

Diversity and Commonality of Environmental *Bacillus*-related Species Revealed by Their Genome Sequences

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A great number of ubiquitous *Bacillus*-related species have been often isolated from various terrestrial and deep-sea environments even from the Mariana Trench at a depth of 11000 m¹⁻³⁾. Some of those *Bacillus*-related species possess various capabilities to adapt to the extreme environments such as high and low temperature, high and low pH, and high salinity. In fact, it is known that *Bacillus*-related species can grow in a wide range of environments, at pH 2-12, with temperatures between 5 and 78°C, in salinity from 0 to 30% NaCl, and pressures from 0.1 MPa (atmospheric pressure) to at least 30 MPa (corresponding to the pressure at a depth of 3000 m)⁴⁻⁵⁾.

We are now intrigued by the questions of how these adaptive capabilities were acquired, as reflected in their genomes, and what intrinsic genomic structure of *Bacillus*-related species has allowed them to adapt to such a wide range of environments. On the other hand, phylogenetically related *Bacillus*-related species share many genes each other although each species has the different capability to adapt to various environments. Thus, it is expected that comparative analysis with *Bacillus*-related species can be a useful approach for extracting candidate genes associated with the mechanisms of adaptation to the environments.

We initiated the genome sequencing project at the beginning of 1998 and have determined the whole genomic sequences of three extremophilic bacilli, alkaliphilic *Bacillus halodurans*⁶⁾, extremely halotolerant and alkaliphilic *Oceanobacillus iheyensis*⁷⁾, and thermophilic *Geobacillus kaustophilus*⁸⁾ until now. We provide the first comparative analysis of the extremophilic bacillar genome with those of 3 other closely related mesophilic and neutrophilic bacilli, *B. subtilis*, *B. anthracis* and *B. cereus*, in order to highlight commonality and diversity of the bacillar genomes.

Through orthologous analyses of the six bacilli, 1257 common orthologous groups composed of 1308 genes were found to be shared among all six bacillar genomes. It was also found that 103 orthologs are shared only between the two alkaliphilic species, *B. halodurans* and *O. iheyensis*, and 1216 orthologs are shared only between the two pathogenic species. Generally, alkaliphilic *Bacillus*-related species cannot grow or grow poorly under neutral pH conditions, but grow well at a pH higher than 9.5. It is known that when the cells of alkaliphilic species are exposed to pH 10.5, the cytoplasmic pH maintains 2 to 3 units lower than outside of the cell and it is thought that a lot of transport systems seem to contribute to keep pH homeostasis. Although 61 genes are functionally unknown in the 103 common orthologs between the two

allaliphilic species, 13 genes were filed into the category 1.2, transport/binding protein and lipoprotein. Mainly, the genes encoding ABC transporter, C4-dicarboxylate transporter and voltage-gated sodium channel are included in this category and these genes were highlighted as major candidates responsible for alkaliphily.

On the other hand, 757 genes were unique to thermophilic *G. kaustophilus*. *G. kaustophilus* can grow up to 74°C but the temperature limit will be dictated by molecular instability. Actually, DNA duplex stability is apparently achieved at high temperatures by elevated salt concentrations, polyamines, cationic proteins and supercoiling rather than manipulation of GC ratios. RNA stability is enhanced by covalent modification and the secondary structure is also probably critical. We could find some genes in a set of unique *G. kaustophilus* genes, which seem to be involved in the stabilization of DNA and RNA, such as protamine, spermidine/spermine synthase, and RNA methyltransferase.

Although insertion sequences such as IS and transposon have not been detected in the *B. subtilis* 168 genome, other extremophilic bacilli were found to contain many insertion sequences. Especially, alkaliphilic *B. halodurans*⁹⁾ and thermophilic *G. kaustophilus*⁸⁾ possess more than 100 IS elements in their genome and some of CDSs were interrupted or truncated by the insertion of IS elements. In addition, it was found that some genes involved in spore formation and germination were horizontally acquired in the *O. iheyensis* genome by Tn3-like transposon¹⁰⁾. In case of the *G. kaustophilus* genome, the housekeeping *recA* gene was interrupted by group II intron. Thus, it is clear that these insertion sequences contribute to diversify the genomes.

In this study, we have attempted to highlight the genes involved in the mechanisms of adaptation to various environments based on comparative analysis of extremophilic *Bacillus*-related species and other non-extremophilic bacilli. Although we were able to find strong candidates responsible for adaptive capabilities to various environments in a common or a unique gene set among all six bacillar genomes, more than half in the gene sets is orphan among the six bacilli or conserved in other organisms but as yet functionally opaque. Therefore, another candidate may be among those genes whose function is not yet known. It will be necessary to do further comparative analysis with other extremophilic and non-extremophilic *Bacillus*-related species in revealing hidden capacity for adaptation to various environments.

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