AUXOTROPH-BASED SCREENING STRATEGIES FOR MODIFIED HETEROCYCLIC BASE METABOLISM

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Purines and pyrimidines are fundamental heterocyclic molecules that emerged early in the evolution of life and therefore are concerved across all three Domains of Life. These compounds play essential role in nucleic acid structure, energy transfer and cofactor function, thereby regulating a wide range of cellular functions. In addition, heterocyclic base derivates are explored for their potential in pharmacological applications, as active ingredients in the theraphy for autoimmune diseases, cancer or viral infections [1]. Auxotrophic bacterial strains provide a powerful *in vivo* platform for metabolic studies, as their growth strictly depends on the restoration of specific biochemical functions. They help to determine the relationship between bacterial cell growth and protein synthesis, and the genes encoding corresponding metabolic functions [2]. During this study, different *Escherichia coli* auxotrophs were used to investigate the metabolism of modified heterocyclic bases using two complementary genetic strategies: metagenomic libraries and transposon mutagenesis.

Environmental genomic DNA isolated from soil samples was used to construct four independent metagenomic libraries in the pUC19 vector. These libraries were introduced into *E. coli ΔguaB::*Km and *E. coli ΔpurH::*Km auxotrophic strains, and growth complementation was assessed on minimal M9 medium supplemented with modified purine bases whose metabolic pathways remain poorly characterized in the literature. In parallel, specific growth phenotypes, including the ability of *E. coli ΔpyrF::*Km to utilize N^4-methylcytosine as uracil source [3] and *E. coli ΔguaB::*Km to grow in the presence of 2-amino-N^6-methylpurine, were investigated using transposon mutagenesis. Individual mutants were screened for loss of growth complementation, and selected candidates were subjected to genome sequencing for further analysis.

Overall, this study demonstrates the applicability of auxotroph-based systems for functional identification of enzymes involved in the metabolism of modified heterocyclic bases. Ongoing analyses aim to identify the genetic determinants underlying the observed growth complementation phenotypes.

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Keywords: Modified heterocyclic bases, auxotrophs, metagenomic libraries, transposon mutagenesis.

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