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A Transcriptional "Scream" Early Response of *E. coli* Prey to Predatory Invasion by *Bdellovibrio*

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Abstract We have transcriptionally profiled the genes differentially expressed in *E. coli* prey cells when predatorily attacked by *Bdellovibrio bacteriovorus* just prior to prey cell killing. This is a brief, approximately 20–25 min period when the prey cell is still alive but contains a *Bdellovibrio* cell in its periplasm or attached to and penetrating its outer membrane. Total RNA was harvested and labelled 15 min after initiating a semi-synchronous infection with an excess of *Bdellovibrio* preying upon *E. coli* and hybridised to a macroarray spotted with all predicted ORFs of *E. coli.* SAM analysis and *t*-tests were performed on the resulting data and 126 *E. coli* genes were found to be significantly differentially regulated by the prey upon attack by *Bdellovibrio*. The results were confirmed by QRT-PCR. Amongst the prey genes upregulated were a variety of general stress response genes, potentially "selfish" genes within or near prophages and transposable elements, and genes responding to damage in the periplasm and osmotic stress. Essentially, the presence of the invading *Bdellovibrio* and the resulting damage to the prey cell elicited a small "transcriptional scream", but seemingly no specific defensive mechanism with which to counter the *Bdellovibrio* attack. This supports other studies which do not find *Bdellovibrio* resistance responses in prey, and bodes well for its use as a "living antibiotic".

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Introduction

Bdellovibrio are small, highly motile, Gram-negative bacteria that act as "living antibiotics" in that they prey upon and kill a wide variety of Gram-negative bacteria; including human and animal pathogens. Despite years of studies in laboratories, there is only one report of resistance to *Bdellovibrio* predation [39] and resistance by most populations is thought to be a plastic (reversible) phenotype by a small minority of a susceptible strain [34] or by a subpopulation that is less susceptible, but not completely resistant to predation [8]. In predation *Bdellovibrio* attach to the outer membrane of prey bacteria and pass through it into the periplasm before they attach to the prey cell cytoplasmic membrane and the prey cell dies after 20–25 min [30, 40]. Here, we investigate the transcriptional response of *E. coli* prey to 15 min of periplasmic invasion by *Bdellovibrio* predators. We find that there is a transcriptional stress response, to the osmotic changes associated with *Bdellovibrio* breaching the outer membrane and the damage caused by having another cell resident in the periplasm, rather than a response specifically to resist *Bdellovibrio* attack. We do not find upregulation of many genes reported to be upregulated in responses to phage attack or ppGpp alarmone systems [17, 37]. That *E. coli* transcriptionally "screams" slightly, but does not resist *Bdellovibrio* predation, bodes well for the potential use of *Bdellovibrio* as a therapeutic agent [35].

Materials and Methods: Culture and Predatory Infection Conditions

E. coli S17-1 and *Bdellovibrio bacteriovorus* 109J were grown as described previously [22]. Semi-synchronous

infections were set up by using centrifugation concentrated *Bdellovibrio*. Control mock-infections of *Bdellovibrio* only and *E. coli* only were carried out concurrently using filtered respective supernatant samples in place of the missing cells. At 15 min post-infection, 4 ml samples of experimental and controls were taken for RNA extraction as described elsewhere [22].

Arrays, cDNA and Hybridisation, Data Acquisition and Analysis

Panorama *E. coli* gene arrays (Sigma Genosys) were used according to the manufacturer's instructions and as described elsewhere [29]. Hybridisation and stringent washes were carried out according to the manufacturer's instructions. Five independent experiments were performed using a total of 4 arrays with array swapping and use of control *E. coli*.

