



***In vitro* gastrointestinal survival of vegetative cells and spores of novel probiotic *Bacillus* strains**

Ayşe Avcı, Fikriye Alev Akçay, Elif Sezer*, Esma Alemdar, Aisha Dahir Ahmed, İnci Cerit

Sakarya University, Faculty of Engineering, Department of Food Engineering, 54050, Sakarya, Türkiye.

Abstract

Probiotics must retain a high level of viability while passing through the gastrointestinal tract, where they are exposed to harsh conditions such as high bile salt concentrations, low pH, and digestive enzymes. The *in vitro* simulated digestion model is based on exposing probiotics to these sequential conditions [salivary juice (SSJ), gastric juice (SGJ), and intestinal juice (SIJ)] mimicking the digestion system. In the present study, the tolerance of both vegetative cells and spores to the *in vitro* simulated gastrointestinal system was evaluated. The vegetative cells or spores were sequentially subjected to SSJ (5 min), SGJ (2 h) and SIJ (2 h). All the reactions were performed at 37 °C, and the samples were centrifuged to remove the liquid phases after each step. The samples were taken after each step to make a bacterial count using nutrient agar plates. Logarithmic survival rate of the microorganisms was calculated. The results showed that both the vegetative cells and the spores were highly resistant to all the digestive system conditions. The minimum viability at the end of the SIJ was 81.8%, while most of the isolates had the survival rates over 90%. Overall, these findings indicated that the tested isolates, in both vegetative and spore forms, possess remarkable resilience to gastrointestinal conditions, highlighting their strong potential as effective probiotic candidates for survival and functionality within the human digestive system.

Keywords: *Bacillus*, Probiotic, *in vitro*, simulated gastrointestinal

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