Pili Formation in Filamentous Cyanobacteria

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Abstract

Pili are flagella-like appendages that can be found on the surface of bacteria. Type 4 pili (T4P) are a form of pili produced that aid in functions such as cell motility, which cyanobacteria rely on due to their lack of flagella. This is carried out through various Pil proteins which are responsible for mechanisms such as pilus assembly, extension/retraction of the pilus rod, along with others working as response regulators for twitching motility. To investigate this, the filamentous species *Chlorogloeopsis fritschii* PCC 6912 was used to observe gene expression for these Pil proteins to understand the phototactic response of pili formation under UV-B and far-red light as these wavelengths of light can be found throughout their natural environment. Under UV-B, 64.29% showed >75% increase in gene expression with 40.0% of these *pil* genes showing significant upregulation. Under far-red, 45.16% showed a >75% decrease with 51.6% showing significant downregulation, specifically impacting the Pil proteins responsible for the formation of the pilus rod. These results suggest a phototactic response of *C.fritschii* PCC6912 with regards to pili formation, however not being triggered by all wavelengths of light that could be found throughout their habitat.

Declarations

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed.... [redacted] .....

Date.....29/09/2022.....

This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

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Abbreviations

ATP – Adenosine triphosphate

DNA – Deoxyribonucleic acid

HEPES – N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid

LED – Light-emitting diode

Log2FC – Log2FoldChange

MAA – Mycosporine-like amino acid

MCP – methyl accepting chemotaxis protein

O_2 – oxygen

RNA – ribonucleic acid

SOLiD –Sequencing by Oligonucleotide Ligation and Detection

T4P – type 4 pili

UV-B – ultraviolet B

1. Introduction

Cyanobacteria are photosynthetic prokaryotes commonly found near the surface of the water where they are exposed to sunlight. They are distinct from other forms of bacteria due to their ability to synthesise chlorophyll a, which is responsible for O_2 production (Whitton & Potts, 2004). Global evolution of ecosystems would not have progressed as such without the presence of these microorganisms due to this influence in primary production (Llewellyn *et al*., 2020). Cyanobacteria can survive in various environments as they are able to tolerate and adapt to stressors such as light intensity, as well as be found in environments with high salinity and water stress (Whitton & Potts, 2004). The filamentous species *Chlorogloeopsis fritschii* PCC 6912 has been found across multiple environments, shown to be terrestrial and found in soils, as well as in freshwater habitats (Llewellyn *et al*., 2020). Certain species even survive within low light environments such as *Prochlorococcus* (Aguilo-Ferretjans *et al*., 2021). They tend to have a higher optimum temperature than eukaryotic algae which assists them to be better suited for extreme environments (Tamulonis, Postma & Kaandorp, 2011). Filamentous cyanobacteria grow in a string of cells as filaments, which can show differentiated cells with separate purposes and functions (Flores & Herrero, 2009). These include heterocysts (N2 fixation), akinetes, and hormogonia (trichomes for motility; Dagan *et al*, 2013). Colonies formed in this way organise into structures comprised of multiple cells, usually in the range of hundreds or thousands (Wilde & Mullineaux, 2015). In microbial mats they are found at higher rates in the upper layers compared to unicellular species (Tamulonis, Postma & Kaandorp, 2011).

One distinction of cyanobacteria compared to other bacterial groups is that these cells lack flagella (Wilde & Mullineaux, 2015). In their place, cyanobacteria develop structures known as pili, which influence factors such as virulence, adhesion, and movement within bacteria (Proft & Baker, 2009). Pili are extracellular structures that are made up of specific pilin proteins (Piepenbrink & Sundberg, 2016). While various types of pili structures have been found, type 4 pili (T4P) is the most extensively studied,

especially in cyanobacteria. T4P have been observed to have different functions such as motility, natural competence, biofilm formation and plant symbiosis (Duggan, Gottardello & Adams, 2007; Nies *et al*., 2020; Wilde & Mullineaux, 2015). These functions are carried out and influenced by different Pil-proteins that work together to extend and retract the pilus (Piepenbrink & Sundberg, 2016; Nakane & Nishizaka, 2017). While extension and retraction are some responsibilities of pili, others are found to be information receptors and for the assembly of the pilus. These Pil-proteins are split between different systems such as the T4P apparatus and MCP-like complex (Fig. 1) to form and control the pilus (Schuergers, Mullineaux & Wilde, 2017).

The role of Pil-proteins in motility for cyanobacteria has been heavily discussed due to different theories being researched in the past; this includes slime propulsion, whip-like motions, and even 'running' movements. Twitching motion from grappling-like movements from seems to be the commonly understood mode of movements (Bhaya *et al*., 2000; Nakane & Nishizaka, 2017; Oeser *et al*., 2021; Okamoto & Ohmori, 2002).

