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A Symbiotic Thermophile, *Symbiobacterium toebii* : Novel Microbial Interaction, Characterization, and Genome Analysis

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There are large numbers of unculturable microorganisms in natural environments that can only be seen under a microscope or detected by molecular ecological methods. A symbiotic thermophilic bacterium, *Symbiobacterium toebii* SC-1, was isolated from hay compost (called as toebii in Korean) in Korea. The new isolate exhibited an obligate symbiotic commensal interaction with a thermophilic partner, *Bacillus toebii* SK-1 and required crude extracts of the *B. toebii* strain SK-1 for growth. The strain SC-1 was non-spore forming, nonmotile rod that was stained gram-negatively. The isolate was a microaerophilic heterotroph and its growth was observed between 45 and 70°C (optimum: 60°C; 2.4 h of doubling time) and between pH 6.0 and 9.0 (optimum: pH 7.5). The genomic DNA had high G+C content (65 mol%), and MK-6 and MK-7 were major quinones. Based on a sequence similarity analysis, the 16S rDNA sequence of strain SC-1 exhibited less than 87% similarity with any other known sequence. Even though above facts, phylogenetic analysis of its 16S rDNA sequence indicated that the strain SC-1 was identified as a Gram-positive, low G+C content, spore forming bacterium within the *Bacillus-Clostridium* subphylum. The molecular analyses such as competitive PCR and terminal restriction fragment polymorphism in soils showed that *S. toebii* SC-1 is widely distributed even in mesophilic soils and might be originated from thermophilic environment such as composts.

The symbiotic growth factors from *B. toebii* SK-1 were irreversibly inactivated by phenol or protease treatment, thereby suggesting that they might be proteins. The factors were aggregated and precipitated at pH 4.0 and renatured by returning to pH 7.0. A wide range of compounds including trace minerals, D-amino acids, vitamin precursors, artificial electron carriers, biopolymers, and proteins were tested as growth stimulating factors. Among compounds tested, some artificial electron mediators such as menadione and benzyl viologen were found to highly stimulate the growth of strain SC-1. These results

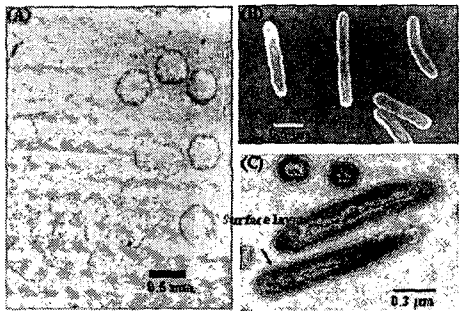

Fig. 1. Microcolony configuration (A) and scanning (B) and transmission (C) electrophotographs of a symbiotic thermophile, *S. toebii* SC-1.

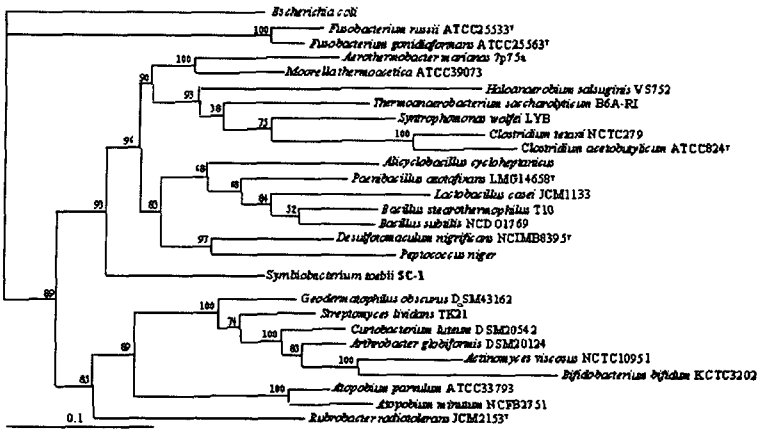

Fig. 2. Phylogenetic position of S. toebii SC-1 using Maximum-Likelihood method.

suggest that the growth factors might be proteins functioning as electron mediators. The size of the growth-stimulating factor was estimated to be approximately 30 kDa by gel permeation chromatography. We are currently trying to elucidate function of the obligate symbiotic factor.

The genomic DNA size of *S. toebii* SC-1 was estimated as about 2.8Mb using pulsed-field gel electrophoresis (PFGE). Understandings of these bacterial interactions help us unravel microbial diversity in natural ecosystem and discover new ubiquitous microbial groups. Therefore, we have sequenced the genome of *S. toebii* SC-1 to identify genes responsible for symbiosis interaction and to advance our development of new microbial sources for industrial applications.

Acknowledgment

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References

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