Antibiotic Resistance Gene: Metagenomic Discovery and Potential Targeting by Small RNAs

Hakmin Mun

Since antibiotics were introduced in society, bacterial acquirement of antibiotic resistance has rapidly out-paced the development of new antibiotics. This necessitates discovery of antibiotic resistance genes to better understand resistance mechanisms and to develop new therapies. The human intestine harbors a large environmental reservoir of antibiotic resistance genes that is accessible to pathogens. To discover antibiotic resistance genes in the human intestine, functional metagenomics, which allowed culturing even uncultureable microorganisms in the laboratory, was applied to construct a library of antibiotic resistance DNA fragments. Then bioinformatics methods were used to identify the individual Open Reading Frames (ORFs) within the antibiotic resistance DNA fragments. Here, ORF is part of reading frame that has the potential to code for a protein or peptide inside its sequence. After that I sub-cloned the individual eighty ORFs in 54 DNA fragments and tested them for the antibiotic resistance. Consequently, 18 ORFs were verified to cause resistance against 7 different antibiotics. In addition, we tried to establish the model of phage therapy for antibiotic resistance. In this model, small RNAs transcribed from designed antisense DNA bind into mRNA of Blue Fluorescent Protein and interfere the protein synthesis. For the model construction, antisense DNA was successfully cloned into bacteriophage plasmid. The cloned DNA will be evaluated for inhibition of fluorescence in the bacterial cell, *Escherichia coli* in the future.