Designing metabolically enhanced next-generation probiotics to eradicate multi-drug resistant bacteria from the gut

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Abstract (max 350 words)

Healthcare-associated infections, especially those caused by multidrug-resistant (MDR) bacteria, are a leading cause of morbidity and mortality worldwide[1]. Gut colonization with MDR bacteria often precedes infections and facilitates patient-to-patient transmission, necessitating costly isolation measures. While antibiotics effectively treat acute infections, their preventive use is limited due to detrimental impacts on the gut microbiota and promotion of further resistance in nosocomial pathogens. Consequently, innovative strategies to selectively eliminate MDR pathogens from the gut are urgently needed.

The human microbiome, a diverse ecosystem of microorganisms, offers a promising source for novel therapeutic agents. Microbiota-based interventions, such as fecal microbiota transplants (FMTs), have shown remarkable success in treating recurrent *Clostridioides difficile* infections (>90% cure rates)[2–4]. However, FMTs are less effective against gram-negative MDR bacteria (GN-MDRs)[5], likely due to the metabolic flexibility of these pathogens, which enables them to persist despite intervention.

Our project aims to address this limitation by developing live biotherapeutic products (LBPs)—defined microbial consortia designed to synergistically close the metabolic niches of MDR bacteria in the gut. LBPs improve upon traditional FMTs due to their controlled composition, reducing safety risks and simplifying production and regulatory approval.

We have identified specific strains within the Enterobacteriales family as critical components of these consortia [6-9]. So far, Enterobacteria were largely excluded from probiotic strain mixtures due to safety concerns but we could prove that careful strain selection and genetic engineering shows that safe enterobacterial can be selected for use in humans. Our findings highlight their essential roles in suppressing GN-MDR pathogens. These strains perform key functions, including metabolizing carbon sources critical for pathogen growth, degrading antibiotic residues and oxygen to support the recovery of anaerobic bacteria, and contributing to down-regulation of intestinal inflammation[6-9].

The efficacy of our LBP candidates has been demonstrated in multiple in vivo models, including immunocompromised mice, humanized microbiota models, and those consuming human-like diets. These results underscore the importance of interdependent interactions between Enterobacteria and

strict anaerobes in restoring colonization resistance. This approach represents a significant advancement in microbiome therapeutics, enabling the development of precise bacterial cocktails to combat MDR pathogens effectively and sustainably.

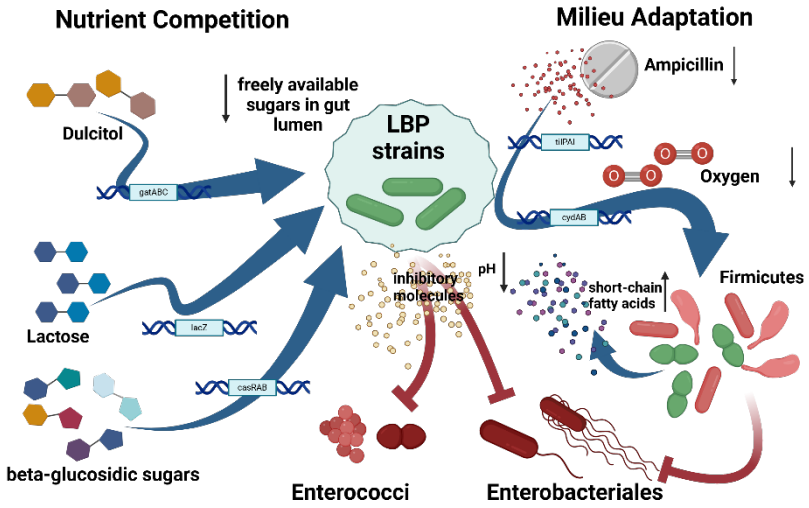

Figure 1: Enterobacterial strains are key components in LBP development fulfilling multi-factorial roles in competition against MDR pathogens

Keywords

Microbiome; Microbiome-based therapies; Microbiota-context; Next-generation-probiotics; ESKAPE pathogens

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