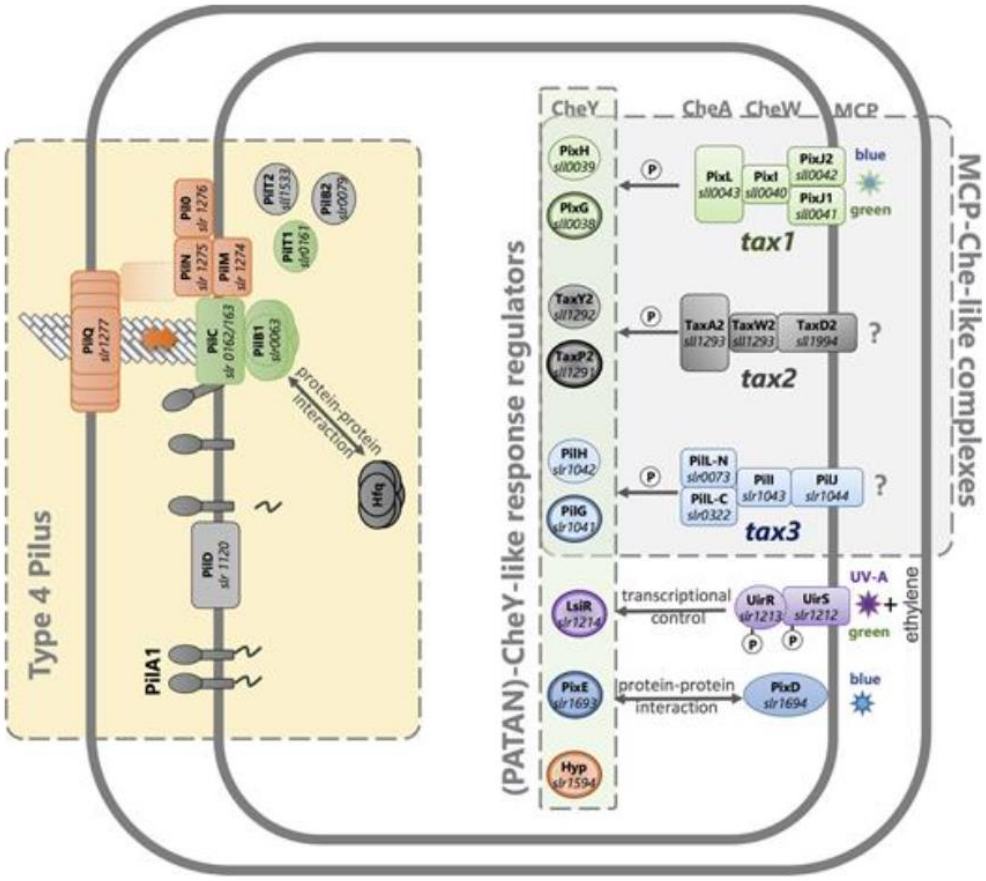

Figure 1. Cellular components of *Synechocystis* PPC 6803 involved in phototaxis. Included are the Pil proteins found in various areas such as the type 4 pilus and the MCP-like complex. Different wavelengths of light are detected around the cell with this information being relayed to the T4P to carry out various functions such as orientation and pilus assembly. Different pil-proteins make up these mechanisms and areas such as the T4P which features PilA, PilB, PilC, PilD, PilM, PilN, PilO and PilQ. There are receptor and response regulator proteins in areas such as the PATAN and MCP-like complexes| (Wilde & Mullineaux, 2017)

Single celled cyanobacteria such as *Synechocystis* sp. PCC 6803 produce a thick and a thin type of T4P. The thin pili tend to be 3-4 nm in diameter and <1 μm in length, with thick pili being ~5 nm in diameter and >5 μm in length, with T4P being the thick pili type. T4P are observed to be responsible for responding to light fluctuations in the cyanobacterial environment (Chen *et al*., 2020). In the filamentous species *Nostoc punctiforme*, this T4P formation has also been observed as a motile response to environmental stressors as mutant *pil* gene cells were seen to be non-motile (Schuergers & Wilde, 2015). The *Nostoc* sp. PCC 7120 has over 150 genes responsible for the coding of these two component signal transduction systems which are involved in cyanobacteria responding to environmental stressors to trigger biofilm formation (Bhaya, 2004; Liu *et al*., 2019). The form of motility using T4P have been observed to differ between forms of cyanobacteria, with twitching motility being a standard form in unicellular species such as *Synechocystis*. In filamentous species gliding motility has also been observed, which is seen in *A. platensis* and in *N. punctiforme* which has been seen to exhibit both types of motility (Fujisawa *et al*., 2010). Understanding how they are formed will assist in the knowledge of how and why cyanobacteria move around their environment (Conradi, Mullineaux & Wilde, 2020).

While many studies focus on unicellular cyanobacterial species such as *Synechocystis* with regards to T4P, this structure and the individual Pil proteins responsible for this have been observed across filamentous species as well such as *Nostoc*, with many species containing genes necessary for pili production within their genome (Fig. 2; Schuergers & Wilde, 2015).

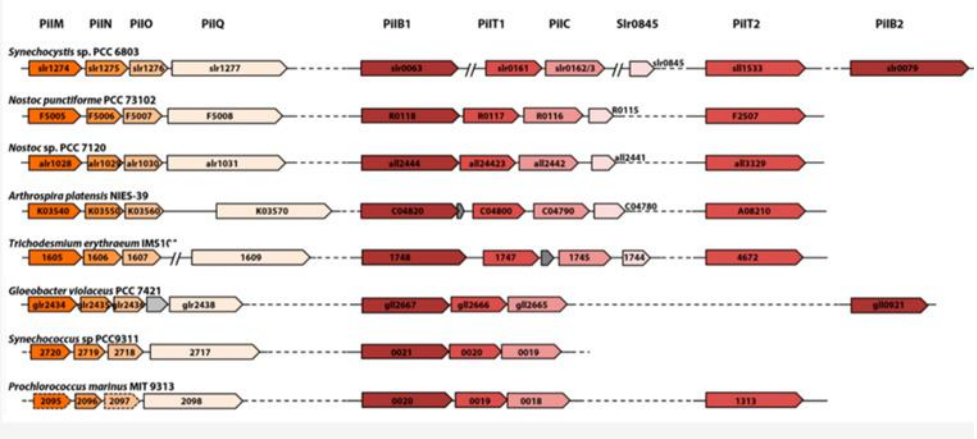

Figure 2. From Schuergers & Wilde (2015), showing the locus with genes responsible for various proteins that make up the type 4 pilus in various cyanobacteria species. The presence of these pil-proteins being found across species indicates the presence of the T4P apparatus in different cyanobacterial species.

Filamentous cyanobacteria have also been observed to produce hormogonia which are shorter piliated trichomes, that present T4P on their surface and shown in figure 3 (Schirmacher, Hanamghar & Zedler, 2020; Schuergers & Wilde, 2015). In *N. punctiforme*, hormogonia are produced by the differentiation of vegetative filaments. This cell differentiation seems to occur from various fluctuation within a species environment such as changes to nutrient concentrations, light availability and quality, or symbiotic plants being present (Campbell *et al*, 2007). The pili on these structures form around the septal junctions; in *N. punctiforme* these grow in rings around these cell poles. (Harwood *et al*., 2021; Khayatan *et al*., 2015). This piliation from the hormogonia assists in biofilm formation due to the surface adherence; as observed in filamentous species such as *Leptolyngbya* and *Scytonema* (Villafani, Yang & Park, 2020).

