UNIWERSYTET WARSZAWSKI

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Warszawa, 15.05.2026

Review of the PhD Thesis of Soroosh Monem, M.Sc., entitled:
"Formation of protein aggregates and glycation products in *Escherichia coli* and *Klebsiella pneumoniae* exposed to desiccation-rehydration stress",
supervised by Prof. Ewa Laskowska,

General assessment

The doctoral dissertation of Soroosh Monem provides valuable insights into the mechanisms underlying bacterial survival under desiccation-rehydration stress, with a particular focus on protein aggregation, glycation, and the protective role of osmolytes. The dissertation addresses bacterial survival mechanisms under desiccation stress by focusing on protein aggregation and glycation in *Escherichia coli* and *Klebsiella pneumoniae*. It investigates how osmolytes—carnosine, glycine betaine, and trehalose—affect bacterial viability, protein aggregation, and glycation during drying and rehydration. The results demonstrate that glycation, rather than protein aggregation, is the primary cause of bacterial death under desiccation stress. Lower concentrations of osmolytes reduced protein aggregation and glycation, enhancing *E. coli* survival, whereas higher concentrations increased aggregation but still inhibited glycation. Additionally, lysine acetylation was shown to prevent harmful glycation products, improving viability. Studies on clinical isolates of *K. pneumoniae* revealed distinct subpopulations differing in capsule presence, biofilm formation, and stress response. Overall, the work suggests that protective protein aggregates form during stress and that controlling glycation is critical for bacterial survival.

The doctoral dissertation of Mr. Sorosh Monem is written in English and represents a coherent and thematically well-organized work consisting of 92 pages, supplemented with 24 figures and 23 tables. The structure is appropriate and typical for experimental studies, containing all necessary components such as an abstract (also in Polish), introduction, materials and methods, aims, results, discussion, conclusions, and bibliography. The dissertation also includes a list of abbreviations and acronyms used.

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Evaluation of individual chapters

The ***Introduction*** is rather brief, covering only eight pages and providing basic background information on the topic. It would be advisable to expand this section by including a broader overview of osmolytes naturally produced by bacteria, not limiting the discussion only to those used in the study and their related research.

The ***Materials and Methods*** chapter raises several concerns. It lacks detailed descriptions of some procedures, which could hinder reproducibility. For instance, the Western blot procedure omits the membrane blocking step and does not specify the secondary anti-mouse antibodies used. The purpose of using anti-EF Tu antibodies is also unclear. The urea gel electrophoresis method is not described. Furthermore, detailed information on the osmolytes—such as their source, physical form, and preparation—is missing. Notably, detailed descriptions of osmolyte application appear only in figure legends rather than in the methods section, which is not appropriate. There is also no mention of the protocol for the long-term desiccation experiment involving carnosine, nor is there information about the medium used for culturing *Klebsiella pneumoniae* to obtain macrocolonies. Consequently, it would be difficult to exactly replicate parts of the experiments based on this chapter alone.

The ***Results*** chapter describes the execution of the four main research aims outlined in the ***Aims*** section: (I) analyzing the effects of three osmolytes (betaine, carnosine, and trehalose) on glycation, protein aggregation, and viability of *E. coli* and *K. pneumoniae* under desiccation and rehydration stress; (II) investigating the effect of lysine acetylation on *E. coli* protein glycation; (III) analyzing viability, aggregation, and glycation in central and ring subpopulations of *K. pneumoniae* macrocolonies; (IV) elucidating mechanisms underlying observed morphological differences.

In the initial experiments, the candidate focused on the viability of *E. coli* cells subjected to 4 hours of desiccation followed by rehydration, comparing conditions with and without carnosine. Simultaneously, the impact of carnosine on protein aggregates and glycated proteins was assessed relative to control cultures. Protein aggregates were isolated and quantified as a percentage of total cellular protein, while glycation products were measured with two methods: fluorescent AGE detection and immunodetection. Based on these data, the candidate identified the porin OmpC as one of the most heavily glycated proteins under desiccation stress. However, this conclusion relies solely on protein size, which is insufficient. The use of additional methods such as mass spectrometry or specific immunochemical tests for unequivocal identification is recommended.

Further in the study, the effects of all three osmolytes, at two concentrations (0.2% and 0.45%), were evaluated during prolonged (20-hour) desiccation stress in *E. coli*. However, survival after rehydration and the levels of aggregates and glycation products post-rehydration were not examined.

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During the defense, I would like to hear answers to the following questions:

- Why did the candidate conduct the first part of the experiments testing only carnosine and short-term desiccation, while the subsequent part involved all three osmolytes at two concentrations under prolonged stress? What justified this experimental design?
- Why were survival and aggregate analyses after rehydration not performed in the long-term experiment?

