

20th Aug, 2021

Dear Tomas,

Please find below my review of the PhD thesis of Marketa Linhartova

Function on the type IV pili proteins in the cyanobacterium Synechocystis sp. PCC 6803.

This thesis presents a body of work related to the understanding of the role of the pili proteins in cyanobacteria. Whereas the role of pili in heterotrophic bacteria is established, the role in photosynthetic microbes is less well understood. In this thesis the candidate uses a series of sophisticated molecular, biochemical and physiological approaches to define the role of pili proteins in cyanobacteria under a range of controlled laboratory conditions.

The thesis is presented as four distinct chapters including both published and unpublished results.

These results present a considerable advance in our understanding of the role of Pili proteins in cyanobacteria. Importantly the results highlight the diverse and distinct role of pili in photosynthetic cells (as opposed to heterotrophic microbes) which is of value to understanding the evolution and function of cyanobacteria in the environment and for biotechnology.

I have no hesitation in recommending an award of PhD for the work presented. It represents a novel and important body of work. Much has been published and I suspect more publications will emerge.

Overall presentation:

The thesis is well presented and easy to read. Results are presented of a quality that has been (or could be) published with appropriate statistical analysis and presentation of methods allowing reproduction of results. The introduction to the thesis shows the candidate has a strong understanding of relevant previous work, and the introduction

enables the reader to assess the results in context. Indeed, the results presented throughout the thesis are consistently done with reference to recent literature. This suggests the candidate has a good understanding of the background and significance of the work.

Could the candidate outline more directly the overall aims/hypothesis of the work?

Could the chapters be presented chronologically as they build on each other well?

Results chapter 1

In this chapter a detailed analysis of the phenotype of pili knockout mutants is presented. The work characterises the impact of knockout cell lines on photosynthesis, growth and assembly of photosynthesis proteins. The work is of high quality and should be published. Importantly the work supports the complex role and interaction of pili in cyanobacteria and the photosynthetic apparatus, with impacts on achieved growth rate. The work on metal limitation is also interesting and potentially implies a connection linking metal availability, Pili and photosynthesis. The integrated analysis of molecular, biochemical and physiological data is impressive.

The chapter starts with a demonstration of the phenotype of a selection of pili knockout mutants. A clear reduction in colouration of reported that is presented as a 'bleaching' phenotype. Further, a change in growth parameter is reported whereby pili knockouts do not reach the same OD 'linear phase' growth. The phenotype is further characterised through metabolite analysis and protein abundance.

Question(s):

Could the result in 3.1 just be the slower growth of pilA cells? Growth curves would be useful?

Some phenotypes shown on agar and some in liquid. Given the role of pili in movement and surface adhesion could phenotype be different depending on how the cell is grown?

PSII:PSI ratio is mentioned – has this been quantified? Protein abundance or 77k?

Conclusion that pili deletion impairs active PSII – measure PSII activity, O₂ evolution per PSII?

Phenotype in low Fe and low Mn conditions. Results are interesting. Define bleaching, had chlorophyll and lost it, or just did not accumulate as much pigment?

Consider issues with comparing phenotypes in liquid/solid; especially in 3.15 and 3.14.

Useful molecular markers of Fe and Mn stress, interesting they seem not to be upregulated. Useful to measure Fe/Mn uptake and composition.

Is there any evidence for *MntCAB* gene expression?

A summary figure of the proposed model linking PSII repair and Mn uptake/transport would be useful.

Results chapter 2 (published)

Study of the impact of *pilD* knockout – shown to accumulate PilA in non-glycosylated form linked to impaired PSII synthesis and assemble. The use of 77k and oxygen evolution (Fig 4) could help the *pilA* work.

Do results suggest more PSI in pilD?

Results chapter 3 (published)

Study not related to interaction of chl biosynthesis and assembly of membrane proteins. Pull down elegant experiment to show interactions of proteins with ChlG. Very nice study highlighting the interactions of chlorophyll synthesis and assembly. Characterisation of functional multi protein complex is an important result.

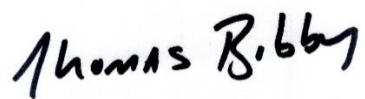
Results chapter 4:

Important study of suppressor mutations. *PilD* mutant causes accumulation of PilA which is thought to be toxic. The cell therefore develops suppressor mutations to compensate. In this study the nature of these mutations is considered. In addition to understanding the mechanism of these reversions and hence greater insight into cyanobacteria, functional mutants are relevant in causing cells to adhere to each other with implications for biotechnology. The study is important as instability of mutations in cyanobacteria is an issue for the field.

General Question:

Are pili likely to function in a similar fashion in other cyanobacteria, i.e. how representative are these results to cyanobacteria in general? Could the role of pili be different in filamentous, marine, pelagic cyanobacteria?

Yours sincerely,

A handwritten signature in black ink that reads "Thomas S. Bibby". The signature is written in a cursive, slightly slanted style.

Prof. Thomas S. Bibby