AN ENGINEERED GASTROINTESTINALLY STABLE MICROBIAL LEUCINE DECARBOXYLASE FOR POTENTIAL TREATMENT OF MAPLE SYRUP URINE DISEASE

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Maple syrup urine disease (MSUD), a rare inborn error of branched-chain amino acid metabolism (IEM), is characterized by toxic accumulation of leucine in the brain, blood, and urine leading to life-threatening episodes of acute metabolic decompensation. Early diagnosis and a life-long leucine-restricted diet can improve patient outcomes and reduce the frequency of acute metabolic decompensation events. Unfortunately, the low protein diet can be difficult to maintain, and some patients have elevated leucine levels despite dietary compliance. To potentially enable greater diet flexibility while maintaining safe leucine levels, we are developing a gastrointestinal (GI)-stable enzyme that degrades dietary leucine prior to its systemic absorption. Starting with a bacterial leucine decarboxylase (LDC), we used the CodeEvolver® protein engineering platform to improve the enzyme's stability to gastric acid, pepsin, and intestinal proteases. Through nine iterative rounds of evolution, we subjected over 16,000 variants to multiple high throughput screening assays. We used either liquid chromatography-mass spectrometry (LC-MS) or RapidFire high throughput mass spectrometry to measure isopentylamine, the decarboxylation product of leucine catalyzed by the LDC. The LDC variants were subjected to different selective pressures (pH, pepsin, intestinal proteases) either independently or sequentially to mimic the conditions the enzyme would be exposed to in the GI tract. In our engineering campaign, we identified several lead variants with improved *in vitro* properties. The wildtype LDC was completely inactive after one hour incubation in the presence of 0.8 g/L pepsin at pH 4 (gastric condition) and in 4 g/L trypsin and 0.5 g/L chymotrypsin (intestinal condition), whereas lead variants maintained >80% activity under similar conditions. Lead variants were evaluated for efficacy in an intermediate MSUD (iMSUD) mouse disease model as well as in healthy non-human primates. Treatment of iMSUD mice with an LDC variant following a high-protein meal led to suppression of plasma leucine area under curve (AUC) by up to 45% compared to vehicle-treated mice ($p<0.001$). LDC variants also suppressed leucine and alpha-ketoisocaproic acid (KIC) AUC in healthy non-human primates following a whey meal in a dose-dependent manner. Collectively these data support the application of CodeEvolver® technology to engineering GI stable enzymes and suggest that engineered LDC variants offer promise as a potential treatment for MSUD patients.