



Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Department of Molecular Sciences

03/01/2025



The Scientific Council of the
Hirsfeld Institute of Immunology
and Experimental Therapy

Review of the PhD Thesis by Katarzyna Pacyga-Prus

I have reviewed the PhD thesis entitled “Biological activity of *Bifidobacterium animalis* ssp. *animalis* CCDM 218 and *Bifidobacterium adolescentis* CCDM 368 surface antigens in complex interactions with the host organism - prevention/treatment of allergy diseases” by Katarzyna Pacyga-Prus for awarding the degree of doctor. I have assessed the scientific level and novelty of the thesis, the impact of the work on the discipline and whether the thesis demonstrates scientific independence of the PhD candidate. My conclusions are summarized below, followed by a final assessment of the PhD thesis.

Scientific level and novelty

The PhD thesis describes a comprehensive investigation of two strains of Bifidobacteria in terms of structural analysis of bacterial polysaccharides and their potential use for treating airway allergies. One of them (Ban218) produced a phosphorylated polysaccharide with a nonasaccharide repeating unit (B.PAT), which was investigated for immunomodulatory functions both in its phosphorylated and dephosphorylated form. Mouse cell lines showed increased immunomodulatory functions when exposed to the dephosphorylated form compared to the phosphorylated form (Paper 2). However, B.PAT or its dephosphorylated form did not stimulate splenocytes from mice with allergic airway inflammation (Supplementary data). Therefore, B.PAT was not investigated further as an anti-allergic agent.

The other strain (Bad368) produced a polysaccharide with a hexasaccharide repeating unit (BAP1; Paper 1). This polysaccharide showed beneficial immunomodulatory properties in mouse cell lines and was later investigated on a mouse model with ovalbumin-induced allergy (Paper 3). Intranasal administration of BAP1 showed reduced allergic inflammation, which makes it a promising candidate for treating airway allergies. In addition, the mechanism of action was investigated from the up- and downregulation of certain genes.

The studies are clearly described in the thesis, with well-presented aims and conclusions. The PhD project has been compiled with a very clear overall aim and

the three papers are clearly connected to each other. There is no doubt on my part that the scientific level of the work is very high, including state-of-the-art structural determination of polysaccharides. The novelty of the work has been pointed out excellently in the thesis by the introduction to the field and by comparing results from the present studies with previous results from earlier work.

Impact on the discipline

The PhD thesis states that postbiotics, defined as a “preparation of inanimate microorganisms and/or their components that confers a health benefit on the host”, have crucial benefits over probiotics. These benefits are associated with easily defined structures of high stability with the possibility to perform structure-function studies, as well as no risk of transferring unwanted bacteria or genes compared to cultures of live microorganisms. Such an approach requires careful characterization of the active components; in the present PhD thesis the components are bacterial polysaccharides. I agree that postbiotics is a beneficial approach for utilizing active components of microorganism, not very different from classical approaches for drug development. However, I would like to emphasize that the knowledge acquired in the present thesis is not only useful for development of postbiotics, but also for a deeper understanding of the action of probiotics, as well as the natural function of Bifidobacteria in gut microbiota. Thus, the present results can be of great importance for investigations on the roles of Bifidobacteria.

There are very few laboratories with instrumentation and expertise for structure determination of polysaccharides. The thesis by Pacyga-Prus is thus a rare contribution to the scientific community, which can be of interest also for others doing similar structure determinations. The combination of polysaccharide structure determination and investigation of immunomodulatory properties is, if not unique, very unusual and of major impact on the field.

Independence of the doctoral candidate

Pacyga-Prus is the first author of all the three papers in the thesis. Declarations of her contributions in Paper 1 and 2 state that she has been active in both conceptualization, method development, investigation (production and purification of polysaccharides, structure determinations, immunological studies), data analysis, and writing of the original drafts. From the listed experience of the doctoral candidate, it is evident that she is already on the way to become an independent researcher, with participation in several grants and already 12 (!) publications in total.

Final assessment

The PhD thesis by Pacyga-Prus fulfills all the criteria assessed in this review. The performance shown by the thesis is excellent in terms of scope and quality. It demonstrates the PhD candidate’s knowledge in biochemistry and immunology and the ability to conduct research independently. The doctoral dissertation is clearly an original solution to a scientific problem.

The doctoral dissertation meets the conditions specified in article 187, paragraphs 1-4 of the Law on Higher Education and Science (i.e., Journal of Laws 2023, item 742, as amended).

Sincerely,



Gustav Nestor

PhD, Associate Professor in Structural Molecular Biology
Swedish University of Agricultural Sciences