

## **Riboflavin-overproducing *Limosilactobacillus reuteri* for biofortification of fermented foods**

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**Introduction:** Up to 50% of the population in developing countries displays riboflavin deficiency with negative impact on energy levels, reproductive function, lactation and pregnancy outcomes. This essential, water-soluble vitamin B2 is important for macronutrient and energy metabolism, and has antioxidant effects. Lactobacilli are promising candidates to simultaneously ferment and fortify fermented foods with vitamins, while additionally producing anti-pathogenic and immunomodulatory bioactive molecules.

**Methods:** Within the Isala citizen science project on women's health, we isolated over 2000 bacterial strains. Here, 74 of these were genomically and functionally characterized for antimicrobial, epithelial barrier and immunostimulatory capacity. Riboflavin production was evaluated in laboratory media as well as in a milk or plant/derived matrices. Active transport riboflavin by intestinal Caco-2 cells, and passive transport in a gastrointestinal dialysis model was also evaluated, as well as survival.

**Results:** *Limosilactobacillus reuteri* AMBV339 showed a riboflavin production of 18.7 µg/mL, the highest described for non-genetically modified lactobacilli, with concomitant increase of riboflavin content in coconut and butter milk. AMBV339 also displayed anti-pathogenic, epithelial barrier-enhancing and immunostimulatory properties. The strain survived for 72h in a gastrointestinal dialysis model while releasing an active and passive stable riboflavin concentration with minimal gut microbiome modulation.

**Discussion:** We establish the properties of an efficient riboflavin-overproducing *L. reuteri* AMBV339 isolated from a healthy volunteer and its potential for fermented food fortification and implementation as a probiotic for the gut and potentially other body niches. Our results suggest that AMBV339 could provide food enrichment and health benefits in humans which could provide cost-effective solution, especially in disadvantaged populations.