FACULTY OF CHEMISTRY AND PHARMACY
UNIVERSITY OF OPOLE

Prof. dr hab. inż. Piotr P. Wieczorek
e-mail: Piotr.Wieczorek@uni.opole.pl

Collegium Chemicum
ul. Oleska 48, 45-042 Opole
www.wch.uni.opole.pl
chemia(@uni.opole.pl

Opole, 2025-09-18

REVIEW OF THE DOCTORAL DISSERTATION BY

Sanling Zuo

entitled

„ Metabolic signatures of probiotic and prebiotic functions in the intestine cells"

The doctoral dissertation of Sanling Zuo, presented for evaluation, was carried out at the Faculty of Health Sciences Collegium Medicum in Bydgoszcz Nicolaus Copernicus University in Toruń under the supervision of Professor Katarzyna Stadnicka and Professor Przemysław Kopsobucki as co-supervisor. The dissertation is a part of the *OVOBIOM* Project, financed by NCN. Thus the aim of this dissertation was studying of the effect of prebiotics and probiotics on gut health regulation, which is the scope of research conducted by the dissertation supervisors.

General aspects

In recent years, more and more attention has been paid to human health problems which largely influences on bowels. Therefore, interest is growing about gut health. Gut health is a general term for the well-being of the digestive system, including balance of the bacterial microflora in the intestines and is a kay centre regulating the functioning of the entire body. It's here the most immune cells are concentrated, and trillions of bacteria and yeasts create a complex microbiome that influences metabolic processes, nutrient absorption and the production of many metabolites including vitamins, hormones or neurotransmitters like serotonin, known as the "happiness hormone". Therefore, it can be characterized by the absence of gastrointestinal complaints and the promotion of proper digestion, metabolism, immunity, and metabolite production. Natural bioactive ingredients can be effective regulators of these metabolic processes and thus gut health. Probiotics and prebiotics show a potential to influence the host intestine health by modification of the intestinal metabolism through microbial and host cell microbiota and the host gastrointestinal environment.

Therefore, taking into account the above, it was decided to investigate haw selected probiotics and prebiotics regulate the gut health of the host at the systemic level by identifying the metabolic signatures of bioactive compounds using the animal and human *in vitro* and *in ovo* models.

Relevance and theoretical framework

The crucial organ in the gastrointestinal system is intestine, which plays a fundamental role in maintaining overall health and well-being. This organ is responsible for a variety of essential functions including nutrient absorption, waste processing, and acting as a barrier against harmful substances. Understanding the diverse functions of the intestine and the factors that can disrupt them is crucial for preventing and managing a wide range of diseases and pathological conditions. Many natural ingredients can constitute sustainable and effective modulators of gut health. Prebiotics and probiotics, among others, have scientifically proven potential to directly or indirectly influence the health, function and structure of the host's intencines. Therefore prebiotics and probiotics play crucial, positive roles in promoting human nutrition and health. Probiotics are microbes that are able to reinforce the intestinal mucosal barrier help maintain and stabilize the intestinal flora, resist pathogenic colonization in the gastrointestinal tract, modulate the immune system, improve vitamin synthesis, absorption of mineral salts, and enhance the production of short-chain fatty acids, bacteriocin, and other antimicrobial substances. Whereas prebiotics are compounds that cannot be digested by the host, but can selectively stimulate the growth and activity of beneficial gut bacteria, such as Bifidobacteria and Lactobacillus. Therefore they promote the reproduction and metabolism of intestinal probiotics for the health of body. It has been confirmed that probiotics have clinical or health care functions in preventing or controlling intestinal, respiratory, and urogenital infections, allergic reaction, inflammatory bowel disease, irritable bowel syndrome and other aspects. Defining and understanding the complex interaction of microbiota with the host gastrointestinal tract environment requires a broad range of multi-disciplinary studies including metabolomics, transcriptomic and metagenomic. Understanding these mechanisms will enable us to identification and determine the usefulness of new candidates for probiotics and prebiotics.

Therefore, it is understandable that the aim of the doctoral dissertation of Sanling Zuo was investigate how 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 vivo* models.

