**Improving the survival of probiotics via in situ re-culture in calcium alginate gel beads**

Fang Zhong1,2,3,4, Yongkai Yuan1,2,3,4, and Katerina Pissaridi5

*1State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, China*

*2Science Center for Future Foods, Jiangnan University, Wuxi 214122, China*

*3School of Food Science and Technology, Jiangnan University, Wuxi 214122, China*

*4International Joint Laboratory on Food Safety, Jiangnan University, Wuxi 214122, China*

*5YIOTIS S.A., 128-130 Kifisou Av., Athens 12131, Greece*

Cell-mediated chemistry is an emerging strategy that leverages the metabolic process of living cells to build advanced materials. Here, a simple yet versatile microbe-mediated in situ re-culture approach using calcium alginate gel beads to improve the survival of probiotics was reported. After re-culture, Lactobacillus rhamnosus GG (LGG) in both liquid and solid core gel beads (LCGB and SCGB) exhibited 100% gastric acid resistance, while the bile salt resistance varied from 59.38% to 92.39%. LGG in LCGB generally showed higher bile salt resistance than SCGB, and the resistance would be further improved with high initial bacterial concentration due to more extracellular polymer secretion. Besides, the re-cultured LGG in beads exhibited survival of 95.02%-96.05% in calcium-supplemented MRS broth within 6 weeks at 4 °C. And the survival of the re-cultured LGG in LCGB was more than 90% in yogurt, milk, milk tea, and juice respectively within 6 weeks at 4 °C, followed by semi-solid jelly (85.81%). The addition of inulin had no adverse effect on the above storage survival of re-cultured LGG in LCGB, indicating the possibility of the construction of synbiotic. Combined transcriptome and metabolome analysis of LCGB implied that the mechanism of LGG damage by gastric acid included 8 pathways, among which, 2 pathways including propanoate metabolism, phenylalanine, tyrosine and tryptophan biosynthesis were responsible for the improved gastric acid resistance of re-cultured LGG. The unique liquid core matrix presents a powerful platform for the formation of probiotics in biofilm state, enabling the integration of probiotics with high density, high gastrointestinal resistance, and commercial production feasibility.