LIÈGE université
Gembloux
Agro-Bio Tech

Le Corps professoral de

Gembloux Agro-Bio Tech - Université de Liège vous prie

de lui faire l'honneur d'assister à la défense publique de la dissertation originale que

Monsieur XUE Dong,

Titulaire d'un diplôme de master of science (microbiology),

présentera en vue de l'obtention du grade et du diplôme de

DOCTEUR EN SCIENCES AGRONOMIQUES ET INGENIERIE BIOLOGIQUE,

le 5 novembre 2020, à 10h00 précises,

en visioconférence par Voovmeeting :

https://meeting.tencent.com/s/yHhZitv691bl.

Meeting ID: 586362560. Code: 987654.

Cette dissertation originale a pour titre :

« Bacterial adaptation to temperature stress: molecular responses in two Gram-positive species from distinct ecological niches ».

Le jury est composé comme suit :

Président : Prof. P. JACQUES, Professeur ordinaire,
Membres : Prof. M. ONGENA (Promoteur), Prof. J. WANG (Copromoteur – CAAS, Chine), Prof. F. DELVIGNE, Prof. M. HANIKENNE, Prof. Y. XU (CAAS, Chine), M. A. ARGUELLES-ARIAS.

UNIVERSITE DE LIEGE – GEMBLOUX AGRO-BIO TECH – SERVICE INSCRIPTIONS ET APPARITORAT
Passage des Déportés, 2 - 5030 Gembloux - Belgique
Téléphone +32(0)81 62 23 45 - Fax +32(0)81 62 21 68 - www.gembloux.ulg.ac.be

Summary

Microorganisms are often affected by various environmental factors. These environmental factors affect their physiological and biochemical functions. Among these environmental factors, temperature plays an important role in the normal physiological activities of microorganisms. To adapt to different temperature environments, bacteria have evolved many adaptive mechanisms to coordinate a range of gene expression and protein activity changes. In this study, we investigated the adaptation mechanisms of two Gram-positive bacteria at different temperatures. The main results of this thesis are as follows:

(1) *Deinococcus radiodurans* is a gram-positive, pink-pigmented, and high G+C bacterium. The heat response of *D. radiodurans* is considered to be a classical stress-induced regulatory system that is characterized by extensive transcriptional reprogramming. In this part, we investigated the key functional genes involved in heat stress that was expressed and accumulated in cells following heat treatment at 48°C for 2 hours (R48). Considering that protein degradation is a time-consuming bioprocess, we predicted that to maintain cellular homeostasis, the expression of the key functional proteins would be significantly decreased in cells that had partly recovered from heat stress (RH) relative to their expression in cells grown under optimal temperature (R30). Comparative transcriptomics identified 15 genes that were significantly downregulated in RH relative to R30, seven of which had previously been characterized to be heat shock proteins. Among these genes, three hypothetical genes (*dr_0127*, *dr_1083*, and *dr_1325*) are highly likely to be involved in response to heat stress. Survival analysis of mutant strains lacking *dr_0127*, *dr_1325*, and *dr_1083* showed a reduction in heat tolerance compared to the wild-type strain. These results suggest that *dr_0127*, *dr_1083*, and *dr_1325* might play roles in the heat stress response.

Based on our RNA-seq data and previously reports, we identified two novel heat-inducible ncRNAs in *D. radiodurans*, named *DnrH* and *dsr11*. Heat tolerance analysis showed that deleting *DnrH* significantly inhibited viability in response to high temperature conditions. Comparative phenotypic and qRT-PCR analyses of a *DnrH* mutant (Δ*DnrH*) and wild-type (WT) suggested that *DnrH* is potentially involved in regulating the expression of the heat shock-related gene *Hsp20*. Microscale thermophoresis and genetic complementation showed that a 28-nucleotide (nt) sequence in the stem-loop structure of *DnrH* (143–170 nt) pairs with its counterpart in the coding region of *Hsp20* mRNA (91–117 nt) via a 22 nt region. *In vivo*, mutation of the 22-nt region in the *D. radiodurans* genome led to a reduction in heat tolerance similar to that observed in the *DnrH*-mutant. Our results show that *DnrH* positively influences heat tolerance by increasing the transcription of *Hsp20* mRNA, demonstrating, for the first time, an ncRNA that directly controls the expression of a heat stress-resistance gene. Similar to *dnrH*, we characterized another ncRNA *dsr11*. Our result showed that the transcription level of *dsr11* was upregulated 4.2-fold under heat stress by qRT-PCR analysis. Heat tolerance assay showed that deleting *dsr11* significantly inhibited the viability under high temperature conditions. To assess the influence of *dsr11* on the *D. radioduans* transcriptome, 157 genes were found differentially expressed in the knock-out mutant by RNA-Seq experiment. Combined RNA-Seq and bioinformatic analysis, we found that *dr_0457* (biopolymer transport protein) was likely to be the direct targets of *dsr11*. Further microscale thermophoresis results demonstrated that *dsr11* can directly bind to the mRNA of *dr_0457*. Our results indicated that *dsr11* can enhance the tolerance to heat stress of *D. radiodurans* by binding to *dr_0457* mRNA.

(2) *Bacillus velezensis* GA1 is a Gram-positive model bacterium for unraveling plant–microbe interactions in Bacilli. In this work, we evaluated the impact of temperature on GA1 and the interaction with tomato roots under cold temperature. Understanding how temperature affects protocooperation between plants and GA1 may enhance the efficacy of plant adaptation in reducing abiotic stress-induced damage. However, little is known about the impact of temperature on plants under abiotic stress adaptation by GA1. Cold temperature negatively impacted the cell growth rate of GA1, reflecting the general reduction in the metabolic activity of cells cultivated at low temperatures. *In vitro* cultures revealed that metabolic productivity changed markedly when the temperature was lowered. We observed that after several rounds of culture on RE liquid medium at 15 and 18°C the growth of GA1 became faster than before. We tested GA1 at 18 °C on tomato, the results showed that the biofilm formation on the root hair was a bit slower than at 22°C, and FCM analysis showed that in cold temperature can influence GA1 form spores on tomato roots.

In summary, this study deeply analyzed the adaptation pathways and mechanisms of two gram-positive bacteria in response to different temperatures. Our study will provide a theoretical basis for future applications in industry and agriculture.