

## **S5-2**

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### **Diversity of Deep-sea Piezophiles and Their Molecular Adaptations to High-pressure Environment**

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#### **Summary**

We have isolated numerous cold deep-sea adapted microorganisms (piezophilic, formerly referred to as “barophilic” bacteria) using deep-sea research submersibles. Many of the isolates are novel psychrophilic bacteria, and we have identified several new piezophilic species, i.e., *Photobacterium profundum*, *Shewanella violacea*, *Moritella japonica*, *Moritella yayanosii*, *Psychromonas kaikoi*, and *Colwellia piezophila*. These piezophiles are involving to five genera in gamma-Proteobacteria subgroup and produce significant amounts of unsaturated fatty acids in their cell membrane fractions to maintain the membrane fluidity in cold and high-pressure environments. Piezophilic microorganisms have been identified in many deep-sea bottoms of many of the world oceans. Therefore, these microbes are well distributed on our planet. One of the isolated deep-sea piezophiles, *Shewanella violacea* strain DSS12 is a psychrophilic, moderately piezophilic bacterium from a sediment sample collected at the Ryukyu Trench (depth: 5,110 m), which grows optimally at 30 MPa and 8°C but also grows at atmospheric pressure (0.1 MPa) and 8°C. We have examined this strain to elucidate the molecular basis for gene regulation at different pressure conditions because this strain is useful as a model bacterium for comparing the various features of bacterial physiology under pressure conditions. In addition, we completed the sequencing of the entire genome of this piezophilic bacterium and we expect that many biotechnologically useful genes will be identified from the genome information.

#### **Piezophiles are adapted to the deep-sea environment**

It has been suggested that life may have originated in the deep sea some 3.5 to 4 billion years ago, where it was protected from the damaging effects of ultraviolet light. The deep-sea is a particularly high-pressure environment and hydrostatic pressure could have been a very important stimulus for early

forms of life. Scientists have proposed that life might have originated in deep-sea hydrothermal vents and thus it appears possible that high-pressure-adapted mechanisms of gene expression could represent a feature present in early forms of life. It has recently been reported that the primary chemical reactions involved in the polymerization of organic materials (i.e., amino acids) could have occurred in such high-pressure and high-temperature environments. Thus, the study of deep-sea microorganisms may not only enhance our understanding of specific adaptations to abyssal and hadal ocean realms but may also provide valuable insights into the origin and evolution of life on our planet.

In 1949, Zobell and Johnson began investigating the effects of hydrostatic pressure on microbial activities. They first used the term “barophilic” to define organisms whose optimal growth occurred at pressures higher than 0.1 MPa or a requirement for increased pressure for growth. Recently, the term “piezophilic” has been proposed to replace “barophilic,” as the prefixes “baro” and “piezo,” derived from Greek, mean “weight” and “pressure,” respectively. Thus, the word “piezophilic” is more suitable than “barophilic” to describe bacteria that grow better at high-pressure than at atmospheric pressure. Therefore, we have opted to use the term “piezophilic” bacteria, meaning high-pressure-loving bacteria. The definitions of “piezophilic” are shown in Fig. 1.

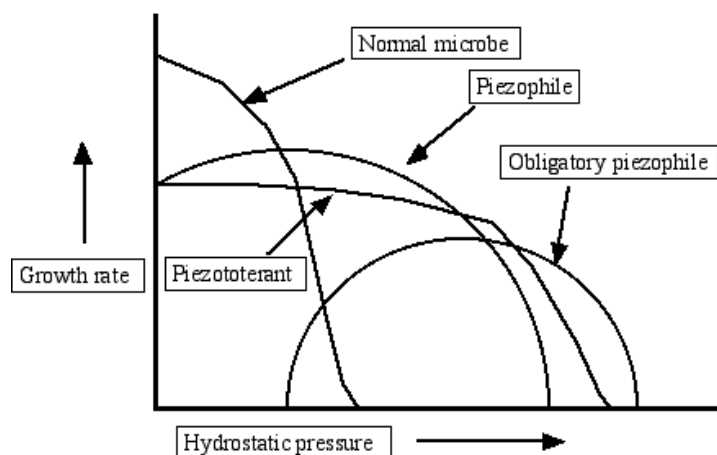


Fig. 1. Characterization of piezophilic growth properties.

### Pressure-regulated promoter of *S. violacea* strain DSS12

The moderately piezophilic *S. violacea* strain DSS12 grows optimally at 30 MPa and 8°C but also at atmospheric pressure (0.1 MPa) and 8°C. Therefore, this piezophilic strain is useful as a model for comparison of various features of bacterial physiology under high and low hydrostatic pressure conditions. An operon identified as a pressure-regulated operon whose the promoter was activated by growth under high pressure, was cloned and characterized from this strain: this operon, which has five transcription initiation sites, is controlled at the transcriptional level by elevated pressure (Fig. 2A). Moreover, transcriptional analysis showed that expression of the genes in the pressure-regulated operon

is positively controlled at the transcriptional level by elevated pressure and further suggested that most transcripts from the operon at atmospheric pressure coincided with expression from initiation site #2 (Fig. 2A). The consensus sequence for the RNA polymerase sigma factor, sigma 54, was found upstream from this operon, and the *S. violacea* sigma 54 was shown to bind to this region. The sigma 54-containing RNA polymerase has been shown to be responsible for the transcription of several genes, e.g., nitrogen metabolic genes such as the *glnA* operon. Glutamine synthetase is one of the enzymes involved in nitrogen metabolism. As shown in Figure 2B, gene expression of the *glnA* operon is also controlled by elevated pressure conditions at the transcriptional level in *S. violacea*, particularly by factor sigma 54 (transcription initiation site #1). These results suggest that sigma 54 might play an important role in pressure-regulated transcription in piezophilic bacteria, although the expression of sigma 54 itself is not regulated by pressure conditions.

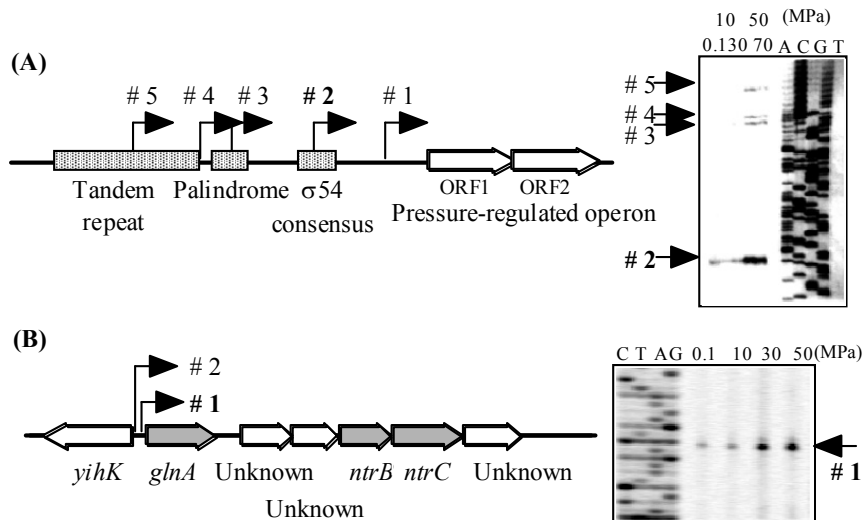


Fig. 2. Diagrammatic representation of the pressure-regulated genes in *S. violacea* strain DSS12.

(A) Pressure-regulated operon. (B) Glutamine synthetase operon. Bold numbers (#2 in "A", and #1 in "B") show the transcription controlled by the sigma 54 factor.