The ArrayVison software was used to analyse the phosphor-images with the template provided by Sigma Genosys for the Panorama arrays. The normalised data were analysed using SAM [36] in a paired test with 1000 permutations and a delta value of 0.86. In order to exclude the possibility that any of the significant genes, called as differentially regulated, were a result of the presence of cross-hybridising *Bdellovibrio* RNA, the pair sample of *E. coli* only control versus *Bdellovibrio* only control were analysed at the lowest possible stringency, i.e. with a delta of 0. This resulted in a false discovery rate of 4.7%. Any genes called in the *Bdellovibrio* control datasets, as well as the experimental infection datasets, were therefore excluded from the analysis. Finally, for further confidence, the data for each significant gene was subjected to a paired *t*-test. Only for 1 gene was the *P* value >0.05 (*minE* $P = 0.051$) showing the robustness of the significant gene lists to different statistical tests. The data discussed are deposited in NCBIs Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) via GEO Series accession number GSE9495.

Quantitative PCR

Quantitative PCR (QPCR) was used for sample genes, to verify the array results using the Statagene Full Velocity SYBR Green QRT-PCR kit as described previously [7] except in two-step reactions, with reverse transcription as described elsewhere [10]. Serial dilutions of the cDNA were used for QPCR with primers designed to specifically anneal to 3 genes that were upregulated in *E. coli* in response to *Bdellovibrio* attack (*treA, cstA* and *creB*) and 3 genes that were not significantly upregulated (*hns, dnaK* and *arcA*). Absolute expression was calculated against a standard curve of a template of extracted PCR product and the ratios of expression in the two samples were compared. At least two independent experiments were carried out and included relevant controls such as no template, and also with *B. bacteriovorus* 109J RNA as a putative "mock" template. A *t*-test was carried out on each set of data to ascertain significance. The PCR products were sequenced to check for the accuracy and specificity of the reactions. Standard semi-quantitative RT-PCR to check for co-transcribed operons was carried out as described previously [22].

Results and Discussion

In order to investigate the response of *E. coli* to *Bdellovibrio* attack, we used the most studied prey and predator strain combination of *E. coli* S17-1 and *Bdellovibrio bacteriovorus* 109J, and macroarrays with all open reading frames of *E. coli* K12 MG1655 spotted. We checked the similarity of the S17-1 and K12 sequences with a Nimblegen tiling array, and discovered that there is a very high level of sequence identity between the strains to allow correct hybridisation between the cDNA from the S17-1 strain tested and the array (data not shown).

Semi-synchronous predatory infections were set up, such that at the 15-min time point, at which the RNA samples for expression studies were taken, greater than 95% of all *E. coli* cells had at least one *Bdellovibrio* cell attached or fully periplasmically invaded, but before prey killing and RNA degradation was extensive. Phase contrast microscopy revealed that 90% of *E. coli* cells had at least one *Bdellovibrio* cell attached to its surface at 15 min. Initial attachment was rapid, within 5 min more than 75% of cells had a *Bdellovibrio* attached. Burnham et al. [4] used a similar semi-synchronous infection process and showed that at 15 min post-mixing, the *Bdellovibrio* cells could not be removed from prey by mechanical agitation and so had started the penetration process; which they monitored by electron microscopy (EM), revealing damage to the prey outer membrane. We have also observed similar invasion by EM at this time point. Thus, very many of the "attached" *Bdellovibrio* would indeed be breaching the outer membrane at the 15-min time point we sampled. This fraction cannot be enumerated by live dead staining as the prey cells are still alive and the extent of the *Bdellovibrio* cell body length that had entered the periplasm of the prey cannot be measured by light microscopy. However, as 5% of the *E. coli* cells at the 15-min time point were rounded, infected bdelloplasts indicating that they had reached the stage of prey cell death, unsuitable for recovery of mRNA it was clear that significant prey penetration had occurred at 15 min and so maximal invasion of prey, with minimal prey cell death was achieved.

