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Profiling of *Brevibacillus borstelensis* transcriptome exposed to high temperature shock

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ABSTRACT

To understand the molecular mechanisms underlying the ability of the bacteria to survive at high temperature, gene expression profile of *Brevibacillus borstelensis* at 55 °C during 5 and 10 min heat shock period was carried out by high-throughput sequencing technology. A total of 2555 non-redundant transcripts were annotated. A total of 575 genes at 5 min and 400 genes at 10 min exhibited significant differential expression in response to temperature upshift from 50 to 55 °C. Genes up-regulated under heat shock were associated with metabolism (*mtnE*), membrane transport, signal transduction, transcriptional regulation (*ycxD*, *codY*) and folding and sorting (*hsp90*). A larger number of genes encoding hypothetical proteins were identified. RT-PCR experimental results carried out on genes expressed under heat shock were found to be consistent with transcriptome data. The results enhance our understanding of adaptation strategy of thermophilic bacteria thereby providing a strong background for in depth research in thermophiles.

1. Introduction

Bacteria have developed complex mechanisms to adapt to high temperature conditions by altering gene expression dynamically [1]. The biggest threat to the cell on exposure to high temperature is protein denaturation which results in the synthesis of the subset of proteins that plays a crucial role in dealing with the stress factor to allow survival. These proteins generally include the molecular chaperones and proteases which maintain protein quality required for normal growth of cells under stress conditions [2]. Besides, heat stress induces changes in the levels of various metabolites such as organic acids, amino acids, and carbohydrates, involved in different pathways.

Till date, the molecular mechanisms underlying the heat shock responses have been investigated in many mesophilic bacterial species, such as *Campylobacter jejuni* [3], *Shewanella oneidensis* [4] and *Listeria monocytogenes* [5]. Even though versatility for adaptation and catalytic metabolism in a wide variety of environmental niches has been studied in the case of thermophilic *Rhodothermus obamensis* [6] and *Geobacillus* sp. NTU 03 under heat stress [7], yet until now heat shock responses in the thermophilic bacteria has not been deciphered specifically at transcriptional level.

Brevibacillus borstelensis is a Gram positive, rod shaped thermophilic bacteria that can grow in a wide range of temperature from 20 °C to 65 °C with the capacity to degrade low-density polythene. The ability of the bacteria to grow under extreme condition makes *B. borstelensis*, a potential candidate for polythene degradation in different environments. Degradation of low-density polythene has become a major challenge particularly in high temperature areas. Hence, in order to understand the adaptability of the bacteria to high temperature conditions, we have studied the heat shock response of thermophilic *B. borstelensis* by analyzing the transcriptome of the bacteria applying short temperature upshift.

Previously, in order to characterize the complete transcriptome of bacteria in response to different growth conditions and environmental stresses [8,9] DNA microarrays were used but in the recent past, high throughput RNA-Sequencing has emerged to be the most promising technology in studying bacterial transcriptomes by quantification and identification of differential gene expression [10].

In the present study, we executed transcriptome profiling of *B. borstelensis* exposed to high temperature shock and identified the differentially expressed genes. The aim of this experiment was to determine global transcriptional response of *B. borstelensis* to heat shock at two different time points. The differentially expressed genes identified in *B. borstelensis* through RNA-Seq technology in this study will further enhance our understanding of the heat shock responses in thermophilic bacteria.

Abbreviations: cDNA, complementary DNA; BLAST, Basic Local Alignment Search Tool; NR, non-redundant; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; GIP, genetic information processing; EIP, environmental information processing; °C, degree centigrade; mRNA, messenger ribonucleic acid; DGE, differential gene expression; HSP, heat shock protein.

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2. Results

2.1. RNA-Seq and assembly

There were 5,435,133, 3,463,212, and 26,144,334 raw reads generated over 5 and 10 min heat shock period and control samples respectively (Supplementary Table 1). The sequencing data was submitted to short read archive (SRA) under the accession numbers SRR900788 and SRR900789. After quality filtration of short (<100 bp in length) and partially overlapping sequences, a total of 2555 non-redundant annotated transcripts were used as a basis for searching of heat shock relevant genes by differential gene expression (DGE).

2.2. Functional annotation of non-redundant transcripts

The non-redundant transcripts were annotated against the non-redundant database (E-value ≤ 1e−06) of NCBI by BLASTX in order to identify the important biological functions. The potential involvement of transcripts in the functional pathways of *B. borstelensis* was identified by KEGG analysis. A number of unique sequences were assigned with KO enzyme codes (Table 1) and the correspondence between the unique sequences IDs and KO enzyme codes is listed (Supplementary Table 2). Among KEGG annotated sequences, 74.41% were classified under metabolism, 11.5% under genetic information processing, 10.1% under environmental information processing and 3.99% under cellular processes. Further, 2007 transcripts were classified under 20 COG categories (Fig. 1), out of which 'amino acid metabolism and transport' (319 members) represented the largest group followed by 'secondary structure' (224 members), 'transcription' (196 members) and 'carbohydrate metabolism and transport' (144 members). The categories 'cell cycle control' (16 members) and 'signal transduction' (9 members) represented the smallest groups. Gene ontology analysis was carried out to find GO terms for the functionally annotated transcripts (Fig. 2). Out of the total nr-annotated transcripts, 1796 were assigned to biological processes, 1693 were assigned to molecular functions and 748 were assigned to cellular components. The highly represented GO categories were 'catalytic activity', 'metabolic process', 'cellular process', and 'binding'.

