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Author: Navid- Azarm

Title: Synthesis and characterization of postbiotic characteristics of *Lactiplantibacillus plantarum* and *Bifidobacterium animalis* enriched with scallion (*Allium schoenoprasum*) hydro-alcoholic extract in cheese whey.

Abstract

Postbiotics are bioactive compounds produced by probiotic bacteria during their metabolic activity in the digestive system, exhibiting beneficial effects on human health, including antimicrobial and antioxidant properties. This study investigated the preparation and characterization of postbiotics derived from *Lactobacillus plantarum* and *Bifidobacterium animalis* enriched with an aqueous-ethanolic green onion (*Allium fistulosum*) extract in a whey-based culture medium. First, the green onion extract was prepared using an aqueous-ethanolic extraction method and then added to the whey medium at concentrations of 1% and 2%. The two probiotic strains used in this study—*Bifidobacterium animalis* (BB12) and *Lactobacillus plantarum* (PTCC 1745)—were activated and cultured in the whey medium, after which their postbiotics were extracted. The antioxidant and antimicrobial activities of the postbiotics were evaluated using DPPH and ABTS assays for antioxidant capacity, as well as agar well diffusion and microbroth dilution methods against pathogenic bacteria, including *Salmonella* Typhimurium (ATCC 14028), *Listeria monocytogenes* (ATCC 19115), *Staphylococcus aureus* (ATCC 6538), and *Escherichia coli* O157:H7 (ATCC 25922). The results of the antioxidant activity assays (ABTS and DPPH) indicated a significant increase in antioxidant potential in postbiotics containing the green onion extract. In both methods, higher concentrations of the extract led to a notable improvement in antioxidant activity. The largest inhibition zones were observed for the treatment combining *Bifidobacterium* and *Lactobacillus* with 1% scallion extract against the pathogens *Listeria monocytogenes* and *Staphylococcus aureus*, measuring 12.25 mm and 24.77 mm, respectively. Similarly, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values demonstrated the strongest inhibitory effects in treatments combining both bacterial strains with the 1% extract. These findings suggest that postbiotics enriched with green onion extract hold promising potential as natural additives in the food and pharmaceutical industries to enhance gut health, improve food preservation, and protect against pathogens. For future research, it is recommended to evaluate the long-term effects of these postbiotics in animal and human models, as well as to explore their molecular mechanisms to optimize industrial applications—particularly in food processing (e.g., meat and dairy products), pharmaceuticals, and even food packaging—to leverage their benefits in preventing spoilage, extending shelf life, and ensuring food safety.

Keywords: Synbiotic, Scallion, *Lactiplantibacillus plantarum*, *Bifidobacterium animalis*, Whey.

