SL304

srf Operon and the Role of *comS* gene in Competence Developement of *Bacillus subtilis*

Oh-Hyoung Lee
Department of Biology, Mokpo National University

srf operon of *Bacillus subtilis* encodes surfactin synthetase complex which catalyzes the nonribosomal biosynthesis of the lipopeptide surfactin using the multienzyme thiotemplate mechanism. The complex is composed, in part, of three subunits SrfA, SrfB, and SrfC, which constitute the seven amino-acid activating domains required for the synthesis of the peptide portion of surfactin. Immunoprecipitation experiments using a hemagglutin(HA) epitope - tagged SrfA derivative and anti-HA antiserum resulted in co-precipitation of these three subunits, indicating that they are working in a complex form. Single amino acid substitution mutations in the active site or intermodule domain showed no effect on this subunit co-precipitation.

The *comS* gene, which is nested in the *srfB* gene and encodes a small, 46 amino acid protein, is required for competence development at early stationary phase. It has been known that ComS antagonizes ComK-inhibitor MecA, thereby activate ComK, a key protein responsible for the expression of late competence genes. Both SinR and DegU are also known to promote competence development. Through the multicopy suppression experiments with plasmid - amplified *comS, sinR,* and *degU* genes, it was found that multicopy *comS* suppresses *sinR* mutation but not *degU* mutation, indicating *sinR* acts at upstream of *comS* and *degU* at downstream, respectively. Experiments testing the efficiencies of *srf-lacZ* and *comS-lacZ* expressions under the *ΔsinR* or *ΔdegU* background showed that only the *comS-lacZ* expression was reduced by *sinR* mutation, while neither *srf-lacZ* nor *comS-lacZ* expression was affected by *degU* mutation. These results suggest that *sinR* is required for optimal *comS* expression but not transcription from the *srf* promoter, and *degU* appears to be in a separate, pararell pathway controlling competence development.