Uniwersytet Gdański | Uczelnie Fahrenheita

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Review

of the doctoral thesis by Akshay Maniyeri Suresh

Regulation of cell envelope homeostasis by Lap proteins in *Escherichia coli*

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Bacteria are microorganisms capable of colonising almost all environments found on Earth. Some bacteria can grow across a wide range of temperatures and pH values, whilst others are adapted to living in environments with high salinity, elevated pressure or high levels of UV radiation. A particular group consists of bacteria that are pathogens of humans and animals. For many of these, the optimum growth temperature is around 30-37 °C, and their functioning is closely linked to the host organism. The ability of bacteria to survive in such diverse, and sometimes extremely adverse, environmental conditions is linked, amongst other things, to the structure of their cell wall. This acts as both a mechanical and physiological barrier, protecting the cell from the adverse effects of external factors whilst simultaneously enabling communication with the environment. Due to differences in cell wall structure, bacteria are divided into two groups: Gram-positive and Gram-negative bacteria. The cell wall of Gram-negative bacteria consists of a cytoplasmic membrane and an outer membrane, between which lies the periplasmic space. The outer membrane has an asymmetrical structure and consists of two lipid layers: an inner layer containing phospholipids and an outer layer composed mainly of lipopolysaccharides (LPS). The biosynthetic pathways of phospholipids and lipopolysaccharides

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are strictly regulated. Maintaining the balance between these two key components of the cell membrane depends, amongst other things, on the activity of proteins involved in the various stages of their biosynthesis, as well as in the coordination of both pathways. Understanding the mechanisms of action of these proteins is therefore crucial to understanding the functioning of the entire system. An imbalance in LPS biosynthesis, which is not accompanied by adequate compensation in the phospholipid biosynthesis pathway, can lead to the disintegration of the cell membrane and, consequently, to the loss of cell integrity.

The research carried out by Akshay Maniyeri Suresh as part of his doctoral thesis is highly relevant to the issues described above.

The doctoral thesis is written in English in the standard format. The main body of the thesis, comprising 124 pages, begins with abstracts in Polish and English, which concisely describe its subject matter and content. This is followed by a very useful list of abbreviations used in the thesis. In my opinion, it lacks explanations of certain abbreviations, particularly those that are less obvious from a chemist's perspective, such as LapB, LapC or LpxC. The first numbered chapter is the 'Introduction', which runs to over 30 pages. In this section the PhD candidate presents the structure of the cell wall of Gram-negative bacteria. He then provides a detailed description of the structure and functions of its basic components: outer membrane proteins, lipoproteins, phospholipids, fatty acids, lipopolysaccharides and peptidoglycan. At the end of this section, the author presents two complementary systems that play a key role in maintaining the integrity of the Gram-negative bacterial cell wall: the RpoE pathway and the two-component Cpx system. The 'Introduction' chapter has been prepared with great care in terms of content and contains numerous, appropriately selected references. Also worthy of note is the inclusion of 23 figures, which significantly facilitate the comprehension and understanding of complex issues, often requiring advanced scientific knowledge. The topics covered in the introduction are closely linked to the research described in the subsequent sections of the doctoral thesis and provide an excellent introduction to it.

The chapter 'Aim and scope of work' outlines the objective of the planned research: to deepen our understanding of the mechanisms that regulate LPS biosynthesis in *E. coli* by determining the molecular functions of the LapC and LapD proteins. These proteins interact with

LapB, a key regulator of LPS biosynthesis that influences cell wall component homeostasis and integrity. The aim of the planned research has been thoroughly justified by presenting the current state of knowledge and precisely identifying existing gaps and areas where the current level of knowledge remains insufficient.

In the third chapter of the doctoral thesis, entitled 'Materials and methods', the PhD candidate lists the strains, plasmids and oligonucleotides used in the research in the form of three comprehensive tables. In tables, he also lists the media, buffers, gels and solutions used in the research, providing a very detailed description of their composition. He then lists and describes the methods employed in the research. It should be emphasised that as many as 26 methods were used, which attests to the very broad range of research techniques utilised during the preparation of the doctoral thesis. Also worthy of note is the detailed description of the procedures employed, on the basis of which it can be concluded that replicating the research or applying the described methods by other researchers should not present any major difficulties.

The fourth chapter, entitled 'Results', is divided into two subchapters devoted to the proteins under study: LapD and LapC. It presents the research findings, some of which are summarised in tables. The chapter is supplemented by numerous figures that clearly illustrate the presented results.

