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Studies on the Growth Characteristics of Unculturable Environmental Microorganisms Using a Syntrophic Bacterium, *Symbiobacterium thermophilum*, as a Model

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Research aims

The commensal relationship between microorganisms is not well studied because of the difficulties in its manipulation techniques, in contrast to the great progress for the microbial symbiosis with animals and plants. This study aims at elucidating the basic molecular mechanisms that underlie the microbe-microbe interaction. Especially, it focuses and carries out precise analysis on the physiological characteristics of *Symbiobacterium thermophilum*. *S. thermophilum* is a unique microorganism, which does not propagate when it is isolated but normally grows when it is cultured with a thermophilic *Bacillus* strain S.[1–3] To identify the factor(s) that support the growth of this organism should provide insight into the molecular basis for the microbial commensalism.

Methods

The strains used were *S. thermophilum* IAM14863,[3] *Escherichia coli yadF*$^-$ mutant and its parental strain[4] and *Bacillus subtilis* ATCC6633. *E. coli* and *B. subtilis* were grown under the standard culture conditions, and *S. thermophilum* was cultivated as described previously.[2,5] In the cultures for the air-phase CO_2 concentration measurement, an airtight 0.5 L Erlenmeyer flask with two side arms was used. For culture media, LB medium (containing 1% Tryptone, 0.5% yeast extract, and 0.5% NaCl) was used as a base medium. The amount of each constituent was changed when needed. For the measurement of cellular concentration, the specific quantitative PCR technique was used for *S. thermophilum*,[5] and the colony-counting method was used for others.

Results

The precise analysis on the culture conditions that induce mono-growth of *S. thermophilum* revealed that this organism performs marked proliferation when CO_2-containing N_2 gas was introduced in the culture medium. Since the organism did not grow when CO_2-free N_2 gas was introduced, we concluded that CO_2 is the factor that induces the mono-growth of this organism. By using the closed cultivation system, the threshold CO_2 concentration for the initiation of proliferation of *S. thermophilum* was determined to be 200 ppm.

Recently, similar phenomena as above were reported as to the phenotypes of *E. coli* and *Ralstonia eutropha* mutants for carbonic anhydrase (CA).[6,7] CA is a widely-occurring enzyme that mediates interconversion between CO_2 and $HCO3^-$. The deficiency of the CA gene in *E. coli* and *R. eutropha* causes lethal effect under normal culture condition but does not affect the growth under CO_2-introduced culture condition. Based on this, we performed cocultivation a CA mutant (*yadF*$^-$) of *E. coli* and *B. subtilis*, and found that the mutant performed marked growth even under the normal culture condition. The threshold CO_2 concentration for the initiation of the CA mutant was determined to be 7,500 ppm.

The genome sequencing of *S. thermophilum*[8] revealed that there is no CA gene in the genome of this organism. The evidence strongly suggests that as observed with the *E. coli* CA mutant, *S. thermophilum* requires CO_2 due to the lack of this enzyme, and it is compensated for by the commensalism with another microorganism.

In order to demonstrate the presence of CO_2-dependent microorganism like *S. thermophilum*, we screened for microorganisms that grow only under the CO_2-introduced culture condition. As a result, about 60 bacterial strains were successfully isolated. The 16S rDNA-based phylogenetic analysis revealed that they contain several strains that are taxonomically very unique.

Conclusion

1) The most critical factor for the initiation of proliferation of *S. thermophilum* is CO_2.

2) *S. thermophilum* can proliferate by utilizing the CO_2 generated by other organisms.

3) The candidate for the genetic background that causes the CO_2 requirement is the deficiency of carbonic anhydrase.

4) CO_2-dependent microorganisms are probably predominant in the natural environment.

5) CO_2-dependent microorganisms may contain unknown strains, since they can not be isolated under the normal culture condition although they grow under commensal cultivation.

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