Doctoral Thesis

Response of deep-sea *Shewanella violacea* to acid stress

(Summary)

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Chapter 1: Introduction

Physical and chemical changes in the environment might cause stress on a microorganism and lead to metabolic disorders (Beales. *Comprehensive Reviews in Food Science and Food* Safety. 3:1–20. 2004). Neutrophilic *Shewanella* species, which can be isolated from a wide range of aquatic environments, exhibit great potential for application to bioremediation of pollutants and as microbial fuel cells (Hau and Gralnick. *Annu Rev Microbiol.* 61:237–258. 2007; Sekiguchi et al. *JAMSTEC Rep Res Dev.* 11:33–41. 2010). Amongst *Shewanella* species, *Shewanella violacea* thrives in extreme environments, and thus it has been extensively investigated in order to understand its biological stress response mechanism.

In this study, *S. violacea* was specifically examined in the aspects of growth rate, organic acid production, and gene expression in order to gain a fundamental insight into its physiological stress response to acidic pH. Atmospheric pressure (0.1 MPa) was adopted throughout this study to examine the pure pH effects on *S. violacea* physiology.

Chapter 2: Growth of *S. violacea* cells under various pH conditions

The experiments aim to elucidate the ability of *S. violacea* to grow at various pH conditions and observe the extracellular pH change during cell growth. *S. violacea* could grow at an initial of pH 5.0–7.0 and the growth rate at initial pH 5.0 was slower than those at pH 6.0 and 7.0. In addition, the pH of the culture initially adjusted at pH 5.0 increased toward neutral during the cell cultivation, which indicates an acid neutralization mechanism. However, genes encoding enzymes for ammonia production, which is highly related to acid neutralization mechanism are not annotated in *S. violacea* genome. Thus, acid neutralization mechanism in this bacterium remains unknown.

Chapter 3: Metabolite production of *S. violacea* cells grown at acidic and neutral pHs

The experiments aim to elucidate the physiological response in *S. violacea* to acid stress through organic acid production. *S. violacea* cells grown at pH 5.0 produced higher concentrations of butyric and isovaleric acids than those at pH 7.0. The ability of *S. violacea* cells to grow at pH 5.0 (Chapter 2) may be related to the production of these organic acids.

Chapter 4: The expression level of genes related to organic acid production in *Shewanella violacea* cells grown at acidic and neutral pHs

The experiments aim to elucidate the expression level of genes related to organic acid production in *S. violacea* cells grown at pH 5.0 and 7.0. The expression level of genes related to the synthesis of butyric acid as the response to acid stress was not entirely detected in this experiment because three genes encoding β-hydroxybutyryl-CoA dehydrogenase, phosphate butyryltransferase, and butyrate kinase are not annotated in the genome of *S. violacea*. As for isovaleric acid, the expression level of three genes encoding key enzymes related to the synthesis, namely branched amino acid aminotransferase, α-keto acid dehydrogenase, and acyl-CoA thioesterase was upregulated at pH 5.0. Accordingly, the production of isovaleric acid is the response of *S. violacea* cells to acid stress.

Chapter 5: Transcriptome analysis of *S. violacea* genes at pH 5.0 and 7.0

The experiment aims to compare the expression level of each gene of *S. violacea* at pH 5.0 and 7.0. The expression level of 81 genes of *S. violacea* is upregulated ≥ 2-fold at pH 5.0 than those at pH 7.0. Amongst the upregulated genes are related to universal stress response, i.e. genes encoding serine endonuclease, the disulfide bond formation family proteins, and heat shock proteins, also genes encoding NADH-dehydrogenase and two-component systems. Meanwhile, other 110 genes are downregulated ≥ 2-fold at pH 5.0 than those at pH 7.0. The most downregulated gene is encoding TonB-depending receptor and other notable genes are encoding porin. These notable upregulated and downregulated genes probably have a specific role in the response to acid stress in *S. violacea.*

Chapter 6: Conclusion and future plan

This study revealed *S. violacea* can survive at the acidic pH of 5.0. Collectively, the present results at least showed that acid stress leads to isovaleric acid production in this bacterium. This information deepens our understanding of the stress response mechanism inherent in *S. violacea* cells. Response mechanism to acid stress in this bacterium should be further studied, especially in the role of each gene found to be upregulated and downregulated in the transcriptome analysis.