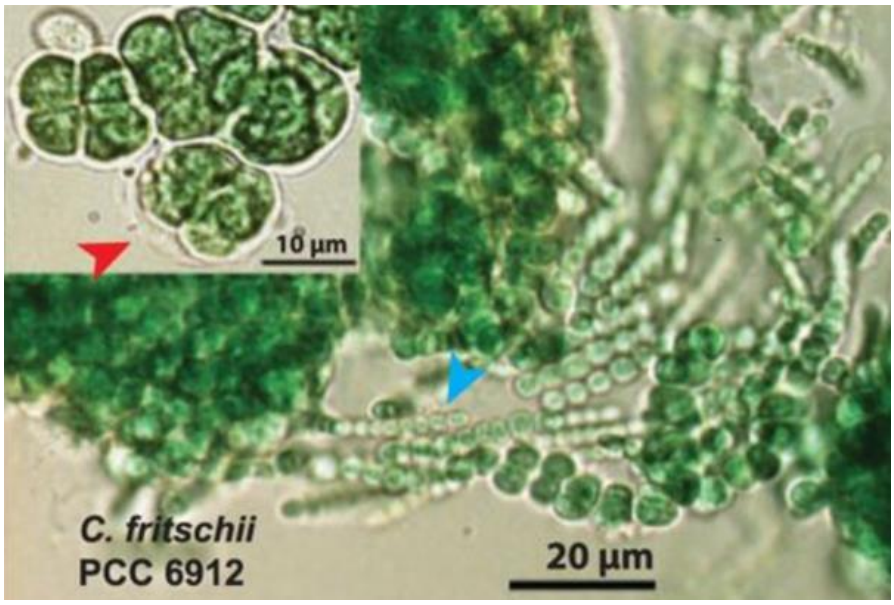

Figure 3. Dagan 2013. Showing the filamentous structure of *c.fritschii* PCC 6912. Heterocysts are shown in the top left image by the red arrow. Hormogonia branches as shown by the blue arrow within the larger section of the image.

1.1. Photoreceptors and the MCP-like complexes

Phototaxis is dictated by photoreceptors that detect various wavelengths of light. There are various types which are responsible for the detection of UV and far-red that include the Cyanobacteriochrome (UV-A to far-red light), Cryptochrome (UV light) and Phytochrome (far-red), with other domains for the detection of other wavelengths (blue/green). In *Synechocystis* sp. PCC6803 there are three major photoreceptors that regulate phototaxis to activate the T4P. PixJ1 (*tax1* operon – MCP-like/PATAN CheY signal transduction system) induces positive phototaxis on detection of blue/green (possibly red light), Uirs (His-kinase – PATAN-CheY signal transduction system) induces negative phototaxis on detection of UV and green light, and PixD (PATAN-CheY signal transduction system) will induce positive/negative phototaxis on detection of blue light (Fig. 1; Wilde & Mullineaux, 2017). These photoreceptors regulate

phototaxis using response regulators that effect binding to the pilus base. Positive phototaxis requires response regulators that inactivate pili on the opposite side of the cell from the light source through phosphorylation, leaving the pili on the side of the light source to remain active. Negative phototaxis uses non-phosphorylatable response regulators that prevent non-phosphorylated response regulators from binding to the pilus base, only allowing phosphorylated response regulators to bind to the pilus base which is localised to the opposite side of the cell from the light source, allowing pilus activation (Schuergers *et al*, 2015; Wilde & Mullineaux, 2017).

As mentioned, PixJ1 can be found in the *tax1* operon as part of the MCP-like complexes. While this operon includes a photoreceptor, there are other operons within these complexes that lack photoreceptor domains (Schuergers, Mullineaux & Wilde, 2017). Figure 1 shows the components of *tax2* and *tax3* with unknown stimuli, however these operons are likely to control T4P motility through chemotaxis-like systems (Harwood *et al*., 2021; Schuergers, Mullineaux & Wilde, 2017)

1.2. The T4P apparatus and components

The T4P apparatus has four main areas: pilus rod, the membrane complexes (comprising of both the outer membrane complex and the cytoplasmic pilis platform), and the secretion ATPases (Schuergers & Wilde, 2015). These areas contain different types of Pil proteins that work together to form the T4P and control the functions of the pilus (Table 1).

Pili apparatus	Pil protein
Pilus rod	PilD
	PilA
Membrane complexes	PilQ
	PilM
	PilN
	PilO
	PilP
	PilF
	PilC
Secretion ATPases	PilB
	PilT

Table 1. Pil proteins that make up each section of the type 4 pilus apparatus, specifically noted within *Synechocystis* sp. PCC 6803 T4P (Schirmacher, Hanamghar & Zedler, 2020; Schuergers *et al*., 2015; Schuergers & Wilde, 2015).

PilA are responsible for the extension of pili through the polymerisation of its subunits. In *N.punctiforme*, hormogonia have concentrated rings of PilA which may aid in co-ordinated movement (Wilde & Mullineaux, 2015). Most cyanobacteria have been observed to have multiple PilA homologs (Schuergers & Wilde, 2015). Not all PilA functions are known, with each being used for certain purposes: some are observed to be important for biofilm formation, some for motility, as well as multiple other functions (Aguilo-Ferretjans *et al.*, 2021). Unicellular *Synechocystis* 6803 has 11 recorded PilA proteins. They have varied functions such as assisting in pilus biogenesis and motility. Certain PilA variants are responsible for both actions, however not all of them are.

Regarding motility, all but three were observed to be linked to motile phenotypes (Schuergers & Wilde, 2015).

PilD carries out N-methylation before filament assembly (Adams & Duggan, 2008). It is responsible for the processing of the PilA subunits to form the pilus rod (Fig. 4; Bhaya *et al*., 2000) When PilD is inactivated in mutants of *N. punctiforme*, they have been observed to be non-motile and produce shorter pili which effects their ability to infect, and their symbiotic growth (Schuergers & Wilde, 2015). This is due to PilD mutants being unable to produce T4P (observed in unicellular *Synechocystis* sp. PCC 6803; Thirumurthy *et al*., 2020).

PilQ is a secretin protein, responsible for assisting pilus subunit transport and part of the outer membrane complex, acting as the pore. (Schuergers & Wilde, 2015; Chen *et al*., 2020). PilMNO (pilM, pilN and pilO) is a cluster of individual proteins found in the membrane complex (as shown in Table 1 and Fig. 4) that assist in pore complex-pilus platform alignment. PilP is thought to be a lipoprotein that connects PilQ to this cluster (Schuergers & Wilde, 2015; Fig. 4). These proteins anchor the base of the pilus in the inner membrane (Wendt & Pakrasi, 2019).