Pre-incubation of bacteria in Ca/HEPES buffer for 3 h prior to infection was to stabilise expression (viability was unchanged during this, as assayed by plaque and colony counts) and a portion of the supernatant from each was 0.22 μm filtered for use in the controls. Experiments were carried out 5 times with duplicate spots on the arrays and the data analysed by *t*-test and SAM. Genes found, by both tests, to be differentially regulated in the infected *E. coli* relative to uninfected *E. coli* control from these analyses were considered, excluding any which flagged as significant in the control of *E. coli* compared to *Bdellovibrio*; giving a final dataset of 126 differentially regulated *E. coli* genes in response to predation.

Upregulated Genes

92 genes were found to be significantly upregulated by *E. coli* in response to periplasmic attack by *Bdellovibrio*. It is very important to realise, in comparison to more usual array experiments for bacteria, that all the small upregulated changes we have tabulated (we have not used a twofold cut-off) are significant and that they are small because a predatory *Bdellovibrio* is acting on the periplasm of the *E. coli,* as the prey cell's transcription is measured—i.e. the *E. coli* is not in an optimum physiological state. Hughes et al. [16] demonstrated how transcriptional profiles with levels of upregulation less than 1.5-fold are very meaningful, even in bacteria that are not subject to insults such as predation. Statistical analyses on genes found to be differentially expressed, shown in the results Tables 1 and 2, confirm that the small changes in expression were significant. Quantitative RT-PCR analysis was carried out on three representative upregulated prey genes (with array fold-change values of 2.22, 1.32 and 1.16) of different functional classes; (*treA, cstA* and *creB*) and on three prey genes that were not significantly upregulated (*hns, dnaK* and *arcA*) (Fig. 1) showing the three upregulated genes to be all significantly upregulated when the three control genes were not. Tables 1 and 2 show up- or downregulated genes, their predicted functions and cellular locations and we discuss them by function below.

Wall and Membrane Repair

Products of upregulated genes in this category include genes encoding enzymes potentially involved in synthesis and/or repair of the peptidoglycan, lipids and membrane bound proteins. The gene upregulation is likely to be an attempt by the prey to repair the damage caused by the physical entry of the *Bdellovibrio* via the outer membrane and peptidoglycan layer and the damage by *Bdellovibrio* degradative enzymes acting on both of these and the prey inner membrane. Interestingly, out of a possible 45+ prey flagellar genes only 3 (*fliG, fhiA* and *flgN*) were significantly upregulated. The prey flagellar driving rods pass from the cytoplasmic membrane to periplasm and then via washers in the cell wall and outer membrane, to the external flagellar filament propellers. The entry of the *Bdellovibrio* into the periplasm very likely prises apart the connection of many of the flagella across the periplasm. The 3 upregulated genes are amongst the first in the cascade to build new flagella and *fliG* encodes the essential rotor of the flagellar motor [25]. Thus, the *E. coli* are inducing genes to rebuild flagella and restore motility.

Interestingly there was very little overlap, (4 genes only, *aroA, guaA, purM and pspB*) between the genes that had altered expression due to *Bdellovibrio* attack and those (over 600) reported to be differentially regulated in *E. coli* in repones to chemical assaults on the cell membrane, by isobutanol in the work of Brynildsen and Liao [3]. We do not think the overlap to be significant.

Stress Response Genes

Amongst the prey genes upregulated in response to stress, were those specifically responding to disturbance to the periplasm by entry of the *Bdellovibrio* through the outer membrane, and its consequent osmolarity impacts on the cell, namely *treA* which encodes a periplasmic trehalase that breaks down trehalose in the periplasm to regulate osmolarity [2] and *betA* which encodes a protein of the choline–glycine betaine pathway involved in compatible osmolyte production [21]. At this point of the *Bdellovibrio* infection the *E. coli* prey cell is still alive and has an electrochemical proton gradient and is trying to restabilise its internal osmolarity after periplasmic invasion.

