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DISSERTATION SUMMARY

Construction and characterization of a multi-deletional *Escherichia coli* strain

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In the past decade, among the first genomes to be sequenced were those of *Escherichia coli* K-12 and *E. coli* O157:H7. Comparison of the data revealed that approximately 70% of their genomes are highly conserved. This is called the backbone sequence, which is interrupted by many strain-specific islands (Perna et al. 2001). Our group's goal was to delete as many strain-specific islands in the K-12 strain as possible, for two main reasons: as a basic scientific research goal, we wanted to see how far we can go with the deletions without impairing the viability of the bacterium under laboratory conditions. Second, we wished to develop a strain that is more useful for DNA-cloning experiments or other biotechnological purposes. Our deletion method is based on a λ-Red type recombination of a PCR-generated linear DNA fragment into the genome, followed by a specific double stranded break-induced intramolecular recombination event. The deletion process leaves no extrachromosomal sequences behind, and can be repeated in the same cell in an unlimited number (Kolisnychenko et al. 2002).

So far we have accumulated 42 deletions in the K-12 strain. Among the deleted sequences were all the mobile genetic elements (IS elements, prophages, Rhs sequences), some damaged genes, putative virulence genes and many genes of unknown function. The DNA-microarray tests made with the multi-deletional strain showed that, although all known IS's were removed, further IS elements were present in the genome. We located three IS1's, one IS2 and one IS5 using inverse PCR, and deleted all five extra insertional sequences.

We compared the growth rates of wild type and multi-deletional strains, and found them to be equal in minimal medium, with the latter growing slightly slower in rich medium. The multi-deletional strain showed elevated electroporation efficiencies in the case of 100 kbp-large BACs, but showed no elevation in the efficiency of small plasmid-electroporation. We compared the mutation rates of the two strains by measuring the inactivation frequencies of the *cycA* gene during growth, and the activation frequency of the *bgl* operon in the absence of growth. Our experiments showed that the inactivation of the *cycA* gene in the wild type strain is caused by IS element-insertions in 20-25% of the cases. We measured the overall inactivation frequency of this gene in the multi-deletional strain to be 20-25% lower than in the wild type. The difference among the two strains can thus be explained by the deletion of IS elements. Also, the *bgl* operon is activated in wild type in 90% of the cases by IS element-insertions into the regulatory region of the operon. The multi-deletional strain has a 90% lower overall activation frequency of this operon, which can also be explained by the lack of IS elements.

The results listed above demonstrate that, by careful planning, 14.1% of the genome of *E. coli* can be deleted without impairing the viability of the bacterial cell. Moreover, our work resulted in a strain that, due to its elevated electroporation efficiency and lower mutation rate, is advantageous for experiments involving téhe cloning of DNA fragments.

References

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