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Review of the PhD thesis of Ms. Anna FIDOR

As a reviewer appointed by the Faculty of Oceanography and Geography, University of Gdansk, I have read and evaluated the doctoral dissertation entitled **Peptides produced by the Baltic cyanobacterium *Nostoc edaphicum* CCNP1411 – structure and biological activity** by Ms. Anna Fidor. The scientific field of the thesis is marine biotechnology/chemistry, and it has been supervised by Prof. Hanna Mazur-Marzec. I would like to make the following statement about Ms. Fidor's PhD thesis:

1. Organization of the PhD thesis

The structure of the thesis follows the usual practice. After Title Page, Acknowledgements and Abstract, a short Introduction to the PhD thesis topic is given. The Introduction is very compact, two pages. It discusses first a few characteristics of cyanobacteria, compounds they produce and some biotechnological/pharmacological potential of cyanobacterial metabolites. The Introduction then continues with a more in-depth description of the structure and activity of non-ribosomal peptides (and polyketides) produced by the cyanobacterial genus *Nostoc*. The cited literature in the Introduction mostly refers to original publications. The Introduction is lacking illustrations and tables but such are used later in the text.

The main Aims of the study have been clearly set and comprise structure elucidation and bioactivity assessment of nostocyclopeptides and cyanopeptolins produced by the Baltic Sea cyanobacterium *Nostoc edaphicum* CCNP1411.

The Materials and Methods section contains four and a half pages. This section is started with an illustrative flowchart presenting the involved scientific methods. The following methods are presented in the text: a) isolation, purification and culturing of the studied cyanobacterium *Nostoc edaphicum* strain CCNP1411, b) genetic analyses, c) extraction and isolation of cyanobacterial nostocyclopeptides and cyanopeptolins, d) LC-MS/MS analysis, e) NMR analysis, f) enzymatic inhibition assays, g) cytotoxicity assays, and h) human 20S proteasome assays. The methods are presented with references to own and others' work, clearly and with sufficient detail. Mastering of the used methods requires skills in e.g. algology, molecular biology, preparative chromatography, chemical structure elucidation and molecular/cellular toxicology, and the candidate gives evidence of understanding the methods by providing the relevant details in a logical manner.

The Results and Discussion are given on six and a half pages. The Results and Discussion are divided in two main sections, Analysis of structure and Biological activity. Within these two sections there is a subdivision in cyanopeptolins and nostocyclopeptides. The strategy of structural analyses by LC-MS/MS and NMR is explained in a logical and detailed way. The candidate's own contribution to structural analyses relates mostly to MS/MS analyses and these are given ample space in the results. While

MS/MS results are partly putative due to isobaric fragment ions and they always leave some space for interpretations, MS-MS is in practice the only way to perform structural analyses of organic compounds present in minute amounts. The identities of some compounds studied in the thesis were further verified by NMR techniques. The nostocyclopeptide results were consistent with the analysis of *ncp* gene cluster. Tables of the identified structures as well as structural drawings are provided. Some selective biological activities, i.e. serine protease inhibition (cyanopeptolins) or human 20S proteasome inhibition (nostocyclopeptides), were identified and there is an attempt to correlate the amino acid composition to the activities. The Discussion compares to results obtained by the candidate to those of others and some therapeutic and ecological connections are identified. A critical discussion of methodological aspects is lacking, e.g. a comparison of pros and cons with LC-MS/MS and NMR methods in structural assessment. The discussion about the future research directions and next steps to be taken is quite minimal.

The thesis also contains Conclusions which are summarized into six statements. The Reference section lists 94 references.

The four original papers together with supplementary materials and coauthor statements are provided in the Reference section. The papers are discussed in detail in the next section.

2. Evaluation of the individual papers included in the thesis

The work underlying the PhD thesis has been presented in four original peer-reviewed scientific papers published in international journals of excellent standing, high visibility, and appropriate scope/readership:

I. Fidor, A., Konkel, R., Mazur-Marzec, H. Bioactive peptides produced by cyanobacteria of the genus Nostoc: A review. Marine Drugs, 2019, 17 (10), 1-16. Impact factor: 6.085.

II. Mazur-Marzec, H., Fidor, A., Cegłowska, M., Wieczerzak, E., Kropidłowska, M., Goua, M., Macaskill, J., Edwards, C. Cyanopeptolins with trypsin and chymotrypsin inhibitory activity from the cyanobacterium Nostoc edaphicum CCNP1411. Marine Drugs, 2018, 16 (7), 1-19. Impact factor: 6.085.

III. Fidor, A., Grabski, M., Gawor, J., Gromadka, R., Węgrzyn, G., Mazur-Marzec, H. Nostoc edaphicum CCNP1411 from the Baltic Sea — a new producer of nostocyclopeptides. Marine Drugs, 2020, 18 (9) 1-18. Impact factor: 6.085.