In general, it appears that prey stress responses to damaged proteins and disturbed periplasm and outer membrane are being elicited. As expected because the *Bdellovibrio,* at the 15-min infection stage, will not have yet secreted DNases into the prey [32]; no transcriptional response to DNA damage was detected as none of the classical prey SOS response genes were found to be upregulated although *yafO*, a toxin of a toxin–antitoxin system, may be indirectly involved in the SOS response [28]. No ppGpp alarmone type responses [37] were detected, probably as our sampling was early, but also it should be remembered that in *Bdellovibrio* predation, prey cell death is early, preceding the possibility of amino acid starvation. Many of the upregulated prey genes are predicted to encode parts of stress response systems including genes involved in responding to carbon starvation (*cstA*); [27], toxic stress caused by paraquat (*pqiB*); [19], potassium tellurite (*tehA*); [38] and pH (*yadF*); [12] as well as a number of genes involved in several different global

Table 1 Significantly up-regulated *E. coli* genes at 15 minutes of *Bdellovibrio* predation

Gene	Putative function	Predicted location	Fold-upregulated	*P* value from *t*-test
Stress response				
cstA	Carbon starvation induced protein	Inner membrane	2.22	0.019816
pqiB	Paraquat inducible protein	Unknown	2.01	0.001174
tehA	Multiresistance efflux pump	Inner membrane	1.46	0.009733
yadF	Putative carbonic anhydrase—possible pH regulation	Cytoplasmic	1.43	0.042138
treA	Trehalase, periplasmic—osmoregulation	Periplasmic	1.32	0.000179
yafO	Toxin of the yafO-yafN toxin–antitoxin system	Cytoplasmic	1.31	0.000402
sufI	Inhibits ftsI and hence septation	Periplasmic	1.28	0.029411
mazG	Regulator involved in amino acid starvation response	Cytoplasmic	1.22	0.038161
ycfQ	Predicted tetR-family response regulator	Cytoplasmic	1.21	0.03176
galR	*lacI*-type transcriptional regulator	Cytoplasmic	1.19	0.033147
betA	Choline dehydrogenase—osmoregulation	Cytoplasmic	1.18	0.019939
glnG	Nitrogen response regulator	Cytoplasmic	1.18	0.030032
iscR	DNA-binding transcriptional repressor	Unknown	1.17	0.019957
creB	Global response regulator	Cytoplasmic	1.16	0.043154
csiE	Global response regulator	Cytoplasmic	1.16	0.005442
Putative wall or membrane repair				
ybaL	Na/H exchanger	Inner membrane	4.41	0.00837
gltJ	Glutamate and aspartate transporter subunit	Inner membrane	1.82	0.042358
phnD	Phosphonate transporter, periplasmic domain	Periplasmic	1.74	0.044892
ydcV	Predicted spermidine/putrescine transporter subunit	Inner membrane	1.70	0.039148
frdA	Fumarate reductase catalytic and NAD/flavoprotein subunit	Periplasmic	1.62	0.02433
yqaA	Putative inner membrane protein	Inner membrane	1.55	0.045115
mdlA	ABC transporter	Inner membrane	1.48	0.004624
kefF	Potassium efflux	Cytoplasm	1.45	0.027868
yjjP	Predicted inner membrane protein	Inner membrane	1.41	0.004595
ydcT	Predicted spermidine/putrescine transporter subunit	Unknown	1.41	0.030283
yihO	Predicted transporter	Inner membrane	1.40	0.032868
flgN	Export chaperone for FlgK and FlgL	Flagellar basal body	1.36	0.049212
yaiW	Putative lipoprotein	Non-cytoplasmic	1.30	0.013018
fliG	Flagellar basal body protein	Flagellar basal body	1.29	0.032728
livJ	Leucine/isoleucine/valine transporter subunit	Periplasmic	1.26	0.021585
plsC	Phospholipid biosynthesis	Unknown	1.24	0.042348
tsr	Methyl-accepting chemotaxis protein	Inner membrane	1.23	0.033582
tnaB	Tryptophan/tyrosine permease family	Inner membrane	1.23	0.01368
cdsA	Cytidylyltransferase	Inner membrane	1.21	0.001263
ygfQ	Permease	Inner membrane	1.19	0.020053
hyfB	Hydrogenase 4	Inner membrane	1.19	0.024532
nrfE	Cytochrome C assembly protein	Inner membrane	1.19	0.002099
hofQ	Predicted fimbrial transporter	Outer membrane	1.18	0.024062
hyfF	Hydrogenase 4	Inner membrane	1.17	0.010739
yaaH	Predicted inner membrane protein	Inner membrane	1.16	0.035842
ybjL	Permease	Inner membrane	1.15	0.02009
ampG	Muropeptide transporter	Inner membrane	1.15	0.042636
fhiA	Flagellar basal body protein	Flagellar basal body	1.12	0.043355
Putative periplasmic content repair				
ykgF	Putative electron transport protein	Cytoplasmic	2.28	0.02889
hypA	Hydrogenase nickel insertion protein	Cytoplasmic	2.09	0.044557

