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Full name of doctoral student: Sanling Zuo

Album no.: 503511

DOCTORAL DISSERTATION ABSTRACT

Scientific discipline: Health sciences

Title of the doctoral dissertation: Metabolic signatures of probiotic and prebiotic functions in the intestine cells

Doctoral dissertation abstract:

Background: Gut health has an important impact on maintaining host health. Probiotics and prebiotics have an direct or indirect impact on gut health by modulating gut microbiota profiles and their metabolism. The novel predictive *in vitro* models are required to efficiently design prebiotic and probiotic formulations with proven bioactivity, for *in vivo* applications.

Objective: This study aimed to investigate as to how the two selected probiotics and prebiotics regulate the gut health of the host at the systemic level by identifying the metabolic signatures of these bioactive compounds using the animal and human *in vitro* and *in ovo* models.

Methods: This study consisted of three parts: 1/ Analysis of the metabolic footprint of the selected candidate probiotics cultivated with supplementation of the selected prebiotics: fish protein hydrolysate (F466Q022) and liquid seaweed extract (A114P252). 2/ *In vitro* characterization of the intestinal cells interacting with *Bifidobacterium lactis* NCC2818 (or its supernatant) and *Bacillus* strain (or its supernatant) 3/ Metabolic footprint and fingerprint of probiotic function in the synbiotic culture. *Bifidobacterium lactis* NCC2818 and *Bacillus* strain were cultivated in a medium containing 2% [v/v] of the two pre-selected prebiotic compounds (prebiotic fish protein hydrolysate and liquid seaweed extract) at 37°C for 24 h.

Results: Metabolite analysis of the supernatant samples was performed by gas chromatography-mass spectrometry (GC-MS). Differential metabolite analysis showed changes in the profiles of specific organic acids: lactic acid, citric acid, acetic acid, phosphoric acid ($p<0.05$). The metabolic pathway enrichment analysis showed that metabolic pathways such as amino acid metabolism, energy metabolism, and two-component signal transduction system were activated. Moreover, the dual species *in vitro* intestinal epithelial cell monolayer model was established to simulate responses of the host intestinal epithelial cells: the new chicken cell line Chick8E11 and the human cell line Caco-2 were co-cultivated with *Bifidobacterium lactis* NCC2818 (or its supernatant) and *Bacillus* strain (or its supernatant) at a ratio of 10:2 (bacteria: cells) for 24 hours. The observed changes in the gene and protein expression levels of tight junction cytokines Villin, Cytokeratin 18, Zonula Occludens 1, and Occludin indicate that *Bifidobacterium lactis* NCC2818 can enhance the tight junction of the intestinal barrier in the *in vitro* intestinal cell co-culture and ensure the normal physiological regulation process of the cells. The identified effect of *Bacillus* strain on intestinal health was apparent by the enhanced adherence of the beneficial species to the intestinal cells. The differential and enrichment analyses showed, that *Bifidobacterium lactis* had a positive effect on the barrier function of intestinal cells, and supported the normal intestinal cells function by regulating amino acid metabolism, protein digestion and absorption.

Conclusion: This study developed a basis to explore the predictive value of *in vitro* intestinal models in metabolomics and microbiology to track the functional footprint and define the potential of novel candidate probiotics and prebiotics separately and in their synergistic combination. Moreover, the results of this thesis have been used for the further *in vivo* (*in ovo*) application, as the part of a vaster research project Ovobiom- National Science Centre *UMO-2019/35/B/NZ9/03186*: Probiotics and prebiotics- their effect on the chicken intestine health and organism performance, from the pre-hatch period to the mature animal, to elucidate the mechanisms of their mode of action through early microbiome modulation.

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doctoral student's signature