Thesis Summary

Identification and Characterization of *Escherichia coli* Chromosomal Genes whose Deficiency in Donor Cells Enhances Bacterial and *Trans*-kingdom Conjugations by IncP1 T4SS Machinery
（細菌間接合と生物界間接合の効率が変異によって向上する大腸菌染色体遺伝子の探索と解析）

Name FATIN IFFAH RASYIQAH

Conjugation in bacteria is a phenomenon that transfers genetic materials from donor to recipient cells through direct cell-to-cell contact between the two cell types. Bacterial conjugation requires expression and regulation of conjugation-related genes, encoded either in conjugative plasmids or integrative and conjugative elements (ICEs). This phenomenon depends on bacterial type IV secretion systems (T4SS). Broad host range (BHR) conjugative plasmids can promote genetic exchange among distantly related bacteria. Some of the BHR plasmids enable gene transfer even to eukaryotes and archaea. The extremely broad range horizontal DNA transfer driven by the conjugative plasmids is called *trans*-kingdom conjugation (TKC). *Escherichia coli* cells having either an IncP1α or IncP1β BHR plasmid can trigger TKC to the model eukaryote, *Saccharomyces cerevisiae* yeast. Although the analysis of the conjugation-related genes encoded on the IncP1-type plasmids toward perspective for conjugation mechanism has been extensively progressed, that on the host chromosome remains limited. To identify the potential chromosomal conjugation factor(s), a genome-wide screening on a comprehensive collection of *E. coli* gene knockout mutants (Keio collection) as donors to yeast as recipients was performed using the T4SS of the IncP1α plasmid (IncP1-T4SS).

In this study, 233 candidate mutants were screened and finally, three “up”-mutant strains (Δ*frmR*, Δ*sufA*, and Δ*iscA*) were found to increase both *E. coli*-*E. coli* and *E. coli*-yeast conjugations by one order of magnitude. The increase was not associated with mRNA level alteration of conjugation-related genes examined. Double-knockout mutants (Δ*frmR*Δ*sufA* and Δ*iscA*Δ*frmR*) did not exhibit any synergistic effects on the conjugation efficiency, suggesting that the three genes independently affect an identical step of conjugation machinery. In addition, the three mutants demonstrated an increase in conjugation efficiency driven by IncP1β-type plasmid, but not that by IncN- and IncW-type BHR plasmids. The importance of the three identified genes was further accessed in another bacterium belonging to a different class in Pseudomonadota. Three *Agrobacterium tumefaciens* knockout mutants, each of which lacks a homolog of the three genes, also showed an increase in TKC efficiency mediated by the IncP1-type transfer system. These results suggest the existence of a specific regulatory system in IncP1 plasmids, that enables the control of conjugation efficiency in different hosts.

Following is one plausible explanation for the whole of the results mentioned above. SufA and IscA work in repressing other target factors (activators) in the *E. coli* donor cells either directly or indirectly. At the same time, the inactivation of FrmR which may also be a repressor of another target factor (activator), derepresses the expression of that particular factor. The unknown target factors of FrmR, SufA, and IscA may form a complex to activate the conjugation at an identical step of IncP1 conjugation machinery.

A promising outcome is expected by identifying and characterizing the presumptive chromosomal gene(s), which could genetically interact with *frmR, sufA,* and *iscA* genes. Further characterization of the interaction between three genes and the presumptive regulatory genes on the IncP1 plasmids will later lead to a better understanding of how the chromosomal genes control the conjugation and TKC mechanisms. Although the native function and regulation are different among the three genes, their ability in repressing conjugation is common. From a practical viewpoint, the three mutants (Δ*frmR*, Δ*sufA*, and Δ*iscA*) could serve as a basis for the development of robust donor strains as gene introduction tools into bacteria, eukaryotes, and archaea.