PilB and PilT are ATPases responsible for pilus growth, depolymerisation, and outer membrane protein transport. They act as a molecular motor by rotating through ATP binding and hydrolysis. The hydrolysis of ATP by PilB causes a pilin subunit to be integrated into the current growing pilus rod which causes pili extension (Schuergers & Wilde, 2015). This extension allows adhesion to a substrate to then take place (Chen *et al*., 2020). PilT powers the retraction of the pili after latching to a surface through depolymerisation of the pilus. This retraction after adhesion creates a pulling motion from the cell (Wilde & Mullineaux, 2015; Chen *et al*., 2020) There have been multiple copies of PilB and PilT-like genes discovered which may have different functional uses for types of movement from the T4P (Schuergers & Wilde, 2015). PilC is found at the base of the pili along with PilB and PilT (Chen *et al*., 2020). PilC is more specifically found in the inner membrane where it anchors the base of the pilus (Wendt & Pakrasi, 2019).

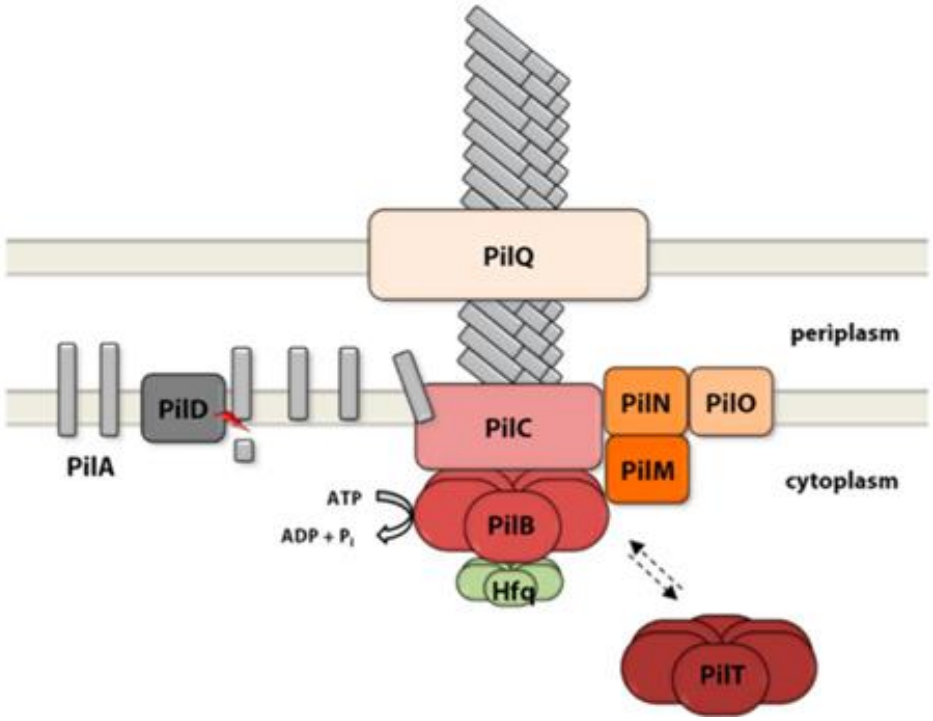

Figure 4. from Schuergers & Wilde (2015) showing a type 4 pilus model in unicellular *Synechocystis* sp. PCC 6803. The different types of pil-proteins are spread among different areas of the cell membrane with some being within the cytoplasm. PilA filaments are used to make the pilus rod that is anchored by PilC. PilB and PilT are interchanged to cause the extension and retraction of the pilus rod. PilQ is a pore complex which is also involved in natural competence.

1.3. T4P Function

Pili are responsible for multiple actions in bacteria, including cell motility (Nakane & Nishizaka, 2017). Motility is important for cyanobacteria as they rely on it to assist in symbiosis and seek out areas of optimal light intensity (Wilde & Mullineaux, 2015). These responses are vital for chemotaxis and phototaxis (Conradi, Mullineaux & Wilde, 2020).

Twitching motion observed in some species can be used for motility from the repetitive grappling-like extensions and retractions of the pili, creating grappling-like motions (Nakane & Nishizaka, 2017). The ability of these motions from the pili filaments is what allows cells and colonies to carry out important functions (Piepenbrink & Sundberg, 2016). Without motility, cyanobacteria would not be able to establish symbiotic relationships with plants and fungi, or be able to seek out the ideal light intensity (Wilde & Mullineaux, 2015). The T4P assists in phototaxis to allow motility for colonies to move towards or away from a source of light. They only move 2-demensionally across a surface, but not when suspended (Jékely, 2009). The secreted polysaccharides create an ideal surface for the colonies to use to move across (Wilde & Mullineaux, 2015).

Filamentous cyanobacteria have been seen to use a gliding motion at times (Bhaya, 2004). They can move as both an individual trichome and as a co-ordinated group. Biddanda *et al* (2015) demonstrated this phototactic response within *Oscillatoria* to understand possible movement within a microbial mat. The *Oscillatoria* was shown to be motile and responsive within in situ conditions. When a light source was used with a foil cut-out as a stencil, positive phototaxis was observed as the cells were congregating under the light having moved away from the shaded areas towards the illuminated areas. Once the directed light source was removed, the filaments disbursed out evenly. This response indicates their ability to react to and move towards a light source (Biddanda *et al*., 2015).

Motility is not the only function of pili. Natural competence involves the uptake of DNA through the type 4 apparatus in pili (Nies *et al*., 2020). Even though it is a common function observed in different bacterial taxa (Schirmacher, Hanamghar & Zedler, 2020), it has only been observed in a few unicellular cyanobacteria, and even less frequently among filamentous species (but has been seen in some such as *Nostoc muscorum* and *C. fritschii* PCC 6912; Nies *et al*, 2020; Nies *et al*., 2022). DNA binds to the filament and is pulled through the pore of the outer membrane/PilQ by pilus retraction and is carried through the type 4 pilus to a separate system (Schirmacher, Hanamghar & Zedler, 2020).

The MCP (methyl accepting chemotaxis proteins)-like complex also contains Pil proteins similar to those needed for chemotaxis. These include CheY-like (PilG and pilH), CheA-like (pilL), CheW-like (pilI), and MCP-like (pilJ) proteins (Fig. 1; Schuergers, Mullineaux & Wilde, 2017). These proteins assist in pilus assembly under chemotactic responses and assist in natural competence (Yoshihara, Geng & Ikeuchi, 2002).