Table 1
Details of enzymes found in KEGG pathway database from *B. borstelensis* transcriptome library.

KEGG categories represented	Number of KO	Unique sequences
Metabolism		
Carbon metabolism	81	93
Carbohydrate metabolism	60	64
Energy metabolism	50	54
Lipid metabolism	23	29
Nucleotide metabolism	34	37
Amino acid metabolism	89	100
Metabolism of other amino acids	22	24
Glycan biosynthesis and metabolism	6	9
Metabolism of cofactors and vitamins	55	58
Metabolism of terpenoids and polyketides	8	8
Biosynthesis of other secondary metabolites	6	6
Xenobiotics biodegradation and metabolism	10	10
Genetic information processing		
Transcription	2	2
Translation	24	25
Folding, sorting and degradation	13	16
Replication and repair	28	32
Environmental information processing		
Membrane transport	31	39
Signal transduction	37	47
Cellular processes		
Transport and catabolism	5	6
Cell motility	13	20
Cell cycle	5	5

2.3. Gene expression profiling

Variations in the gene expression profile between control and treated samples were analyzed by NOISeq R package. The RPKM values for control and treated samples following heat shock at 5 (Fig. 3) and 10 min (Fig. 4) intervals are plotted in the expression data graph. Differentially expressed genes were identified based on the NOISeq score data. Considering the applied criteria (q ≥ 0.9), we identified 575 and 400 significant DEGs, out of which, 564 (98.09%) and 358 (89.5%) DEGs were up-regulated and 11 (1.91%) and 42 (10.5%) DEGs were down-regulated at 5 and 10 min respectively. Comparatively larger numbers of up-regulated genes were observed at both the time points suggesting that the expression of these genes have been induced as an adaption to high temperature stress.

The DEGs were mapped to different terms in GO database for determining specific functions. Genes with altered expression as a result of heat shock were largely found to be associated with metabolic process, membrane transport, signal transduction and stress response (Table 2). Similarly, the differentially expressed genes mapped to KEGG pathways were found involved in carbon metabolism, amino acid metabolism, energy and lipid metabolism, signal transduction, folding and sorting (Supplementary Table 3). Approximately, 38% of the transcripts of *B. borstelensis* encoding hypothetical proteins were annotated out of which only 13% and 7% transcripts exhibited significant differential expression, and comparatively greater number of transcripts (12.9% and 5.8%) were significantly up-regulated in response to heat shock at 5 and 10 min respectively.

2.4. Major pathways associated with DEGs in response to heat shock

To understand the functional role of the transcripts involved in the heat shock response of *B. borstelensis* subjected to two different time points of heat shock, we evaluated the significant DEGs associated with the major processes and pathways. The stress responsive genes involved in folding and proteolysis *viz.*, heat shock protein 90 (*hsp90*), oligoendopeptidase, aminopeptidase *ampS* and extracellular protease displayed significant up-regulation. Besides, most of the genes expressed under heat shock at both the time points were associated with different metabolic activities. The genes associated with carbon (2-isopropylmalate synthase), carbohydrate (glycosyl transferase) and amino acid metabolisms (N-methyltryptophan oxidase, methylthioribose kinase, aminotransferase *mtnE*) were significantly up-regulated. The gene encoding glycine dehydrogenase commonly involved in carbon and amino acid metabolisms as well as acetyl-CoA synthetase associated with carbon and methane metabolisms exhibited significant up-regulation in response to heat shock at 5 and 10 min. Similarly, the genes involved in sulfur (phosphoadenosine phosphosulfate reductase, sulfate adenylyltransferase), nitrogen (nitropropane dioxygenase) and lipid metabolisms (gamma-glutamyltranspeptidase) were significantly up-regulated. Further, significant up-regulation was also observed for NADPH dehydrogenase I, N-methyltryptophan oxidase, acetyl-CoA synthetase and nitropropane dioxygenase that participated in the oxidation–reduction processes of the cell. Other genes involved in the metabolic processes of the bacteria *viz.*, mannose-1-phosphate guanyltransferase, propionyl-CoA carboxylase, 5-methyltetrahydrofolate–homocysteine methyltransferase, amidohydrolase and cysteine desulfurase were significantly up-regulated.

Significant up-regulation was observed for transcriptional regulators (*ycxD* and *codY*), DNA-binding response regulators and sporulation proteins (Stage IV and V sporulation protein) under heat shock. The genes associated with membrane transport (ABC transporter substrate-binding protein) and signal transduction (two-component response regulator) was also up-regulated. On the other hand, methyl-accepting chemotaxis protein was found to be down-regulated in response to heat shock at both the time intervals. Clearly, this data indicates that the heat shock response of *B. borstelensis* is largely affected

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