



Stress Responses Within *Bacillus* Species Under Heat Shock

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Abstract

Bacterial homeostasis under heat stress has widely been observed especially in *Escherichia coli* cells. However, the survival potential of *Bacillus* spp. under heat shock is known to some extent; while the mechanism of cellular protection is still obscure. Current micro-review briefly stated such stress responsive events in *Bacillus* spp. The bacterium is known to possess 6 classes of heat shock proteins (HSPs), of which HrcA, the first gene product of the *dnaK* operon, and GroE chaperon of the class I category are presumed as the cellular thermometer of homeostasis. Class II category is regulated by the alternative sigma factor σ^B , transcribing *rsbV*, *rsbW*, and *rsbX* genes at elevated temperatures. The RsbR protein of the σ^B regulon has been noticed to sense the elevated temperature with the concomitant activation of the sporulation specific sigma factor σ^F . Class III heat shock genes are known to be responsible for DNA binding and heat sensing. The heat shock gene *htpG*, although not involved in thermal resistance, is categorized into class IV. Two heat shock genes *htrA* and *htrB* are categorized into class V, which functions as the stress regulator and sensor, respectively. The class VI heat shock genes have not been functionally characterized yet.

Keywords: Heat stress; *Bacillus* spp; heat shock proteins (HSPs); sigma B (σ^B); sigma B (σ^F)

Introduction

A wide range of bacteria are known to activate different transcriptional regulatory network (TRN) at the stationary phase, improving the potential of stress defensive response [1-6] against an array of physicochemical stress transmitting stimuli including temperature, pressure, irradiation, pH, osmolite/salt concentration, elevated level of reactive oxygen species (ROS), imbalance in redox-state and the toxic chemicals [7-9]. Different types of multi-genes systems have been reported to be either up-regulated or down-regulated in response to a variety of stress stimuli [6,10-14]. Under specified stressed conditions, a portion of the cells of *Esche-*

richia coli, the model bacterium for the study of stress response, have often been found to lose their ability to grow on standard nutrient agar plates at the early or mid stationary phase [15-18]. Such cells may be dead, or in the viable but non-culturable (VBNC) state [9,19-21]. The remaining bacterial population are suggestively known to evolve protective responses employing the chaperon proteins like the heat shock proteins (HSPs) with the concomitant up-regulation of σ^S and σ^E regulon genes against stresses like nutrient depletion, heat shock or the elevated ROS [14,18,22-25].

A nearly similar fashion of defense has also been noticed in *Pseudomonas* spp. [26-28], *Salmonella* spp. [10,29,30], and in *Vibrio* spp. [19,31,32]. Apart from these bacterial species, the survival ability of *Bacillus* spp. reveals an artifice expedient to survive upon high temperature [12,33-36]. However, there is no ascertaining indication of a master regulator in *Bacillus* cells upon heat shock so far. Varieties of *Bacillus* spp. are recognized to be industrially important and have been studied extensively for more than 50 years [11,37-40] and unraveled that the environmental stress stimulon of *Bacillus* species is so far the most complicated ever described in bacteria, which is regulated by 10 sigma factors based on their cellular state (vegetative and spore forms) [11,38,41]. To portray the role of the sigma factors of *Bacillus* species in 1995, Haldenwang [11] classified all the identified sigma factors (σ^A , σ^B , σ^C , σ^D , σ^E , σ^F , σ^G , σ^H , σ^K and σ^L) into 2 categories within two cellular states as illustrated in Figure 1. However, extensive research on the stress responsive events in *Bacillus* cells compared to that of *E. coli* have been found to be paused since 2003 [38,41].

psychrophiles (grow at 0°C but optimum is about 15°C) [42,43], Psychrotrophs (grow at 0°C also but optimum is 20 to 30°C) [43], mesophiles (grow best around 37°C) [43], thermophiles (optimum growth at around 60°C) [43] and hyperthermophiles (optimum growth at around 80°C or higher) [43]. *Escherichia coli* (mesophiles) is usually found to grow over a range of 24.3°C to 44°C; however, the optimal growth temperature has been recorded at 37°C [15,44]. Besides, *Pseudomonas* spp., *Salmonella* spp. and *Staphylococcus* spp. have been found to grow over a range of 30°C to 40°C, 6°C to 46°C and 25°C to 40°C, consecutively; but all of them grow best at 37°C, and hence all the bacterial species may be categorized into mesophiles group [45,46]. Apart from these bacterial species, *Bacillus* cells have been found to exhibit remarkable temperature diversity (20°C to 70°C) between their species for their optimal growth (table 1).