Many studies investigate phototaxis in white light, however interests in *C.fritschii* PCC 6912 have recently increased amongst researchers due to its reaction to far-red light (Llewellyn *et al.*, 2020). In this paper, the formation of pili in filamentous cyanobacteria shall be explored, specifically those found in *C.fritschii* 6912. Influence from different light conditions (UV-B, far-red and white light) on the changes of pili formation is the primary focus to further understand how they may react to these different light conditions in their natural environment. In a microbial mat, UV-B light (280 – 320 nm) would be found to be more prevalent at the surface, while far-red light (700 – 800 nm) is still prevalent in darker areas of an aquatic environment (Castenholz & Garcia-Pichel, 2012; Conradi, Mullineaux & Wilde, 2020; Soulier, laremore & Bryant, 2020). Due to the damage that UV light has on cyanobacteria, it is expected T4P formation and activation may increase with the upregulation of pil-protein genes, due to the cyanobacteria possibly needing to move to find safe areas (Donkor & Häder, 1991). As far-red light is found in darker areas, gene upregulation may also be observed for the cyanobacteria requiring motility to move to areas with brighter light (Ohkubo & Miyashita, 2017). Using this we may further understand the formation and purpose of pili with regards to phototactic movement.

2. Methodology

2.1. UV-B: Cyanobacterial cultivation

Inoculation of *C.fritschii* PCC 6912 was carried out at a dilution of 1:50 of a grown master culture to a mix of BG11 media (2 L) with 10 mM HEPES. The inoculate was kept at 38 °C with 1% CO2 under white light from Grolux fluorescent tubes (wavelength of 410 – 750 nm, 60 µmol photons m−2 s−1). Cells were harvested during the exponential growth phase and placed into nine separate flasks with a 0.44 g L−1 solution (pH) of BG11 medium (200 mL) with 10 mM HEPES. All flasks were further exposed to the white light (410 – 750 nm) for 100 hours. Following this, the nine flasks were divided into three groups of three flasks. The first group of three were exposed to only white light for a further 100 h. The next three were exposed to both white light and UV-B (300 – 310 nm, 3 µmol m−2 s−1 Philips UV-B tubes), with the UV-B exposure for the final 4 h. The final three flasks were exposed to both white light and UV-B, with the UV-B exposure occurring each day for 4 h over the four days.

2.2. UV-B: RNA sequencing

Samples from the flasks were then centrifuged at 3000 g while being kept at 4 °C. SOLiD sequencing (Sequencing by Oligonucleotide Ligation and Detection) was carried out at the Centre for Genomic Research at the University of Liverpool returned around 49,034,856 sequences (sample average of 57,516,996.44 sequences, 50 bp).

Bowtie version 0.12.7 was used to align sequences against the reference *C.fritschii* PCC 6912 genome (Dagan *et al*, 2013), with a 47.67% average of unambiguous alignments. With the limma package in R, the ‘loess smooth’ method was used to produce normalised gene counts. The EdgeR package in R was then used on this data using the ‘GLM’ model for differential gene expression analysis and to calculate Log2 fold change and adjusted p-values. Values with log2 fold change outside of the bounds of

− 1.3 to 1.3 and adjusted p-value below 0.05 showing significant changes in gene expression.

2.3. Far-red: Cyanobacterial cultivation

C.fritschii PCC 6912 inoculation was carried out to create three flask cultures of 800 mL with a 750 nm optical density. These were left to cultivate for six days under white light (410 – 750 nm, 60 μmol photons m−2 s−1) using LED lights.

Three 50 mL samples were taken from each flask and placed into a 50 mL centrifuge tube. These were left as the white light control samples. The remaining cultures in the flasks were exposed to far-red light (710 nm, 18 μmol photons m−2 s−1) using LED lights for 24 hours. 50 mL samples were taken from the flasks and transferred to 50 mL centrifuge tubes for the far-red light samples.

Centrifugation was carried out for RNA preparation. The samples were spun at 3500 rpm for 15 minutes (at 4°C), then again at 5000 rpm. The supernatant was removed and the pellets were frozen and stored at −80 °C. The pellets were suspended in Trizol and homogenised (0.5mm beads) at 6500 rpm. The samples were then put back in the centrifuge for 15 minutes at 12,000xg (at 4°C). The upper phase was mixed as a 1:1 with ethanol (70%) for a PureLink RNA Mini Kit spin cartridge, followed by PureLink DNAse (used in accordance to the manufacturers instructions). RNA concentrations were measured using spectrophotometry (NanoDrop ND-1000) and fluorometry (Qubit 3.0).

2.4. Far-red: RNA sequencing

The collected 50 mL samples (three control white tubes, three far-red tubes) were centrifuged at 3500 rpm for 15 minutes at 4 °C. The samples were transferred into new 50 mL centrifuge tubes and centrifuged at 5000 rpm for 15 minutes at 4 °C. The supernatant was removed and the pellet frozen at – 80 °C. Trizol reagent was used to

resuspend the4 collected pellets and homogenised (6500 rpm). The samples were left to incubate for 5 minutes then extracted and centrifuged at 12,000xg (at 4 ℃ for 15 minutes). Using the PureLink RNA Mini Kit spin cartridge and Purelink DNAse, the sample was prepped following the manufacturer's instructions.

RNA concentration was measured using a spectrophotometer and fluorometer. DNA concentration was measure using a fluorometer, with DNA contamination being removed using TURBO ™ DNase and TURBO ™ DNA-free Kit following the manufacturer's instructions. DNA was confirmed to be removed before RNA samples were sequenced. A RiboZero bacteria kit was used to purify the samples from ribosomal RNA from bacteria. A MiSeq platform was used by the Swansea University Sequencing Facility which returned 99,448,410 sequences between all samples (from multiple V3 2×75 bp paired-end sequencing).

Rockhopper 2 was used for de novo assembly to identify absent transcripts from the genome. All of the sequences were mapped against the *C.fristchii* genome (Dagan *et al*, 2013) using Subread aligner, followed by the use of featureCounts for read summarisation. The DeSeq2 R package was used to find the differential gene expression from the un-normalised read count summarisation. Log2 fold change values and adjusted p value was calculated using the normalised dataset from DeSeq2 R, with any values with log2 fold change outside of the bounds of -1.3 to 1.3 and adjusted p-value below 0.05 showing significant changes in gene expression.

Disclaimer: all work for the UV-B and far-red experiments was carried out by others for previous publications, with the produced data being worked with for the results of this project. The data collection for UV-B was done during a different time period and carried out with differences to the data collection for far-red, so data plotting for these experiments were kept separate. For further information regarding the methods and background information behind the data collection, refer to Llewellyn *et al* (2020) '*Mycosporine-like amino acid and aromatic amino acid transcriptome response to UV and far-red light in the cyanobacterium Chlorogloeopsis fritschii PCC 6912'.* The data

from the original study can be found on NCBI BioProject (accession number reference: PRJNA545395).