Table 1 continued

Gene	Putative function	Predicted location	Fold-upregulated	*P* value from *t*-test
hybA	Hydrogenase 2 4Fe-4S ferredoxin-type component	Periplasmic	1.85	0.001521
hycA	Formate hydrogenlyase regulatory protein	Unknown	1.62	0.025271
hypF	Hydrogenase maturation protein	Unknown	1.58	0.048483
hypB	Hydrogenase isoenzyme nickel incorporation protein	Cytoplasmic	1.54	0.011291
hycH	Hydrogenase maturation protein	Cytoplasmic	1.42	0.030986
ycjS	Putative oxidoreductase	Unknown	1.33	0.003842
glmU	Peptidoglycan biosynthesis; lipopolysaccharide biosynthesis	Cytoplasmic	1.28	0.021109
hycI	Hydrogenase maturation protease	Cytoplasmic	1.23	0.002523
fhlA	Formate hydrogenlyase transcriptional activator	Cytoplasmic	1.22	0.001627
Putative phage or transposon genes				
ykfC	Reverse transcriptase	Unknown	1.40	0.000281
insL	IS186/IS421 transposase	Cytoplasmic	1.31	0.02964
insD	IS2 insertion element transposase InsAB′	Cytoplasmic	1.20	0.005684
Biosynthesis/metabolism				
ansA	Asparaginase	Cytoplasmic	1.82	0.010869
metJ	Methionine repressor	Cytoplasmic	1.82	0.015532
malQ	4-alpha-glucanotransferase	Cytoplasmic	1.68	0.00488
aidB	Acetyl CoA dehydrogenase	Unknown	1.60	0.004193
hemC	Hydroxymethylbilane synthase	Unknown	1.42	0.040394
paaA	Catabolism of phenylacetic acid	Cytoplasmic	1.41	0.005881
paaH	3-Hydroxybutyryl Co-A dehydrogenase	Inner membrane	1.38	0.001737
yeiT	Pyridine nucleotide-disulphide oxidoreductase	Cytoplasmic	1.37	0.000155
tktA	Transketolase	Cytoplasmic	1.31	0.019749
dfp	Flavoprotein	Unknown	1.28	0.001176
ilvY	DNA-binding transcriptional dual regulator	Cytoplasmic	1.24	0.016897
aroA	5-Enolpyruvylshikimate-3-phosphate synthetase	Unknown	1.23	0.019383
fadA	Fatty acid oxidation	Cytoplasmic	1.23	0.02383
dxr	1-Deoxy-D-xylulose 5-phosphate reductoisomerase	Unknown	1.23	7E-05
hemB	Porphobilinogen synthase	Cytoplasmic	1.21	0.017421
guaA	GMP synthetase	Cytoplasm	1.20	0.036066
abgA	Aminobenzoyl-glutamate utilisation protein	Unknown	1.18	0.028809
rfaG	Glucosyltransferase I	Cytoplasmic	1.16	0.021738
deoD	Purine-nucleoside phosphorylase	Cytoplasmic	1.15	0.021485
ugd	UDP-glucose 6-dehydrogenase	Cytoplasm	1.13	0.031243
purT	Phosphoribosylglycinamide formyltransferase 2	Cytoplasmic	1.13	0.025227
Others				
minE	Cell division topological specificity factor	Cytoplasm	2.38	0.05137
b2973	Unknown function	Unknown	1.16	0.035317
rph	RNase PH exoribonuclease	Unknown	1.61	0.003009
ynjH	Unknown function	Unknown	1.53	0.036073
rtn	Resistance to phage N—this is an EAL domain protein	Inner membrane	1.44	0.03824
yhfV	Phosphotriesterase	Cytoplasmic	1.26	0.014469
yejH	Type III restriction endonuclease and helicase	Cytoplasm	1.21	0.046185
b2345	Unknown function	Unknown	1.18	0.039688
yahG	Conserved protein of unknown function	Inner membrane	1.18	0.033265
yoaF	Unknown function	Unknown	1.18	0.037459
ybiU	Conserved protein of unknown function	Cytoplasm	1.17	0.024414
dnaA	Chromosome replication initiation	Cytoplasmic	1.17	0.004566

