Isoleucyl-tRNA synthetase carrying antibiotic resistance cannot support sporulation and biofilm formation in Bacillus megaterium

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V. Zanki[I], B. Božić[I], I. Gruić-Sovulj[I]

[I]Faculty of Science, University of Zagreb, Zagreb, Croatia

Isoleucyl-tRNA synthetase (IleRS) catalyzes covalent coupling of isoleucine and $tRNA^{Ile}$ for protein synthesis. Several *Bacilli* species have two distinct types of *ileS* gene. While *ileS1* seems to be following species phylogeny tree, *ileS2* distribution among species is best explained by horizontal gene transfer. To understand requirements for having both *ileS* genes, we used *B. megaterium* as a model organism and performed kinetic analyses on the isolated enzymes and in vivo analyses on the constructed knockout strains. We found that BmIleRS2 exhibits 25-fold higher K_M for isoleucine than BmIleRS1 and at the same time a 1000-fold higher K_i for antibiotic mupirocin. Interestingly, while mupirocin acts as a fast-on/fast-off competitive inhibitor of BmIleRS2, it displays a slow-tight binding type of inhibition with BmIleRS1. Our data further indicate that interaction of BmIleRS1 and BmIleRS2 with isoleucine is modulated by $tRNA^{Ile}$ but in a different way. Whether and how distinct mupirocin interaction with BmIleRS1 and BmIleRS2 is related to the observed different K_M values of these enzymes toward isoleucine is still not clear, however, it may suggest that aminoacylation mechanisms could have distinctly evolved in these enzymes to account for the trade-off between mupirocin resistance and amino acid affinity. Knockout strains lacking either *bmileS1*(*ΔileS1*) or *bmileS2* (*ΔileS2*) were created to address cellular demands for both *ileS* genes. Although both knockout strains were viable, *ΔileS1* exhibited slower growth, inefficient sporulation and deviation in biofilm formation compared to the wild-type strain. In a minimal medium, *ΔileS1* strain is outcompeted by *ΔileS2* strain, suggesting *bmileS1* in under constant selective pressure to remain in the genome. These results show that *bmileS2*, carrying antibiotic resistance, can complement *bmileS1* as a sole housekeeping gene, however, BmIleRS1 is essential for overall fitness of *B. megaterium*.