Supplementation of Citrulline with Lactic Acid Bacteria Protects Foodborne Pathogens Adhesion and Improves the Cell Integrity on the Intestinal Epithelial Cell

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Abstract: Lactic acid bacteria (LAB) have shown the beneficial effects on human gastrointestinal tract, such as protects diarrhea induced by lactose intolerance or enteric pathogens. Citrulline is a non-protein amino acid and also the precursors of arginine and nitric oxide, it has shown to enhance intestinal barrier function. Citrulline has shown to improve the growth of some strains of LAB, it is important for LAB to have a sufficient cell concentration to contribute the effects. Therefore, the aims of this study were to investigate the effect of combining citrulline with LAB on the anti-adhesion effect against pathogens and the effect on the cell integrity. The effect of citrulline on selected LAB was determined by incubating in 0%, 0.1% or 0.2% citrulline enriched MRS broth for 18 h. The adhesion ability of LAB and the anti-adhesion effect of LAB and citrulline against pathogens were performed on IPEC-J2 cell line. Transepithelial electrical resistance (TEER) assay was used to measure the tight junction (TJ) integrity. TJ proteins (claudin-1, occludin and zonula occluden-1 (ZO-1)) were determined by western blot analysis. It found that the growth of Lactobacillus helveticus ASCC 511 was significantly stimulated by 0.2% citrulline compared with control during 18 h fermentation. The adhesion of L. helveticus ASCC 511 and Lactobacillus delbrueckii ssp. bulgaricus (L. bulgaricus) ASCC 756 was increased when supplemented with citrulline. Citrulline has shown significant inhibitory effect on the adhesion of Escherichia coli PELI0480 (O157:H7), Shigella sonnei ATCC 25931, Staphyloccocus aureus CMCC26003 and Cronobacter sakazakii ATCC 29544. The anti-adhesion effect of L. helveticus ASCC 511, L. bulgaricus ASCC 756 and Lactobacillus paracasei ASCC 276 against Cronobacter sakazakii ATCC 29544 was significantly enhanced with citrulline supplementation. Treatments with citrulline and LAB were able to maintain the TEER of IPEC-J2 cell and shown the positive effect on the TJ proteins. In conclusion, citrulline had stimulating effect on some strains of LAB and determined to improve the adhesion of LAB on intestinal epithelial cell, to enhance the inhibitory effect on enteric pathogens adhesion as well as had beneficial effects on maintaining cell integrity. It implied LAB supplemented with citrulline might have advantageous effects on gastrointestinal tracts.

Keywords: citrulline, lactic acid bacteria, amino acid, anti-adhesion effect, cell integrity

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