The studies presented in Sanling Zuo thesis are devoted to the analysis metabolic footprint of selected two prebiotics. As a prebiotics she chose fish protein hydrolysate and liquid seaweed extract. In vitro characterisation the intestinal cells interaction with two probiotic bacteria strain and their supernatants (*Bifidobacterium lactis* and *Bacillus*), and also metabolic footprint and fingerprint of probiotic function in the symbiotic culture of these bacteria strain and two selected

prebiotic compounds (prebiotic fish protein hydrolysate and liquid seaweed extract). As a model intestinal cells monolayer for simulate *in vitro* responses the host she selected new chicken cell line Chick8E11 and human cell line Caco-2. The research has established a translational model linking in vitro and in ovo effects of probiotics and prebiotics, and the obtained results allowed to identified key probiotic metabolic signatures and mapped the affected pathways in amino acid metabolism, energy production and antioxidant defence. These functional changes translate into improved barrier function, improved energy metabolism, redox balance and an enhanced microbiome. However, the research from *in ovo* results show the synergistic effects of combination *B. lactis* with Astragalus polysaccharides, and between *Bacillus* with protein hydrolysate.

The results of these in vitro studies can be used to predict the effects of novel ingredients in the host, developing metabolomics strategies for future in vivo applications to specifically modulate gut health. Gut health modulation is a promising, strategic preventive measure for sustainable regulation of animal health to eliminate outbreaks of foodborne pathogens that pose a threat to public health. The application of sensitive and responsive cell models such as Caco-2 and Chic-8E11 contributes to the development of safe and effective combinations and doses for in vivo probiotic-based treatments.

Overall, the obtained results are very interesting and may have a number of applications, including improved probiotic screening methods, further validation in post-hatch chicken, and the potential development of personalized probiotic formulations tailored to specific needs.

Methodology

Due to the nature of the project Sanling Zuo used and optimized a wide variety of research methods from many fields, which required the author's interdisciplinary competence. She optimized the cell and probiotic culture growth condition and established cell monolayer and spheroid as intestinal co-culture models. The obtained models was characterized using MTT colorimetry, confocal microscope and fluorescent staining. For evaluation the changes in tight junction related gene expression on epithelial intestinal cells co-cultures with probiotic used qPCR. The metabolites were analysed by gas chromatography with mass detector (GC-MS), while for short chain fatty acids she optimized specific sample pretreatment procedure with derivatization step. GC-MS was also used for metabolic footprint and fingerprint determination and metabolomic analysis of chicken intestinal contents after *in ovo* injection of two candidate probiotics, prebiotics and synbiotics combination. To compare the metabolite analysis, it is necessary to use appropriate statistical methods, which enabled the determination of differences for individual samples. For this purpose, the PhD student used principal component analysis (PCA) to observe the overall

distribution between samples and supervised partial least square analysis (PKS-DA) and orthogonal partial last squares analysis (OPLS-DA). Additionally for extract the major information from a large number of sample data she used One way Anova for generate the general conclusions from the sample data, by applying the interval estimation and statistical hypothesis testing,

Bibliography

The literature section is supported by numerous current and well-selected citations from the literature. Based on the literature review, Sanling Zuo formulated the goal of the work.

Discussion and conclusions

The discussion of the results of the experiments and conclusions, that have been drawn, are also very nicely presented in the thesis. In my opinion, the summaries of professional accomplishment were very well prepared, with a comprehensive literature review of the subject and detailed discussion of the results.

In summary, I'm impressed by the broad scope of the research and the results achieved. Sanling Zuo demonstrated extensive knowledge of numerous aspects of various disciplines, from chemistry, through biological and medical sciences, to the application aspects of the research. The work is interdisciplinary in nature and required knowledge of many different topics, which She mastered admirably.

The thesis are written very well. I found just a few minor mistakes of typographical errors which are not even worth mentioning.

In conclusion, I would like to state that the dissertation presented for review represents a high level of research and contains many elements of scientific novelty. The scope of the research, the experimental results contained in it, the manner of interpretation and inference indicate that Sanling Zuo, has demonstrated the ability to conduct scientific research independently and has made a significant contribution to the development of research in the Health Sciences, especially improved methods for probiotic and prebiotic influence on health. Having examined the dissertation of Sanling Zuo **I conclude that the presented dissertation meets all the requirements set forth in the Act on Academic Titles and Degrees for doctoral theses and I request its admission to further stages of the doctoral dissertation procedure.**

Taking into consideration above I am assessing the replaced work very good, and I propose to award it a ***summa cum laude*** distinction.

P. Ulicznych