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### Review

of the PhD thesis entitled “Deciphering the diversity and functionality of fungal endophytes associated with *Salicornia europaea* L.” elaborated by MSc Bliss Ursula Furtado

The presented PhD thesis was done in the Department of Microbiology Nicolaus Copernicus Faculty of Biological and Veterinary Sciences University in Toruń under supervision of Prof. dr hab. Katarzyna Hryniewicz and co-promoters Prof. Torben Asp, Aarhus University, Denmark and Dr. Niels Roulund, DLF Seeds A/S, Denmark.

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The evaluated thesis addresses the questions of fundamental importance to microbial ecology related to the host–microbiome interaction and the role of microbiome in the response of plant to stresses. Studies in this field are of great importance since the plant microbiome play an important role in conferring abiotic stress tolerance to their plant hosts.

The main part of the dissertation consists of three following publications:

1. Furtado B. U., Nagy I., Asp T., Skorupa M., Gołębiewski M., Tyburski J., Hulisz P., Hryniewicz K. 2019. Transcriptome profiling and environmental linkage to salinity across *Salicornia europaea* vegetation. *BMC Plant Biology*, DOI: 10.1186 / s12870-0192032-3 (IF: 3.67)
2. Furtado B. U., Gołębiewski M., Skorupa M., Hulisz P., Hryniewicz K. 2019. Bacterial and fungal endophytic microbiomes of *Salicornia europaea*. *Applied and Environmental Microbiology*, Vol. 85 Issue 13 e00305-19. DOI: 10.1128/AEM.00305-19. (IF: 4.077)



3. Furtado B. U., Szymańska S., Hrynkiewicz K. 2019. A window into fungal endophytism in *Salicornia europaea*: deciphering fungal characteristics as plant growth promoting agents. *Plant and Soil*, DOI: 10.1007/s11104-019-04315-3 (IF: 3.259)

All these papers are co-authored but in all of them MSc Bliss Ursula Furtado is the first author showing her leading role in conducting the laboratory experiments, analysing some of the data, searching for the relevant literature and writing the papers. I would like to underline that publications in the high-ranked journals mentioned above prove high value and novelty of the presented dissertation.

Apart of these publications the PhD thesis includes Table of Contents, Abstract, Introduction, Aim of the research, Hypotheses, Research objectives, General conclusions, Future outlook, Main findings and Data submission resources. Additionally, the CV of MSc Bliss Ursula Furtado is also attached.

In well-written Introduction Author gives background of her study. In the following parts of this chapter she presents problem of soil salinity as one of the important abiotic factor affecting the plant growth and describes the features of halophytes i.e. plants that are able to cope with salinity stress. She paid special attention on the fungal endophytes that might exert the positive role for plants by promoting their growth, controlling phytopathogenic microorganisms and increasing tolerance to abiotic stress including salt stress. The review of the literature presented in Introduction led the readers to the clearly stated aims of the study.

The main goal of MSc Bliss Ursula Furtado study was to characterize the bacterial and fungal endophytes isolated from *S. europaea* growing in soil with different salinity gradients. The second aim was to check if selected fungal endophytes may be potentially used as plant growth promoting agents in non-host plant *Lolium perenne*. In my opinion one more aim of the study should be mentioned on page 25. PhD student studied and presented results also on the correlation between soil salinity level and gene expression of *S. europaea*. Plant selected for research is very interesting as glasswort is a non-mycorrhizal halophyte, one of the most salt-accumulating plant that is thought to be a pioneering plant in saline environments.

To achieve the aims of the study MSc Bliss Ursula Furtado compared the transcriptome profiles of *S. europaea* obtained from plants growing in soil with salinity gradient, determined the genetic structure of endophytic bacterial and fungal communities of host plant, assessed fungal



mechanism responsible for plant growth as well tested the usefulness of selected fungal *Salicornia* endophytes in enhancing growth of non-host *L. perenne*.

During her study MSc Bliss Ursula Furtado used a broad spectrum of methods that could capture the soil and plant physicochemical parameters, differences in gene expression, diversity of glasswort microbiome and fungi that exerted beneficial effect on plant development. Analyses of soil and plant properties were conducted with commonly used method. Advanced molecular and bioinformatics methods were applied for the determination of transcriptomic profiles of *S. europea* and genetic structure of microbiome assemblages. These methods included sequencing of plant cDNA (Illumina MiSeq), functional annotation of some genes and RT-qPCR of selected DEG. Sequencing of 16S rRNA and ITS gene amplicons was used to determine bacterial and fungal diversity taxon, respectively. For the characteristic of culturable fungi that potentially may act in non-host plant as the plant promoting agents their ability to synthesis of siderophores, indole-3-acetic-acid, polyamine and selected enzymes was assessed. This part of the dissertation is of high practical importance as members of the inner microbiota can be exploited as inoculants to improve plant growth in agriculture and horticulture or used for removal of contaminants from soil or water, especially in phytoremediation approach.

All the experiments were properly designed and the obtained results were clearly presented and documented. The strong point of the study is the statistical analysis of data. The results were subjected to the multitude of different statistical tools that made the results valuable and reliable. Some of the important results of MSc Bliss Ursula Furtado studies show that: (1) *Salicornia europea* gene expression is influenced by seasonal variations but not by salt; (2) diversity of endophytic bacterial and fungal communities depended on the sampling site; (3) diversity of bacterial microbiome was significantly higher as compared to fungal one; (4) inner glasswort tissues are colonized by plant growth promoting fungi that can act as effective tool for foster plant development in non-host plant; (5) functionality of fungal inocula in a new host plant greatly is influenced by existing microbiome. Moreover, the study allowed to identify the genes that might be involved in the acclimatisation of *S. europea* to season-dependent changes in salinity. In my opinion it is an important finding because the understanding of salt tolerance mechanisms enable to enhance the tolerance of crop plants to abiotic stresses.

The general conclusions as well discussions in published papers were well laid out and presented in a logical sequence. MSc Bliss Ursula Furtado carefully draw the conclusions and the

claims are supported by the results. The Author explained how the results related to hypotheses and have discussed the contribution of other researchers in the field of her study.

***Final conclusion:***

I have found Ms Bliss Furtado dissertation to be a very good one. It presents new interesting results that enhance our knowledge on the biodiversity of endophytic communities of halophytes *S. europea* exposed to various level of salt stress. It should be again underlined that her studies apart from the basic scientific value have also applicable aspect and the results may be useful for the improvement of crop and bioremediation techniques.

In my opinion the doctoral dissertation “Deciphering the diversity and functionality of fungal endophytes associated with *Salicornia europea* L” by MSc Bliss Ursula Furtado fulfils all requirements set for PhD theses in science as specified by Polish law (*Ustawa o stopniach i tytule naukowym oraz o stopniach i tytule w zakresie sztuki z dnia 4 marca 2003 roku z późniejszymi zmianami*, Dz. U. z 2017 r. poz. 1789). It contains new valuable data, proposes original solutions to important scientific and practical problems and proves candidate’s general knowledge of her study area and her ability to organize and conduct the experiments. The high-quality research publications support the scientific recognition of the PhD student in the field of microbiome diversity and microbial-plant interactions. Based on the above, I conclude that MSc Bliss Ursula Furtado should be admitted to the next stages of the PhD procedure.

Taking into account the wide range of the study and methods applied, novelty and the importance of presented results MSc Bliss Ursula Furtado should be awarded a prize for the best doctoral dissertations.

*2p/egct*