Abstract

Lactic acid bacteria (LAB) have garnered significant attention for their remarkable probiotic potential, which plays a vital role in promoting gut health and enhancing overall well-being. Beneficial microorganisms, particularly LAB, are primarily present in traditional fermented foods, which represent a rich source of diverse strains of LAB. Each strain possesses distinct functional properties that can positively influence human health. The aim of this study was to evaluate the anti-obesity and anti-gastric ulcer activity of probiotic lactic acid bacterium isolated from traditional fermented foods. Considering this, a comprehensive isolation process was conducted, resulting in the isolation of 12 distinct lactic acid bacterial colonies from a variety of fermented products, including *idli* batter, *dosa* batter, and *haria* (a rice-based fermented beverage). The standard plate method was employed for LAB isolation using Rogosa SL agar media (supplemented with 0.132% acetic acid). The morphological analysis confirmed that all strains were rod-shaped, Gram-positive, non-motile, and non-spore-forming. The negative test for Indole, Methyl Red, Voges-Proskauer, and citrate utilization confirmed their reliance on carbohydrate fermentation. The enzymatic analysis revealed that the isolates might exhibit significant α-amylase, glucoamylase, and protease activity.

Further *in vitro* probiotic characterization of the isolated LAB was conducted. Based on the cumulative probiotic score, we selected the E2_MCCKT strain for further investigation. After 120 min of incubation, the E2_MCCKT strain exhibited the highest survivability at different pH levels. The survival rate of the E2_MCCKT strain was 98.4% at pH 2, 99.3% at pH 3, and 100% at pH 6.8, which was statistically nonsignificant. Similarly, the E2_MCCKT strain demonstrated significant resistance to bile salts and could endure 0.3% and 2% bile salt concentrations for 120 min. The survival rate of the strain was 98.8% in 0.3% bile and 97.69% in 2% bile salt after 120 min of incubation. Concurrently, the E2_MCCKT strain exhibited moderate autoaggregation ability (52.52%) and surface hydrophobicity (38.08%), which was higher in comparison to other isolates. The antibiotic susceptibility tests indicated that the bacterium had intermediate sensitivity to ampicillin and chloramphenicol. The bacterium was identified as *Lactiplantibacillus plantarum* E2_MCCKT through 16S rRNA gene sequence analysis. E2_MCCKT exhibited non-haemolytic activity, making it a safe candidate for probiotic applications.

Subsequently, E2_MCCKT was tested on obese mice to demonstrate its potential anti-obesity effects. The initial body weight for all male albino mice was 15.39±0.19g, and they were acclimated for 10 days in standard conditions (32±2°C and 50% humidity) with a 12-hour

light/dark cycle. The mice were provided with regular food and free access to water. Mice were randomly selected and divided into three groups (n=5) based on the food and treatment. The first group, known as the normal diet (ND), was fed a meal consisting of foods with a ratio of carbohydrate, protein, and fat (64.2:22.3:13.5). The second group was the high-fat diet (HFD), which received lab-made food with a ratio of carbohydrates, protein, and fat (38.9:22.2:38.9). The high-fat treatment group (HFT) was the final group; it received a high-fat diet for 8 weeks and probiotic *Lp. plantarum* E2_MCCKT (10^9 CFU/ml) for the final 4 weeks. The HFT group received treatment for the probiotic bacteria four weeks following the onset of obesity.

The final average body weight and BMI were found to be high in HFD mice after the eight-week investigation period. Furthermore, the HFD group had the greatest levels of total serum cholesterol, triglycerides, LDL, and VLDL when compared to the other groups. In contrast, the *Lp. plantarum* E2_MCCKT treatment was able to reduce these parameters drastically, and the results were comparable to those of the ND group. The HFD group had higher SGOT and SGPT levels, which may be a sign of harm to the liver cells or fat buildup and adipocyte proliferation. The bacterial therapy increased mRNA levels of lipolytic transcription factors, including peroxisome proliferator-activated receptor-α, potentially leading to higher expression of fatty acid oxidation genes like acyl-CoA oxidase and carnitine palmitoyl-transferase-1. Alongside, the down-regulation of the genes for sterol-regulatory element-binding protein-1c (2.23-fold), acetyl-CoA carboxylase (2.54-fold), and fatty acid synthase (1.61-fold) represented the simultaneous stoppage of both adipocytogenesis and fatty acid synthesis. E2_MCCKT significantly reduced the expression of pro-inflammatory cytokines [IL-1Ra (1.19-fold) and TNF-α (2.26-fold)] while increasing IL-10 (1.07-fold) in the protein expression investigation.

Moreover, the gastroprotective effects of *Lp. plantarum* E2_MCCKT was investigated using a cold-induced gastric ulcer mice model. Gastric ulcer is a chronic gastrointestinal illness characterized by a significant disruption in the mucosal barrier. The adult male mice (28.4±1.62 g) were randomly divided into three groups (n = 10): normal (N) group, positive control (PC) group, and *Lp. plantarum* E2_MCCKT treatment (T) group. The oral supplementation of the *Lp. plantarum* E2_MCCKT strain (10^9 cells daily with a standard diet) significantly reduced cold-induced gastric erosion in the stomach mucosa after 30 days. The histopathological study clearly showed that probiotic *Lp. plantarum* E2_MCCKT strain could protect the stomach epithelial cells from cold injury. Moreover, *Lp. plantarum* E2_MCCKT strain might regulate

relative mRNA expressions corresponding to gastric inflammation, such as up-regulation of IL-10 (2.31-fold) and down-regulation of IFN-γ (2.17-fold), IFN-λ (10.05±0.03-fold), and IL-12 (1.31-fold). In the context of protein expression study, *Lp. plantarum* E2_MCCKT significantly increased IL-10 (1.06-fold) and PPAR-α (1.13-fold) expression while decreasing inflammatory cytokines [IL-6 (1.18-fold), and PPAR-γ (1.11-fold)] expression compared to PC group, which indicated the anti-inflammatory activity of the strain during cold-induced gastric ulcer.

In conclusion, this study highlighted the promising probiotic potential of *Lp. plantarum* E2_MCCKT, in combating obesity and gastric ulcers. *In vitro* analysis showed strong survival in acidic and bile salt conditions and beneficial autoaggregation and hydrophobicity properties. *In vivo* studies in obese mice indicated that *Lp. plantarum* E2_MCCKT reduced weight gain, improved lipid metabolism, and lowered inflammation by modulating relevant transcription factors and inflammatory markers. A gastric ulcer model also demonstrated significant protection by reducing gastric erosion and inflammation. Hence, the probiotic *Lp. plantarum* E2_MCCKT might be an alternative therapeutic agent for obesity and cold-induced gastric ulcer management.

Keywords: Probiotics, lactic acid bacteria, obesity, gastric ulcer, inflammation