Table 1 continued

Gene	Putative function	Predicted location	Fold-upregulated	*P* value from *t*-test
clpX	Protease	Cytoplasm	1.15	0.011204
holA	DNA polymerase III, delta subunit	Unknown	1.13	0.00926

Table 2 Significantly down-regulated *E. coli* genes at 15 minutes of *Bdellovibrio* predation

Gene	Putative function	Predicted location	Fold-downregulated	*P* value from *t*-test
rdgB	dITP/XTP pyrophosphatase	Unknown	0.89	0.001542
msrA	Methionine sulfoxide reductase A	Unknown	0.88	0.005384
lsrB	AI2 transporter	Periplasmic	0.87	0.004696
dusC	tRNA-dihydrouridine synthase C	Cytoplasmic	0.86	0.002917
ydfH	Predicted DNA-binding transcriptional regulator	Cytoplasmic	0.86	0.001975
sdaC	Predicted serine transporter	Inner membrane	0.86	0.003618
yieK	Predicted 6-phosphogluconolactonase	Unknown	0.85	0.003358
yfiD	Pyruvate formate lyase subunit	Cytoplasmic	0.85	0.005335
pepE	Aspartyl-dipeptidase	Cytoplasmic	0.85	4.38E-06
malY	Bifunctional beta-cystathionase, PLP-dependent/regulator of maltose regulon	Unknown	0.84	0.007903
hpf	Hibernation promoting factor	Cytoplasmic	0.84	0.000864
insG	KpLE2 phage-like element; IS4 predicted transposase	Unknown	0.84	0.004375
yhdZ	Predicted amino acid transporter subunit	Cytoplasmic/inner membrane	0.84	0.004034
puub	Gamma-Glu-putrescine oxidase, FAD/NAD(P)-binding	Cytoplasmic	0.82	0.005585
yjdA	Conserved protein with nucleoside triphosphate hydrolase domain	Cytoplasmic	0.81	0.002824
cmoB	Predicted S-adenosyl-L-methionine-dependent methyltransferase	Cytoplasmic	0.80	5.69E-05
ilvH	Acetolactate synthase III, thiamine-dependent, small subunit	Cytoplasmic	0.80	0.002524
katE	Hydroperoxidase HPII(III)	Cytoplasmic	0.79	0.003291
sacY	Preprotein translocase membrane subunit	Inner membrane	0.78	0.007958
talB	Transaldolase B	Unknown	0.78	0.003563
yfcQ	Predicted fimbrial-like adhesin protein	Unknown	0.77	0.004262
glpG	Predicted intramembrane serine protease	Inner membrane	0.76	0.003553
ygcE	Predicted kinase	Unknown	0.74	0.000554
yebS	Conserved inner membrane protein	Inner membrane	0.74	0.001472
pspB	Phage shock protein B	Cytoplasmic/inner membrane	0.74	0.000354
pfkA	6-Phosphofructokinase I	Cytoplasmic	0.74	0.000155
surA	Peptidyl-prolyl *cis–trans* isomerase	Periplasmic	0.71	0.00257
secG	Preprotein translocase membrane subunit	Inner membrane	0.70	0.011531
yfhL	Predicted 4Fe-4S cluster-containing protein	Cytoplasmic	0.69	0.01222
nrdR	Transcriptional repressor of ribonucleotide reductase genes	Cytoplasmic	0.69	0.010921
rutA	Predicted monooxygenase	Unknown	0.66	0.003513
srmB	ATP-dependent RNA helicase	Cytoplasmic	0.64	0.00445
tolB	Periplasmic protein	Periplasmic	0.63	0.004886
purM	Phosphoribosylaminoimidazole synthetase	Unknown	0.51	0.010088