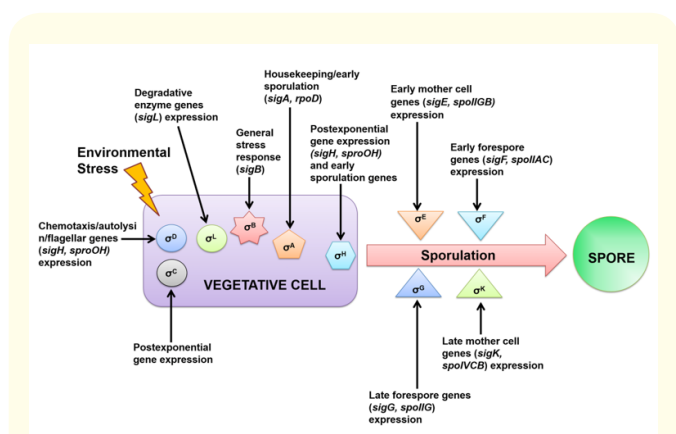


Figure 1: Sigma factors of *Bacillus* species involved in heat shock response. The illustration shows the transformation of the vegetative cells into a spore through the sporulation mechanism, wherein the sigma protein σ^A , σ^B , σ^C , σ^D , σ^H and σ^L are posited into vegetative state and sigma protein σ^E , σ^K , σ^G and σ^F are posited into sporulation mechanism. Their functions along with structural genes are also pictorial via arrow.

Growth of bacteria along with the variation in temperature

All bacteria have a most favorable degree of temperature (optimum temperature) at which they exhibit their highest growth and survival rates. Notably, bacteria also have a minimal and critical growth temperature. Minimal growth temperature is a degree of temperature below which bacteria are metabolically inactive and the critical growth temperature (also referred to as the maximal growth temperature) is a degree of temperature above which it is no longer possible for bacteria to survive. Based on their temperature adoption diversity bacteria can be categorized into

Species	Optimum growth temperature (°C)	Minimum growth temperature (°C)	Critical growth temperature (°C)
<i>Bacillus brevis</i>	35-55	10-35	40-50
<i>Bacillus caldolyticus</i>	67	10 and below	72-75
<i>Bacillus cereus</i>	33-39	2-4	41-47
<i>Bacillus coagulans</i>	40-55	25-40	55-65
<i>Bacillus fumarioli</i>	40-55	25-40	55-65
<i>Bacillus infernus</i>	40-55	25-40	55-65
<i>Bacillus insolitus</i>	20	0 and below	25
<i>Bacillus licheniformis</i>	25-40	5-20	63-68
<i>Bacillus macquariensis</i>	42	inconclusive	48-50
<i>Bacillus megaterium</i>	25-30	13-18	35-55
<i>Bacillus methanolicus</i>	40-55	25-40	55-65
<i>Bacillus okuhidensis</i>	40-55	25-40	55-65
<i>Bacillus schlegelii</i>	55-70	37 -50	65-75
<i>Bacillus smithii</i>	40-55	25-40	55-65
<i>Bacillus sphaericus</i>	38	inconclusive	45
<i>Bacillus stearothermophilus</i>	55	35	71-76
<i>Bacillus subtilis</i>	46	11	53-58
<i>Bacillus thermoamylovorans</i>	40-55	25-40	55-65
<i>Bacillus thermocloacae</i>	55-70	37 -50	65-75

Table 1: Optimum and critical growth temperature for growth and survival of vegetative cells of *Bacillus* species [50-54].

How does *Bacillus* respond to heat shock?

A range of earlier research on the bacterial stress response unraveled the comparative stress resistance of *Bacillus* spp., possibly due to the involvement of stressosome [18,39,47]. Indeed, as with other general stresses, several species of *Bacillus* exhibit sustainable growth a wide range of temperatures starting from 31°C to 76°C [39]. Upon a shift from optimum to high temperatures, *Bacillus* and other bacteria have been found to transiently increase the rate of synthesis of the HSPs [38,41]. *Bacillus* spp. have been known to possess 6 classes of heat shock genes, wherein class I is controlled by *hrcA* gene encoding a transcriptional repressor protein called HrcA, which in turn may negatively control the heat shock operons *dnaK* and *groES/ groEL*. However, inactive HrcA repressor proteins are activated by the GroES/ GroEL chaperon system, which then bind to a class I gene operator called CIRCE (controlling inverted repeat of chaperone expression) to repressed its activity [7,41]. Nevertheless, during heat shock the GroES/ GroEL chaperon system is titrated by nonnative proteins, increasing the amount of inactive HrcA and lower the repressor activity [7,41]. For this expedient modulating activity, HrcA and GroES/ GroEL are presumed as cellular thermometer of class I heat shock genes.

