

## ENGINEERING THE MICROBIOTA TO TREAT METABOLIC DISORDERS

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Inborn errors of metabolism (IEM) are a family of more than 500 potentially lethal congenital genetic disorders that cumulatively affect 1 in 1000 newborns. In many IEMs, pathologies manifest as a result of improper metabolism of nutrients in food. In Phenylketonuria (PKU) for example, elevated levels of phenylalanine and the accumulation of aberrant metabolic intermediates in the system lead to acute and chronic toxicities. Resultantly, many disorders within this group are generally treated through lifelong nutritional management due to the lack of alternative and pharmacological options. Longitudinal studies have indicated that even with strict adherence to a diet of synthetic supplements, patients experience chronic issues like frailty, delayed growth, and intellectual disabilities. Recently, enzyme-replacement therapies (ERT) have demonstrated promise in pre-clinical and clinical settings by providing a metabolic sink for phenylalanine in PKU. As an enhancement to traditional ERT, we are developing a novel therapeutic for IEMs associated with amino acids by expressing metabolic enzymes in lactic acid bacteria (LAB) that natively colonize the human gastrointestinal (GI) tract. Starting with an enzyme under clinical development for PKU, phenylalanine ammonia-lyase (PAL), and by promoting the intestinal adhesion and colonization characteristics, the engineered LAB will intervene before amino acid absorption occurs in the small intestines during digestion. To engineer new enzymes with activities required for treating IEMs, we have developed a novel facile selection and screening methodology. This can potentially be utilized to enhance enzymatic properties or identify mutants with altered substrate specificity, creating a spectrum of PALs that can be used to treat IEMs associated with other amino acids. Here we describe the methodology, development, and optimization of this method. To characterize and engineer microbial adhesion to intestinal mucus, we developed a novel assay that is able to capture the quantitative and mechanistic binding thermodynamics of cells to mucus. We will discuss the development of this assay and its implementation for engineering improved mucus binding. The platform technologies discussed here will be instrumental in realizing microbiota-based therapeutics as an emerging and urgently-needed treatment for IEMs that currently have inadequate or no options.