responses to stress (*creB*; [14], *sufI*; [33] *csiE*; [26] *yafO*; [28] *iscR*; [44] *glnG*; [43] *mazG*; [23]). In some cases, such as for *pqiB*; [19], *tehA*; [38] and *yadF*; [12] the single gene products may have a function alone without other co-transcribed genes in their operon being expressed.

"Selfish DNA" Phage or Transposon Genes

A number of chromosomally integrated phage and transposon genes were transcribed by *E. coli* in response to *Bdellovibrio* challenge of prey. One, *ykfC* is predicted to

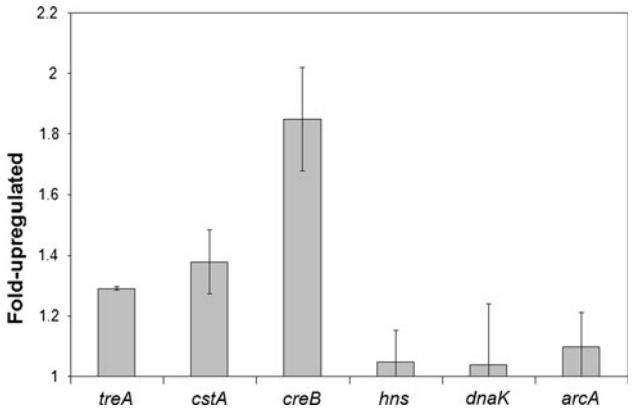

Fig. 1 QRT-PCR results shown as fold increase in expression in the test infection sample compared to the *E. coli*-only control. *treA, cstA* and *creB* are all significantly upregulated whilst *hns, dnaK* and *arcA* are not significantly upregulated (or downregulated). These results agree with the array data and *t*-test on the QRT-PCR data showed that it was significant for the former, but not the latter genes. *Error bars* represent one standard deviation above and below the mean

encode a reverse transcriptase of the CP4-6 prophage and is also near IS elements [9], *insD* is an IS2 insertion element transposase [31] and *insL* is an IS186/IS421 transposase [6]. Induction of phage and transposable elements has been demonstrated in a number of studies of stressing bacteria [13, 20, 29, 41] and it is thought that it is a generic stress response whereby the transposable elements are in some way, detecting adverse conditions and are attempting to "jump ship". Not all phage and transposon genes were upregulated; this has been seen before [13] and probably represents the fact that many prophage genes are no longer functional in the *E. coli* genome.

Biosynthesis and Housekeeping in the Periplasm

A significant number of genes encoding apparently housekeeping functions of prey were also upregulated upon attack by *Bdellovibrio*. 39 of these are predicted to encode periplasmic or membrane bound products, or those involved in production of periplasmic proteins (see Table 1) and so are likely induced to repair the damage caused to the prey by *Bdellovibrio*, by regenerating the membrane and periplasmic contents. The genes *fhlA, hycA, hycH, hycI, hypA, hypB* and *hypF* were all called as upregulated whilst the *hypCDEFG* and *hycBCD* genes within the same apparent operons were not called, but were just below the chosen significance threshold. It is likely therefore that both of these operons were synchronously upregulated in order that all of the gene products may act together, probably in an "attempt" to produce different hydrogenases in response to changing pH levels within the prey periplasm as a result of the invading *Bdellovibrio* [1].