Class II category is regulated by the alternative sigma factor σ^B , which transcribes three subsets of genes, *rsbV*, *rsbW*, and *rsbX*, which are expressed upon heat shock [47,49]. However, in σ^B regulon, RsbR protein senses the elevated temperature, wherefore it might be presumed as the second cellular thermometer [38]. Notably, *Bacillus* species exhibits the regulation of the sporulation specific sigma factor σ^F at the elevated temperatures [38]. Class III heat shock genes, i.e., *clpC*, *clpE*, *clpP*, *ctsR*, *mcsA* and *mcsB* of CtsR regulon, are responsible for DNA binding and heat sensing. Interestingly all σ^A dependent promoters are negatively controlled by the CtsR repressor protein. Notably, most of the genes of the CtsR regulon are expressed at optimum growth temperature (~37°C), which are strongly de-repressed upon exposure to extended temperatures [38]. Heat shock gene *htpG*, which is not involved in the development of thermotolerance are categorized into class IV [41,49]. Two heat shock genes (*htrA* and *htrB*) are categorized into class V, responsible for controlling the stress regulator and sensor [38]. Finally, the class VI category consists of a total of 10 heat shock genes whose regulatory mechanisms have not been chalked out [38]. Nevertheless, the activation of each of these classes (I - VI) of heat shock genes especially depends on specific temperature [38,41] as illustrated in Figure 2.

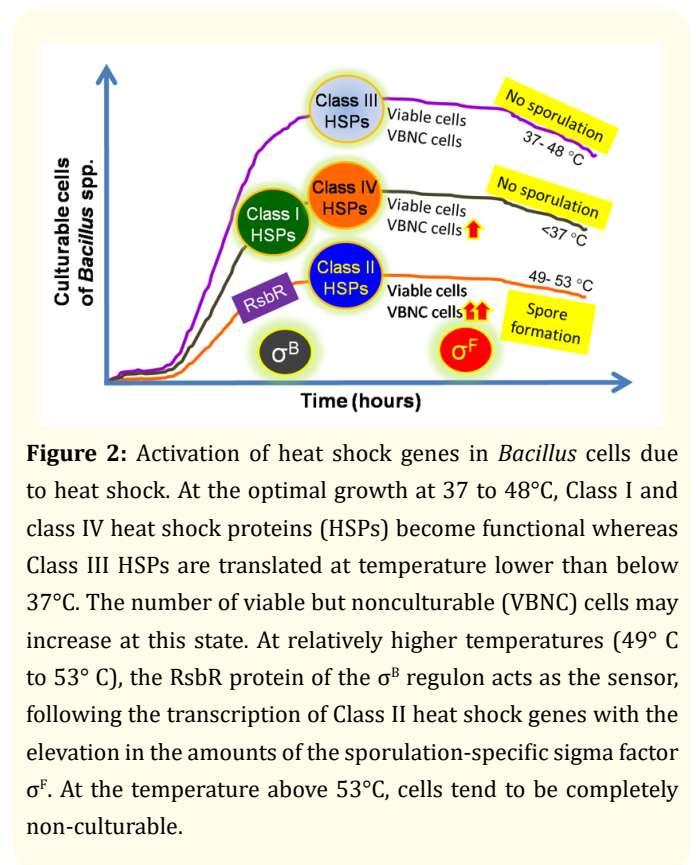


Figure 2: Activation of heat shock genes in *Bacillus* cells due to heat shock. At the optimal growth at 37 to 48°C, Class I and class IV heat shock proteins (HSPs) become functional whereas Class III HSPs are translated at temperature lower than below 37°C. The number of viable but nonculturable (VBNC) cells may increase at this state. At relatively higher temperatures (49° C to 53° C), the RsbR protein of the σ^B regulon acts as the sensor, following the transcription of Class II heat shock genes with the elevation in the amounts of the sporulation-specific sigma factor σ^F . At the temperature above 53°C, cells tend to be completely non-culturable.

What does protect *Bacillus* cells upon heat shock?

In *Bacillus* cells, σ^B with its regulon genes is considered as the major component for general stress response [12]. Notably, the dispensability of σ^B is most clearly illustrated during heat shock as *sigB* null mutants no longer keep temperature sensitive compared to the wild-type strains, hence imparting no impairments in the vegetative growth or the sporulation of *Bacillus* cells [33]. Surprisingly, the housekeeping σ factor, σ^A (σ^{70}) and the class I gene operator CIRCE dependent promoter have been noted to be strongly activated in *sigB* null mutants during heat stress and through this promoter switching the loss of heat-inducibility at σ^B dependent promoter can be compensated in those mutants [12].

Conclusion

Current review illustrated the possible mechanism of cellular resistance against heat stress employing the different classes of HSPs and projected the orchestration between the HSPs and σ^F in course of spore forming event under heat shock. From all the published data on *Bacillus* stress responsive events against heat shock

discussed above, it can be concluded that *Bacillus* species might exhibit momentous growth retardation due to the extreme high temperature; however, they can protect their cells through the well defined sporulation mechanism. Further investigation is imperative to unravel the regulatory mechanisms of heat shock genes that are apparently seem to be non-functional at present.

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Conflict of interest

Authors have no potential conflict of interests

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