Promoter Activities of Hypothetical *ileZ-argN-argO* Operons and Expression and Function of *ileZ* in *Escherichia coli* O157:H7 Sakai

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Enterohemorrhagic *E. coli* (EHEC) O157:H7 is an important foodborne pathogen, notorious for its low infectious dose and its potential to cause severe diseases such as hemorrhagic colitis and hemolytic uremic syndrome. For example, a massive outbreak in primary schools in Japan caused by prototypical strain Sakai caused approximate 12,680 clinical cases. Most of the well-characterized virulence-related EHEC genes such as the Shiga toxin (Stx) and the locus of enterocyte effacement were acquired by horizontal gene transfer (HGT). Whole genome sequencing of Sakai and other EHEC strains indicated that the genes encoded within HGT regions utilize the rare codons [AGG (*arg*), AGA (*arg*), CGA (*arg*) and ATA (*ile*)] more often than those encoded in the backbone genome.

In strain Sakai, 7 lambda-like prophage, including the one that encodes *stx2*, carry hypothetical *ileZ-argN-argO* operons, which are predicted to recognize ATA (*ileZ*), CGA (*argN*) and AGA/AGG (*argO*). Blast result identified 5 to 8 copies of *ileZ-argN-argO* in 7 fully sequenced O157 and non-O157 EHEC strains, but 0-3 copies in all other sequenced *E. coli* strains. These observations suggested that *ileZ-argN-argO* might be involved in the regulation of EHEC virulence genes. However, the function of these operons remains to be investigated.

We performed *in silico* analysis to calculate the frequencies of ATA, CGA, AGA and AGG in Sakai genome. Among all isoleucine codons, the fraction of ATA is used 9.53%, and the arginine codons CGA, AGA and AGG are used 6.97%, 5.33% and 3.44%, respectively. These codon usages are remarkably higher in virulence genes. The activities of *ileZ-argN-argO* operon promoters were determined by β-galactosidase assays. All 7 promoter fusions gave relatively high β-galactosidase activities (varied from 26 to 303 Miller Units) and a variation in promoter strength was observed. Also, the expression of *ileZ* in Sakai was confirmed by reverse transcription qPCR. In order to determine the activity of *ileZ*, we constructed a model expression system by integrating tandem ATA codons into the 5' end of a His-tagged *hfq* cloned into pET28 plasmid. Our result indicated that over-expression of the *hfq* derivative with 2 tandem ATA codons was only seen when *ileZ-argN-argO* was supplied *in trans*.

Therefore, we confirmed that the 5' promoters of all 7 *ileZ-argN-argO* are functional and that the *ileZ* encodes for a functional $tRNA_{Ile}$(ATA). The characteristics of other tRNA genes will be investigated in the future.