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Evaluation of PhD thesis by
Adrianna Aleksandrowicz

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Honoured by the task of evaluating Adrianna Aleksandrowicz's PhD thesis, I here deliver the external referee statement.

Scope and structure of the thesis

In the dissertation entitled "The role of *sanA* in *Salmonella* pathogenicity", Adrianna Aleksandrowicz presents original experimental work in the form of two manuscripts (chapter 4-5), which investigate the phenotypes linked to, and the properties of, the *Salmonella* protein SanA. The research manuscripts are placed into context by a focused introduction chapter (chapter 1), a summarizing chapter called "summary and future prospects" (chapter 6), and shorter chapters that crystallize the research objectives, hypotheses, and main conclusions of the studies. The clear structure and layout of the thesis makes it easily accessible to a scientific readership.

Scientific findings, novelty and productivity

The thesis builds around two original research chapters. The first one is recently published (Aleksandrowicz et al 2024, *Frontiers in Microbiology*; DOI: 10.3389/fmicb.2023.1340143), with the second one presented as a manuscript that has reached a mature state, but is not yet published. Both research chapters focus on phenotypic and biochemical studies of the *Salmonella* SanA protein. The following specific findings are supported by the data presented in the thesis.

Chapter 4, manuscript 1:

- Deletion of *sanA* results in altered *Salmonella* susceptibility to a wide array of antibiotics (49/240 tested), with both cases of decreased and of increased susceptibility observed across the major classes of antibiotics.
- *Salmonella* Δ *sanA* bacteria exhibit increased membrane permeability.
- Deletion of *sanA* affects the electrochemical/biophysical properties of the bacterial envelope.
- *Salmonella* Δ *sanA* replicate, or survive, better within macrophages than their wildtype counterparts.

Chapter 5, manuscript 2:

- SanA localizes to the inner bacterial membrane, as judged by co-fractionation with well-established markers proteins.
- SanA expression, broadly speaking, trails that of *Salmonella* Pathogenicity Island-1 (SPI-1) expression, with highest expression around the late-exponential to early-stationary phase transition in liquid broth, but SanA expression is also noted during late stage infection in macrophages.
- *Salmonella* Δ *sanA* are hyper-invasive in epithelial cell line culture infections.
- Deletion of *sanA* correlates with enhanced expression of a SPI-1 reporter.

The studies fall short of uncovering a direct molecular function(s) for the SanA protein and to formally explain through which mechanism(s) its deletion results in the observed phenotypes, particularly i) increased/decreased susceptibility to various antibiotics, ii) hyper-replication in macrophages, and iii) enhanced expression of SPI-1 and epithelial cell hyper-invasiveness. Nevertheless, the thesis includes rigorous biochemical characterizations and phenotypic studies of *Salmonella* strains having or lacking SanA, which together provide novel and important insights towards teasing apart the mechanism-of-action for this previously poorly known bacterial membrane protein. Taken together, these findings will be of significant value to the research community, particularly in the fields of microbiology, microbial pathogenesis, and antibiotics research.

Adrianna Aleksandrowicz is the first author on both of the manuscripts included in the thesis, and has had a leading role in the experimental work, analysis and statistical analysis, as well as the drafting, editing, and revision of the final manuscripts. This is proof that she is capable of taking on all the relevant roles expected of a PhD-level scientist. The candidate has beyond the two manuscript chapters in the thesis also contributed as a co-author to an additional four published original research articles, and as first author to two research overview articles, which can be found in recognized publication databases (e.g. PubMed, Google Scholar). These additional publications all fall within the broader research field of the thesis, with the contribution to Kolenda et al 2021 Applied Environmental Microbiology (DOI: 10.1128/AEM.02177-20) particularly relevant to the thesis itself. In summary, the total scientific output from the PhD thesis period passes the bar for scientific productivity by a substantial margin.

Methodology and analysis

The experimental work has been conducted by genetic, microbiological, biochemical and tissue-culture based approaches customary to this field of research. When it comes to stringency, it is particularly worth pointing out that the candidate has throughout used plasmid complementation to ensure the accuracy of the various *sanA*-linked phenotypes. This represents golden-standard practice in microbiology, and it honors the candidate to so consistently have adhered to this high level of stringency in the experimental work.

The data presentation and analyses in figures and tables also appear largely appropriate, although there are a few examples where the presentation of data could be further improved. For example; i) the use of truncated y-axes in Figs 4-5 of chapter 4 (manuscript 1) makes for suboptimal representation of the results where relatively small differences appear larger than

they are; and ii) in the bar charts of chapter 5 (manuscript 2) it is often not clear how many biological replicates have been included since individual data points are not plotted. These minor notes aside, the methodology and analyses applied appear largely sound throughout the body of the thesis, and matches the accepted procedures of the research field.

Scientific reasoning and discussion of relevant literature

The thesis contains a concise, well-written, and focused introduction section that prepares the reader for the two original research chapters. In an international comparison, I note that the scope of the introduction is somewhat narrower and shorter than often seen, but this seems in accordance with Polish thesis traditions. Similarly, it could have been helpful to further broaden the summary discussion section (chapter 6) and extend the reasoning and speculation around the new findings in the thesis. At the same time, the discussion sections included in the two manuscripts are very extensive and thoroughly cover the literature pertinent in each case. In both the introduction chapter and the manuscript discussion sections the relevant literature is amply and thoroughly referenced, with >100 references covered by the overview chapters alone. This, combined with the coherent and stringent reasoning in the discussion sections of the manuscripts demonstrates the PhD candidate's ability to distill the essence from a large bulk of scientific literature and put her own results into an appropriate context.

Concluding remark

In this thesis, Adrianna Aleksandrowicz presents a substantial body of novel microbiological research findings, acquired by appropriate methodology, and put into a relevant scientific context in the introduction chapter and the discussion sections of the two included manuscripts. After thorough scrutiny of the thesis, I conclude that the doctoral dissertation meets the requirements of art. 187 ustawy Prawo o szkolnictwie wyższym i nauce (tj. Dz.U.2023.0.742 ze zm.), as these requirements have been described to me. I therefore propose to admit the doctoral candidate to the next stages of the doctoral procedure at the Wrocław University of Environmental and Life Sciences.



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