The section on lysine acetylation's protective role against glycation, especially against carboxymethyllysine (CML), is particularly notable. The in vitro and in vivo experiments using the *E. coli* strain and a triple mutant lacking acetylation capability demonstrate that acetylation may significantly enhance cell survival during desiccation and rehydration.

For the *Klebsiella pneumoniae* part, the approach is logical and consistent. Effects of individual osmolytes at 0.45% concentration on viability, protein aggregation, and glycation in strain MKP103 after desiccation-rehydration stress were analyzed. However, data analysis, including Figure 13, shows minimal differences between treatments and controls, and the lack of statistical analysis impedes evaluation of significance. The reported decrease in dead cells after rehydration in the presence of trehalose is surprising and requires further explanation.

Questions for the candidate to consider:

- Was statistical analysis performed for viability and aggregation data in *K. pneumoniae*?
- How does the candidate explain the biologically unlikely differences in dead cell levels post-desiccation and rehydration in trehalose-containing cultures?
- Is carnosine a naturally occurring bacterial osmolyte? What is its physiological relevance in the studied strains?

The candidate also investigated two subpopulations within *K. pneumoniae* 577-BA macrocolonies: a mucoid, encapsulated center and a non-mucoid, non-capsular ring. Genomic comparison revealed an Insertion Sequence, IS5 in the *wbaP* gene linked to the non-capsular phenotype and correlated with increased biofilm formation. Differences in viability, AGE levels, and protein aggregation between subpopulations highlight bacterial adaptive complexity.

Remaining questions include:

- What might be the biological and evolutionary significance of mutations leading to capsule loss in macrocolonies?
- How reproducible is this phenomenon—would similar mutations appear in every newly formed macrocolony? Can the candidate suggest a straightforward method to verify this reproducibility?
- How can the larger colony size on trehalose-containing medium compared to other conditions be explained?

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- The candidate states that carnosine's protective effects are most prominent during rehydration. Is this conclusion justified when carnosine was only added during growth, not during the stress phase? Would adding osmolytes to rehydration media better assess their role in cell recovery? How does the candidate view this?

The candidate applied a comprehensive range of methods including microbiological techniques (culturing, detection of live, dead and VBNC cells, fractionation, capsule detection), biochemical assays (aggregate isolation, AGE measurements, lipid peroxidation, SDS-PAGE electrophoresis, immunoblotting), and statistical and bioinformatic analyses. This variety demonstrates strong technical competence.

In the ***Discussion***, the candidate thoroughly analyzed and critically discussed the results in the context of existing literature. The discussion offers innovative insights into the protective function of protein aggregates and the key role of glycation during desiccation and rehydration stress. However, many conclusions rest on general correlations and hypotheses that require further molecular-level investigation, such as precise aggregate composition and functional analyses.

The exploration of glycation's complexity and its multifaceted effects—including specific porin glycation—is well presented, though sometimes speculative and in need of confirmatory experiments. The relationship between acetylation and glycation is an intriguing direction but remains largely hypothetical and demands stronger empirical support, especially in bacterial systems.

The role of osmolytes, particularly carnosine, is convincingly illustrated, although the underlying mechanisms are not fully elucidated, as the candidate prudently notes. The differential effects of osmolytes in various bacterial strains, such as *K. pneumoniae*, highlight the complexity of cellular responses.

The section on *K. pneumoniae* 577-BA macrocolonies is detailed and credibly links phenotypic changes to genetic background and pathogenic features, though interpretations regarding aggregate functions require further study.

In summary, the discussion is well-prepared, contextualizing results within current knowledge and emphasizing novel findings on protective protein aggregates and glycation's significance, while acknowledging the need for additional confirmation.

The doctoral dissertation by Sorosh Monem successfully advances our understanding of bacterial survival under desiccation and rehydration stresses, highlighting glycation as the main determinant of cell death and suggesting a protective role for protein aggregates. The study demonstrates a high level of both scientific content and methodology, though some methodological descriptions warrant refinement and certain hypotheses require further investigation. Overall, this work constitutes a valuable and solid contribution to the fields of microbial stress physiology and protein chemistry.

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Despite my critical comments, I am convinced that the scientific achievements of Soroosh Monem, who is applying for a doctoral degree, meet the requirements specified in Article 187, paragraphs 1 and 2 of the Act of 20 July 2018 – The Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended). Therefore, I request that the Research Discipline Council of Biological Sciences at the University of Gdańsk accepts Soroosh Monem for the further stages of doctoral proceedings in the field of exact and biological sciences, within the discipline of biological sciences.

Podpisany elektronicznie przez
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