

The SIMPSONS Project: Screening and Induction of Microbial Probiotics for Sweetener-Optimized Novel Strain

Jung, Hyunwoo (School: Sejong Academy of Science and Arts)

Ahn, Jihoo (School: Sejong Academy of Science and Arts)

Artificial sweeteners are widely used sugar substitutes, and their effects on gut microbiota remain controversial. This study investigates the antimicrobial effects of artificial sweeteners and explores the mechanisms by which specific probiotics acquire resistance. Previous studies have shown that artificial sweeteners can inhibit the growth of certain gut bacteria, but whether probiotics can develop resistance through prolonged exposure remains unclear. To address this, the antimicrobial effects of Acesulfame potassium (Ace-K) and Saccharin were evaluated, along with the potential for resistance induction in *Lactobacillus casei* and *Lactiplantibacillus plantarum*. The minimum inhibitory concentration (MIC) experiment revealed that the MIC of Ace-K was 1.7%, while that of Saccharin was 0.17%. Serial passaging experiments showed that *L. casei* gradually recovered growth over generations when exposed to Ace-K. RT-PCR and qRT-PCR analyses demonstrated a 2.5-fold increase in *dnaK* (a stress response gene) expression in *L. casei* treated with Ace-K, indicating gene regulation related to artificial sweetener resistance. This study demonstrates that artificial sweeteners not only exhibit antimicrobial effects on beneficial gut bacteria but may also induce resistance in certain probiotic strains through continuous exposure. Additionally, selecting resistant probiotic strains could facilitate the development of formulations capable of maintaining gut balance in environments with artificial sweeteners. These findings highlight the importance of further research on gut microbiota balance and the long-term safety of artificial sweeteners while laying the groundwork for functional probiotic development.

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