ	D-tagatose-1,6-bisphosphate aldolase subunit GatZ	Component of the tagatose-1,6-bisphosphate aldolase GatYZ that is required for full activity and stability of the Y subunit. Could have a chaperone-like function for the proper and stable folding of GatY. When expressed alone, GatZ does not show any aldolase activity. Is involved in the catabolism of galactitol. [27]

Discussion

Temperature is one of many environmental conditions, which can be measured by bacteria precisely. However, most systems do not register the temperature sensor, but respond to immediate consequences of a change in temperature, for example, to the accumulation of unfolded proteins to a heat shock RNA thermometers, however, are temperature controlled mRNA sensors, measure the thermal signal directly and implement them in gene expression. RNA to form complex structures which control access to the ribosome binding site (RBS). In most cases, the translation will be prevented at low temperatures. Increasing the temperature leads to the melting of the structure, thereby exposing the RBS and translation is enabled. [3] The bacteria detect elevated temperature using a molecular thermometer. The alternative σ factor σ 32, which is encoded by the rpoH gene in the establishment of the heat shock response, plays a central role. The rpoH mRNA is pronounced secondary structure at low temperatures of, in which the initiation code and the ribosome binding site (RBS) are not accessible. Don't bind the ribosome, there is no translation instead. When the heights temperature dissolves secondary structure, the RBS is available for translation, and the content of protein σ 32 increases. The stabilization of the protein σ 32 is especially important in the heat shock. The key is dnak -chaperone that performs two functions; it is involved in the renaturation of the denatured proteins, in the heat shock protein binds denatured dnak together with the DnaJ and GrpE chaperone machinery. dnak is caught in this way, does not bind to σ 32, and can not thus infiltrate into the Proteolysis cycle. This is the case when there is no heat shock. Then you bind to σ 32 and destabilize the protein. σ 32 serves as a substrate of the protease and is degraded FtsH. [28] In this study the gene expression of E. coli was examined by heat shock. It observed that classes of proteins or genes were over expressed by heat. The HSPs (heat shock proteins). These have large homologies in amino acid sequence and function. That are named and classified according to their molecular weight and also be induced by transcriptional activator σ 32. Among the HSPs-70 (70 kDa) and HSPs-90 (90 kDa), for example, include the examined proteins in this study DnaK, DnaJ (70 kDa) and GrpE (90 kDa). HSPs most of either a chaperone function such as (DnaK, DnaJ, GrpE, IbpA,

IbpB) or a proteolytic function (such as HPTx). Chaperones are proteins that help newly synthesized proteins to fold correctly. The name was chosen because they preserve immature proteins from harmful contact. In stressful situations it comes to increased denaturation of proteins. Denatured proteins are a signal for increased formation of chaperone protein, proteolysis also another property of proteins to remove the misfolded proteins. Typically the heat shock response in *E.* coli is of the induction of proteases, its overproduction leads to degradation. HPTx is a protein which has such properties and is especially over expressed in the experiment. The heat shock proteins at 37 ° C less than ribosomal proteins (RPID, RPSG, RPMd, RPIC, RPIB, and RPIW) expressed or proteins that play a role in the respiratory chain (CyoB, CYDA, CyoB, or NuoM) and the generally reflect the normal growth of the cell. The dnaJ, IbpA both heat shock proteins have not been over-expressed in 50 ° C, although having a chaperone function and playing a role in the heat shock response.

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