Interestingly, the *rtn* gene, annotated for resistance to phage N [11], was upregulated in our study. That this gene renders *E. coli* resistant to phage in experiments exposing *E. coli* to phage [5] suggests that the prey cell may be detecting damage caused by infection and attempting to respond, however, the response which could be effective against phage (the details of which are as yet unknown in the literature) has no preventative effect on the invading *Bdellovibrio*. Interestingly, other genes that were reported to be upregulated upon phage infection were not upregulated in our study showing that there are different responses to phage and to *Bdellovibrio* infections [18]. Genes reported to be initially upregulated by phage infection are *nagE* and *dhaK,* involved in initial steps of cellular energy generation, with later genes such as *rraA* and *rof* involved in transcription processes and so these are likely useful for phage processing that is not necessary for *Bdellovibrio*. Particularly significant is the fact that the phage shock proteins are not upregulated by *Bdellovibrio* invasion [17, 24]. The phage shock protein (Psp) F regulon response in *E. coli* is thought to be induced by impaired inner membrane integrity and an associated decrease in proton motive force, although the mechanisms by which the Psp system detects the stress signal and responds have so far remained undetermined. PspA and PspG respond to a variety of inducing stimuli by switching the cell to anaerobic respiration and fermentation and by downregulating motility, thereby subtly adjusting and maintaining energy usage and proton motive force. It is surprising given the apparent response to periplasmic damage upon *Bdellovibrio* infection that the Psp system is not induced, but perhaps the damage induced by *Bdellovibrio* is too little at this point for a transcriptional response by the phage shock system, as after 15 min of infection there is little interference of *Bdellovibrio* with the prey inner cytoplasmic membrane (and thus the prey cells are still viable and transcriptionally active as intended and required by our experimental design). Intriguingly, *pspB*, an activator of the Psp regulon [42] appears to be downregulated (Table 2), giving rise to speculation that the *Bdellovibrio* may be actively repressing the Psp regulon by some unknown interference mechanism to prevent restoration of the prey proton-motive-force which is shortly deliberately disrupted by the *Bdellovibrio* as the infection proceeds [30].

Downregulated Genes

34 prey genes were significantly downregulated upon predation by *Bdellovibrio* and in most cases they do not seem to be of any obvious use to the prey cell. In some cases, the downregulated genes are repressors and so their downregulation could result in de-repression of operons. One such example is *malY,* which is a repressor of the *mal*

operon and so its downregulation could result in the upregulation of the mal operon [45]. Another is phage shock regulator *pspB,* mentioned above; where it is interesting to speculate as to whether the invading predator has somehow repressed expression of a potentially repairing response of the prey to aid in quick killing.

In conclusion, periplasmic invasion of prey by *Bdellovibrio* elicits transcriptional responses by the *E. coli* prey cell which seem to be an attempt to repair the physical damage to the periplasm caused by the presence of a *Bdellovibrio* penetration and to stabilise the osmolarity. It seems unlikely that the products of the genes transcribed would help the cell to defend itself against the *Bdellovibrio* attack; rather that it is a transcriptional "scream" responding to damage caused by the *Bdellovibrio*. This transcriptional response is short-lived as the *Bdellovibrio* go onto kill the prey in the subsequent 10 min after our RNA sampling. That we have been able to profile the response to *Bdellovibrio* binding and invasion by *E. coli*, a susceptible prey bacterium, may allow future gene expression comparisons to non-susceptible prey, to confirm the long held hypothesis that prey range in *Bdellovibrio* is determined by the ability to bind productively to prey outer membrane surfaces rather than being a result of the presence or absence of prey defensive gene expression as is the case for defence against phage [15].

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