solution(126g/*l*) at 80℃.

A Symbiotic Thermophile, *Symbiobacterium toebii*: Characterization, Genome Analysis and Industrial Application

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The extremophilic nature of many bacteria has stimulated intensive efforts to probe the potential biotechnological applications of their stable enzymes. It is difficult to isolate some microorganisms because of their interaction with other organisms. *Symbiobacterium toebii* is a symbiotic thermophile which grows only with *Geobacillus toebii*. For its axenic culture, the cell free extract of *Geobacillus toebii* have to be supplied into the medium.

Its genome consists of a circular chromosome of 3,474,049 base pairs containing 3,942 predicted protein coding sequences (CDs) and there was not an extra-chromosomal element (ECE). An average G+C content of the genomic DNA was 68.8 mol%. Of these protein-coding sequences, 1,546 (40 %) protein coding sequences have functional assignments and 2,396 (60 %) have no match to any protein database. Of these protein-coding genes, a lot of gene families were repeatedly expanded by duplication. The genome contains at least 26 transposases and insertion sequence (IS) elements, indicating that transposases have played an important evolutionary role by horizontal gene transfer. The genome of *Sym. toebii* has the complete respiratory apparatus using nitrate as an electron acceptor and eight signal peptidase genes, as well as several genes for the secretory machinery. It also contains at least 70 putative ATP-binding transport proteins and more than 83 putative transcriptional regulators. Comparison with other sequenced microorganisms revealed that lots of gene families in *Sym. toebii* were closely related

with those of *B. subtilis* and *B. halodurans*. To elucidate growth mechanism of *Sym. toebii*, we tried to the analysis of proteom of *S. toebii*. As a result of 2D-PAGE, we found more than 20 proteins which were only expressed at the exponential growth phase.

Genome analysis revealed that *Sym. toebii* had almost genes encoding enzymes that could be found in other microorganisms except the genes related to branched amino acid-tRNAs synthetases. The structural genes encoding 25 thermostable enzyme families were first searched in the genomic library of *Sym. toebii* for industrial use of genome information of *Sym. toebii*. These enzymes contained 9 different peptidases, 6 esterases, 5 phophatases, 5 oxidases, and 3 catalases, and so on.

Especially, although *Sym. toebii* did not show any lipase activity in the culture broth and cell-free extract, it had two silent genes encoding lipases. This fact indicated that industrially valuable thermostable enzymes could be obtained from genomic library of the *S. toebii* whether the structural genes was expressed or not.

Among these useful thermostable enzymes, the genes encoding a thermostable tyrosine phenol-lyase (TTPL) and a thermostable tryptophan indole-lyase (TTNA) were cloned from the genomic DNA of the *S. toebii* by PCR and overexpressed in the recombinant *E. coli* BL21. These two enzymes were used as biocatalysts for the production of L-tyrosine and L-tryptophan.

The TTNA, which catalyzes in vivo degradation of L-tryptophan to indole, pyruvate, and ammonia, was used as a biocatalyst for producing L-tryptophan with a reverse reaction of TTNA. L-Tryptophan used for treatment of mental disorder like depression, anxiety, and premenstrual syndrome, etc. Optimal conditions for the production of L-tryptophan were 37 ℃, pH 8.5. After 24 hr of reaction in above optimal condition, 188 g/L of L-tryptophan (productivity = about 8 g/L/hr) was synthesized from indole, pyruvate, and ammonium chloride.

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