2.5. Analysis

Using the data provided (Llewellyn; 2020) which was referenced against the *C.fritschii* PPC 6912 genome by Dagan (2013), the Pil-proteins involved in pili formation and function were extracted from the dataset and the given accession numbers were run through NCBI BLAST. Pil proteins as well as others found to relate to pilus formation were searched for within documented genomes on NCBI BLAST from similar species (such as filamentous *n.punctiform* and some unicellular cyanobacteria such as synechocystis). These were then run back through BLAST to find any that are similar to sequences in the *c.fritschii* genome. Those accession numbers were then checked against the dataset to find any other notable proteins that may initially have been missed. Out of 6690 far-red and 3199 UV-B genes from the original dataset, 31 far-red and 14 UV-B were used for analysis.

The gene expression of these found in the far-red and UV-B data were used to produce figures to show differentiation to their respective control white results. The given Log2Fold change values were used to produce volcano plots to show any upregulation or downregulation of *pil* genes. Results with a -log10p-values <1.3 and p-value >0.05 were insignificant. As the means were already calculated for the gene expression within the dataset that was passed over by Dr Llewellyn, standard error could not be calculated and used for gene expression counts and was only available for the Log2FoldChange data for analysis.

Figures were produced using Rstudio (transcript used for figures within the results can be found in the appendix)

3. Results

Many genes relating to pili formation and function were found, but not everything found in the sequences from the far-red experiment were found in the UV-B experiment. The UV-B data does not include PilA1, PilB, PilC, PilF1-2, PilG1-2, PilT3-5, PilT7-10 or PilW. There were regions showing gene clusters discovered such as the PilMNOQ, PilBTCA and PilGHIJ (Table 2). The PilMNOQ gene cluster is shown to be predominantly responsible for pilus assembly. Amongst many being genes coding for proteins related to pili assembly, the majority showed to be related to be related to the twitching function of the pili. The PilBTCA cluster showed to predominantly be responsible for pilus assembly while the PilBT section also being responsible for the twitching motility of the pilus. The PilJIHG cluster showed to be predominantly responsible for signal and response mechanisms. There was a possible second cluster of PilGHIJ which is in reversed order in the genome to the previous mentioned cluster, however there was no PilI found as there seemed to be a missing accession number. Within the dataset, there looked to be an accession number that would fit between PilGH and PilJ: accession number WP_016876362.1 (Table 2). When run through NCBI BLAST, it returned the CheW protein. It is unknown why this was not found within the sequencing carried out within the methodology.

As the two experiments were carried out at different times, the anomalies of genes found that were linked to pili formation in one dataset whilst being absent within the other may have been caused (Table 2).

Product accession number	Identifier in the text	Protein	Function/process	P-value (UV-B; 3.s.f)	p-value (far-red; 3.s.f)
WP_016872478.1	PilO1	PilO (ribbon-helix-helix domain-containing protein)	Polypeptide and nucleotide binding/ pilus biogenesis	1.98 x 10-8	6.01 x 10-9
WP_016872930.1	PilT1	PilT (type II toxin-antitoxin system VapC family toxin)	Twitching motility	n/a	0.229
WP_016873320.1	PilT2	PilT	Twitching motility	6.36 x 10-5	8.01 x 10-11
WP_016873630.1	PilT3	PilT (type II toxin-antitoxin system VapC family toxin)	Twitching motility	n/a	0.215
WP_016874001.1	PilT4	PilT (type II toxin-antitoxin system VapC family toxin)	Twitching motility	n/a	2.37 x 10-2
WP_016874078.1	PilQ	PilQ (AMIN domain coding protein/ type 4 secretin family)	pilus assembly/ twitching motility	3.21 x 10-10	3.86 x 10-74
WP_016874079.1	PilO2	PilO (hypothetical protein)	Pilus assembly	2.08 x 10-9	4.87 x 10-75
WP_016874080.1	PilN	PilN (PilN domain coding protein)	Fimbrial/ pili assembly	1.12 x 10-10	8.84 x 10-73
WP_016874081.1	PilM	PilM	Pilus assembly	8.91 x 10-16	5.94 x 10-71
WP_016874123.1	PilD	PilD (A24 family peptidase)	prepilin peptidase	5.85 x 10-6	7.96 x 10-2
WP_016874200.1	PilB	PilB (Type II/IV secretion system protein)	Pilus assembly/ motility	n/a	1.32 x 10-74
WP_016874201.1	PilT5	PilT	Twitching motility	n/a	2.94 x 10-61
WP_016874202.1	PilC	PilC Type II secretion system F family protein	Fimbrial assembly/ pilus biosynthesis	n/a	1.59 x 10-67
WP_016874203.1	PilA1	PilA (Hypothetical protein)	Pilin	n/a	4.51 x 10-37
WP_016875341.1	PilT6	PilT (PilT/PilU family type 4a pilus ATPase)	Twitching motility	1.38 x 10-2	6.23 x 10-7
WP_016875933.1	PilT7	PilT (PIN domain-containing protein)		n/a	2.52 x 10-3

WP_016876360.1	PilG1	PilG (two-component response regulator)	Twitching motility response regulator	n/a	2.49 x 10-6
WP_016876361.1	PilH1	PilH (CheY-like response regulator)	Twitching motility response regulator	5.7 x 10-4	3.52 x 10-62
WP_016876363.1	PilJ1	PilJ (Methyl-accepting chemotaxis protein)	Twitching motility	3.48 x 10-3	3.12 x 10-67
WP_016877190.1	PilT8	PilT (PIN domain-containing protein)		n/a	0.450
WP_016878569.1	PilT9	PilT (type II toxin-antitoxin system VapC family toxin)	Twitching motility	n/a	1.57 x 10-4
WP_016878612.1	PilW	PilW (hormogonium polysaccharide secretion pseudopilin HpsC)	Pilus assembly/ prepilin-type N-terminal cleavage	n/a	2.65 x 10-14
WP_016878846.1	PilT10	PilT (PIN domain-containing protein)	Twitching motility	n/a	0.730
WP_016879164.1	PilJ2	PilJ (Methyl-accepting chemotaxis protein/ CheW-like protein)	methyl-accepting chemotaxis sensory transducer with GAF sensor	3.24 x 10-3	1.13 x 10-35
WP_016879165.1	PilI	PilI (purine-binding chemotaxis protein CheW)	Cell motility/ Signal transduction mechanisms	2.56 x 10-2	2.00 x 10-26
WP_016879166.1	PilH2	PilH (CheY like response regulator)	Twitching motility response regulator	n/a	3.35 x 10-27
WP_016879167.1	PilG2	PilG (two-component response regulator)	Twitching motility response regulator	n/a	7.76 x 10-43
WP_016879293.1	PilA2	PilA (Type 4 pilin-like G/H family protein)	prepilin	1.95 x 10-3	2.09 x 10-32
WP_016879352.1	PilF1	PilF (tetratricopeptide repeat protein)	Pilus assembly	n/a	5.93 x 10-2
WP_016879562.1	PilJ3	PilJ (Methyl-accepting	Chemotaxis/ twitching motility	8.59 x 10-6	0.0247