IV. Fidor, A., Cekała, K., Wieczerzak, E., Cegłowska, M., Kasprzykowski, F., Edwards, C., Mazur-Marzec, H. Nostocyclopeptides as new inhibitors of 20S proteasome. Biomolecules, 2021, 11 (10), 1-10. Impact factor: 6.064.

A rule sometimes applied (in Finland) for the original papers in a PhD thesis is that the sum of impact factors of the papers should exceed 10. In Ms. Fidor's case the sum is more than 24 indicating that the rule is satisfied with a wide margin.

While there are three to eight authors in the publications (which is not unusual in natural sciences) the contribution of Ms. Fidor appears central in the papers as evidenced by three first author positions and one second author position. The statements of co-authorship estimate Ms. Fidor's contribution to 33-60% of the work in the papers. Her contributions have been detailed in the co-authorship statements and the published papers. Ms. Fidor's role relates to (paper I) planning, discussing and writing; (paper II) LC-MS/MS analyses, and acquisition and interpretation of data, and manuscript preparation; (paper III) formal analysis, investigation, writing and visualization, and (paper IV) conceptualization, LC-MS/MS, extraction, fractionation and isolation of compounds, 20S inhibition assay, writing and

visualization. It is evident that the quantitative criteria set for a doctoral candidate are satisfied by the scientific project, the original papers and the PhD thesis of Ms. Fidor.

I have absolute no reservations towards the scientific contents of the four individual papers published in the respected peer-reviewed journals Marine Drugs and Biomolecules. All the methodology has been described in adequate details and the conclusions drawn are justified. The review paper I is comprehensive and in my understanding up-to-date at the time of publishing. I consider papers II and III, and the structural analyses performed within these, the core contents of the PhD project and thesis. The bioactivity assays performed in II and IV are useful as such but are bound to show only a part of the pharmacological/therapeutic/biotechnological potential of the studied cyanopeptolins and nostocyclopeptides. The nature of chemical work is simply more finished than that of the biological work which can continue with other biological targets.

The primary merits of the original papers are following:

Paper I: comprehensive review of bioactive peptides produced by the cyanobacterium *Nostoc*

Paper II: identification of thirteen cyanopeptolins including twelve new variants in *Nostoc edaphicum* CCNP1411 and correlation of structure with inhibitory activity on trypsin and chymotrypsin

Paper III: six new nostocyclopeptides identified in *Nostoc edaphicum* CCNP1411, whole genome sequencing and *in silico* characterization of the biosynthetic gene cluster of nostocyclopeptides

Paper IV: inhibition results of the human 20S proteasome, some results on structure vs. activity.

3. Coherence of the individual papers

The individual papers are well linked and support each other in solving a bigger research question: the structure and activity of nostocyclopeptides and cyanopeptolins in the cyanobacterium *Nostoc edaphicum* strain CCNP1411. The papers form very logical steps in the research process. There was a literature survey (paper I) in the early phase of the study. In the later papers II-IV the work has evolved in the direction of structural chemistry and assessment of biological activities of *Nostoc* peptides. While it is unusual in Finland to include a review paper among the individual papers in a PhD thesis, the choice underlines the fact that a PhD candidate must have the necessary background knowledge of the relevant literature in order to successfully pursue the PhD degree.

The candidate's research on cyanometabolites from *Nostoc edaphicum* strain CCNP1411 has made the following conclusions possible:

a) *N. edaphicum* CCNP1411produces nostocyclopeptides (Ncps) and cyanopeptolins (Cps).

b) Some selective biological activities, i.e. serine protease inhibition (cyanopeptolins) or human 20S proteasome inhibition (nostocyclopeptides), were assigned to the isolated compounds. There were significant activity differences between analogues belonging to the same group of compounds.

c) *N. edaphicum* CCNP1411 produced Ncps mainly in the linear aldehyde form whereas other *Nostoc* strains have been reported to produce cyclic Ncps analogues.

d) The described Ncps activity against the h20S proteasome was pioneering work.

e) There is some potential in the characterized molecules to be used as lead compounds in drug development.

f) The structural diversity and the related selective biological activity of Ncps and Cps isolated from *Nostoc edaphicum* CCNP1411 shows potential for biotechnological applications.

It can be concluded that the main aims related to the PhD project, i.e. structure elucidation and bioactivity assessment of nostocyclopeptides and cyanopeptolins produced by the Baltic Sea cyanobacterium *Nostoc edaphicum* CCNP1411, have been successfully met.

4. Conclusion

As detailed above I have carefully examined the doctoral dissertation by Ms. Anna Fidor. I conclude that the doctoral dissertation is of high scientific quality according to internationally accepted academic standards and fulfills the criteria established by Faculty of Oceanography and Geography, University of Gdansk. The original papers have been published in high-ranked peer-reviewed journals. **I respectfully and without any hesitation propose acceptance of the work as PhD thesis. The work could be possibly considered for an outstanding grade but I will give my suggestion for the grade after hearing the defence by the candidate.**

Turku, 28.7.2022

Yours sincerely,

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