Another important chapter is the 'Discussion', in which the PhD candidate comprehensively synthesises and compares the results of the research with the current state of knowledge. Given the complexity of the analysed issues, the diversity of the employed research methods and the extensive scope of the obtained results, this chapter has been written in a clear, logical and accessible manner. Particular mention should be made of the manner in which the discussion is conducted, demonstrating the PhD candidate's excellent knowledge of the subject matter, academic maturity, and considerable research experience.

The final chapter, entitled 'Conclusions', is equally professional and clear. In it, the PhD candidate summarises the most important conclusions arising from the research and results in a concise, logical manner.

Summarising the results obtained, the discussion conducted and the conclusions drawn, it can be unequivocally stated that the PhD candidate has fully achieved the set research objective. The careful planning and consistent conduct of research utilising a broad and appropriately selected range of methods has enabled the identification and characterisation of new molecular functions of the LapC and LapD proteins. This has significantly expanded our knowledge of the mechanisms that regulate LPS biosynthesis in *E. coli*.

The most significant scientific achievements include:

- demonstrating that the LapD protein is a central component of a multiprotein complex that links the main pathways of cell wall biogenesis. This is evidenced by its physical interactions with proteins involved in the biosynthesis and transport of LPS, fatty acid synthesis, and phospholipid metabolism. The loss of LapD also has functional pleiotropic consequences, including temperature sensitivity, increased outer membrane permeability, abnormal cell morphology, and a specific reduction in LpxC enzyme levels,
- explaining the functional relationship between the LapD and LapC proteins,
- proposing a dynamic model for the regulation of cell membrane biogenesis in *E. coli*, in which the LapB/C/D protein complex plays a key role.

The final section of the doctoral thesis comprises a bibliography containing 320 entries. The selection and citation of references demonstrate a very good understanding of the subject matter and should therefore be rated as very good. The thesis also includes a list of 42 figures and 13 tables. At the end of the thesis are four publications, of which the PhD candidate is a co-author and the first author of one of them. These publications present, amongst other things, the research results obtained as part of this doctoral thesis.

I read the thesis with great pleasure. It is written in a logical and clear manner. The excellent and consistent formatting of the text is also worthy of note.

The work under review is a well-planned and carefully executed research project. Reading it has provided me with many new scientific insights, as well as raising a number of questions arising from natural scientific curiosity. However, the comments set out below do not affect my

very positive assessment of the project. I would ask the candidate to address these comments during the defence.

- The research described in the doctoral thesis is fundamental in nature, concerning the structure and function of the cell wall of Gram-negative bacteria. Given the significance of these microorganisms, particularly the pathogenic ones, I would be grateful if the candidate could elaborate on the general statement in the thesis about the potential practical applications of the obtained results.

- Figure 41 shows three MS spectra recorded for LPSs isolated from a wild-type strain and two of its mutants. Based on their virtually identical profiles, it was concluded that the introduced mutations do not affect the structure of the LPSs. To what extent is such a statement justified from the perspective of a geneticist who knows the exact nature of the genetic modifications introduced and can predict their potential consequences for LPS biosynthesis and modification? Conversely, to what extent is this conclusion justified from the perspective of a chemist who observes virtually identical MS spectra when analysing the three samples? In both cases, should further steps be taken to clarify any doubts? If so, what steps should be taken?

In conclusion, I would like to emphasise that the present study is a valuable and well-planned research project, which has been carried out correctly and consistently. It contains numerous elements of scientific novelty and makes an original contribution to expanding our understanding of the influence of the LapB/C/D protein complex on the biogenesis of lipopolysaccharide and other components of the *E. coli* cell wall. The results obtained represent a valuable addition to existing knowledge in this field and may serve as a starting point for further research into the mechanisms regulating the formation and function of Gram-negative bacterial cell walls. The PhD candidate has demonstrated extensive knowledge of the scientific literature, which has been appropriately and skilfully utilised in the thesis. He is proficient in a wide range of research techniques and methods used in molecular biology, biochemistry and chemistry. His scientific output, which is impressive given the current stage of his academic career, comprises four papers published in high-quality journals indexed in the JCR (IJMS, IF = 5.6, 2025).

In summary, the doctoral thesis submitted for assessment meets the statutory requirements for doctoral theses as set out in the Act on Higher Education and Science of 20 July 2018, as amended. In view of the above, I hereby request that the Council for the Discipline of Chemical Sciences at Gdańsk University of Technology admit Akshay Maniyeri Suresh to the subsequent stages of the procedure for the award of a doctoral degree in the field of exact and natural sciences, in the discipline of chemical sciences.