		chemotaxis protein)			
WP_016879735.1	PilF2	PilF (tetratricopeptide repeat protein)	Pilus assembly	n/a	0.280

Table 2: Proteins found in the dataset that are involved in the formation of pili in *C.fritschii* PCC 6912. The product accession number was put through BLAST to identify the protein, protein length and function. P-values for both the UV-B and far-red experiment were calculated prior to dataset being handed over for analysis. n/a values show the genes which were not returned during the bioinformatics of the UV-B data.

3.1. Gene expression

When *C. fritschii* PCC 6912 was placed under UV-B light, PilJ1, PilJ2 and PilO1 decrease in gene expression compared to the control white samples (Fig. 5A&B). All others observed to increase in gene expression under UV-B light. 71.43% of the observed genes showed >50% increase in expression, 64.29% showed >75% increase, and 42.86% showed >90% increase (these same genes also showed >100% increase). The PilMNOQ gene cluster showed an 84.79 - 129.72% increase in expression.

When samples were placed under far-red light, most of the results showed a major decrease in gene expression except for PilF1, PilJ3, PilO1 and PilT3. 64.52% of the observed genes showed a >50% decrease in expression, 45.16% showed a >75% decrease, and 35.48% showed a >90% decrease. The PilMNOQ gene cluster showed a 91.24% - 95.12% decrease in expression. The PilBTCA gene cluster showed a 91.51 > 94.13% decrease. The PilJIHG cluster showed a 73.28% - 80.91% decrease.

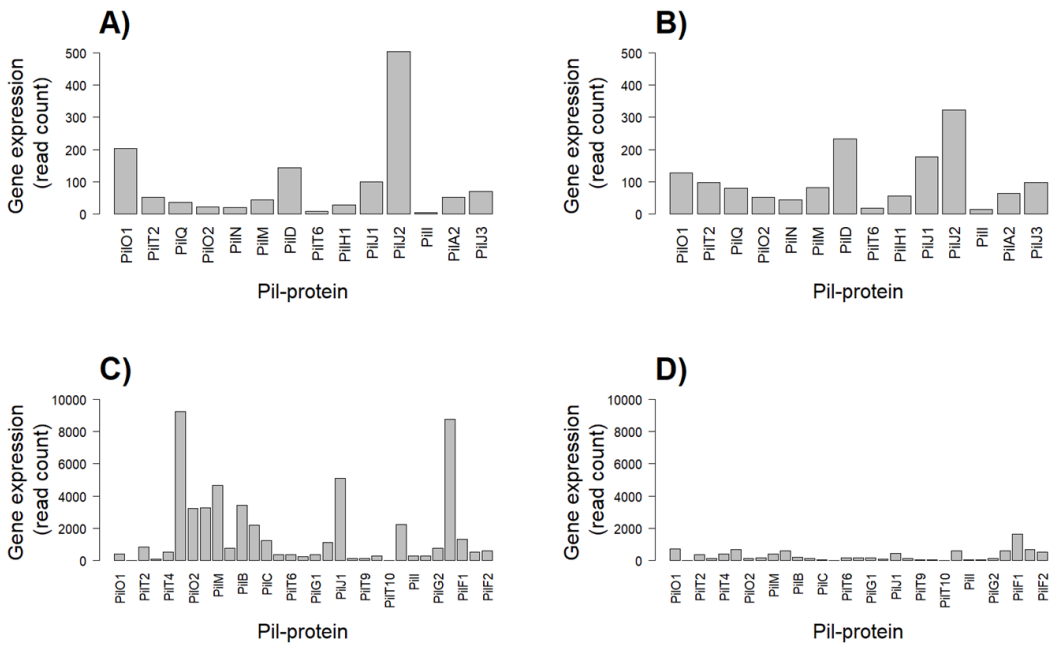

Figure 5. The gene expression count for each observed Pil-protein found in *C. fritschii* PCC 6912 under UV-B and far-red, with a control white plot for each respectively. These Pil-proteins are plot in order of accession number (refer to Table 2 for the gene accession number for each produced protein). A) Control white data for UV-B experiment. B) UV-B data. C) Control white for far-red data. D) Far-red data. The control white light samples were used as a baseline for pili production within *C.fritschii*. PCC 6912.

3.2. Upregulation/downregulation

Pili were observed to increase in gene expression under UV-B compared to white light with 6 genes showing significant upregulation and 8 genes showing no significant change as shown by the upregulation seen in figure 6A. All -log10p-values were above 1.30, showing statistical significance, however not all were differentially expressed. Only PilI, PilM, PilN, PilO2, PilQ and PilT6 show significant upregulation, with no values showing significant downregulation. The PilMNOQ cluster (accession numbers WP_016874081.1, WP_016874080.1, WP_016874079.1 and WP_016874078.1 respectively; Table 2) was highly upregulated under UV-B. PilM showed a Log2FC of

3.20 ± 0.19 (SE, 2.d.p), PilN 3.63 ± 0.23, PilO 2.87 ± 0.23 and PilQ 2.13 ± 0.20. There were two datapoints that showed a negative Log2FC which implies downregulation which were PilJ3 and PilO1. However, these values are non-differentially downregulated as they fall within the cut off boundary between -1.3 and 1.3 to show differentiation, with PilJ showing a Log2foldFC of -1.22 ± 0.27 (SE, 2.d.p) and PilO with -1.06 ± 0.14.

Under far-red light, pili were observed to decrease in gene expression compared to white light with 16 genes showing significant downregulation and 15 genes showing no significant change as seen in figure 6B. Not all values were statistically significant as there are -log10p-values below 1.30, with others also not being differentially expressed as they have a Log2FC value that falls between -1.3 and 1.3. Those that showed significant downregulation were: PilA1, PilA2, PilB, PilC, PilG2, PilH1, PilH2, PilI, PilJ1, PilJ2, PilM, PilN, PilO1, PilQ, PilT5, and PilW show significant downregulation, with no values showed significant upregulation.

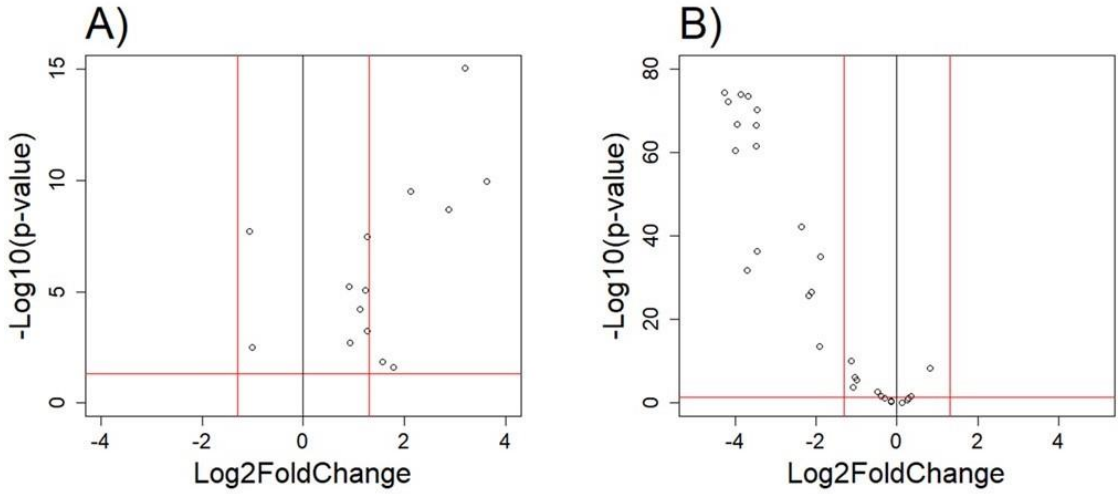

Figure 6. Volcano plots showing the Log2FoldChange (Log2FC) for genes related to pili formation in *C.fritschii* under A) UV-B light compared to white light . B) far-red compared to white light. Data points on both figures with a Log2FC < 0 show downregulation while data points with a Log2FC > 0 show

upregulation. Data points with a -log10(p-value) < 1.3 are insignificant. Values with a Log2FC value between -1.3 and 1.3 are not differentially expressed.

The PilMNOQ cluster is highly downregulated under far-red light. PilN and pilO2 were highly downregulated with a Log2FC <-4 which can be seen in figure 6B. PilN showed a Log2FC of -4.18 ± 0.23 (SE, 2.d.p), while PilO showed a Log2FC of -4.27 ± 0.23. PilM showed Log2FC -3.46 ± 0.19 and PilQ showed -3.68 ± 0.20. The PilBTCA cluster showed downregulation, with PilB showing a Log2FC of -3.87 ± 0.21 (SE, 2.d.p), PilT5 with -3.99 ± 0.24, PilC with -3.94 ± 0.23, and PilA1 with -3.47 ± 0.27. PilA2 was also differentially upregulated with a Log2FC of -3.70 ± 0.31 (SE, 2.d.p). The PilJIHG cluster also showed downregulation with PilG showing a Log2FC of -1.89 ± 0.15 (SE, 2.d.p), PilH showed -2.18 ± 0.21, PilI showed -2.12 ± 0.20, and PilJ showed -2.37 ± 0.17. The PilJIHG cluster while showing downregulation that was differentially significant, it was not as downregulated as the other clusters.

PilT3 did show slight upregulation, however it was non-differentially upregulated. All other forms of PilT showed downregulation with only PilT5 falling between the bounds of -1.3 and 1.3 for Log2FC. PilB showed downregulation with Log2FC -3.87 ± 0.21 (SE, 2.d.p) and falling in the bounds of significance. PilC showed differential downregulation with Log2FC -3.94 ± 0.23 (SE, 2.d.p). PilW (HspC) was differentially downregulated with a Log2FC of -1.91 ± 0.25. The remaining *pil* genes were non differentially expressed and/or were non-significant.

Under UV-B, PilO1, PilT2, PilD, PilH1, PilJ1-3 and PilA2 were non-differentially expressed. While under far-red, PilO1, PilT2, PilT4, PilT6-7, PilG1, PilT9 and PilJ3 were non-differentially expressed, with PilT1, PilT3, PilD, PilT8, PilT10, PilF1 and PilF2 being insignificant values.

4. Discussion

Pil proteins responsible for pilus assembly were shown to be expressed at high rates compared to other forms under white light, suggesting that light may be an important trigger for the formation of the pilus. Schuergers, Mullineaux & Wilde (2017) mentions this within their research on the unicellular cyanobacteria *Synechocystis,* with active T4P on the side of a cell facing the light source and inactive T4P on the side facing away from it (Fig.7). There were different gene clusters found which included PilMNOQ, PilJIHG and PilBTCA. These clusters have been found across various cyanobacterial species. The PilMNOQ cluster was found in *C.fritschii* and has been found across other cyanobacterial species such as *Synechocystis* sp. PCC 6803 (Schuergers & Wilde, 2015). This cluster in the genome is commonly involved in pili biogenesis and the alignment of the pilus for T4P in cyanobacteria (Fujisawa *et al*., 2010; Oeser *et al*., 2021). The PilBTCA cluster that has been found in *C.fritschii* has been linked to other cyanobacterial species. A PilBTC cluster has been observed within the genome of another filamentous cyanobacterium *Anabaena* sp. PCC 7120. These were also found in unicellular species such as *Synechocystis* sp. PCC 6803, however the PilB was split in the genome from the others (Yoshihara *et al*., 2001).

PilGHIJ is part of the MCP-like complex, separate to the T4P region observed by the other clusters. They are not a component of the T4P, but interact with it (Schuergers, Mullineaux & Wilde, 2017). These proteins are not as understood in cyanobacteria as they are in other bacteria. They are understood to not be assembly proteins for T4P but have influence on the adhesion from the pilus when observed in *Neisseria meningitidis* when interacting with human cells. This suggested that this cluster is linked more heavily to the function of T4P rather than the formation (Carbonnelle *et al*., 2006).

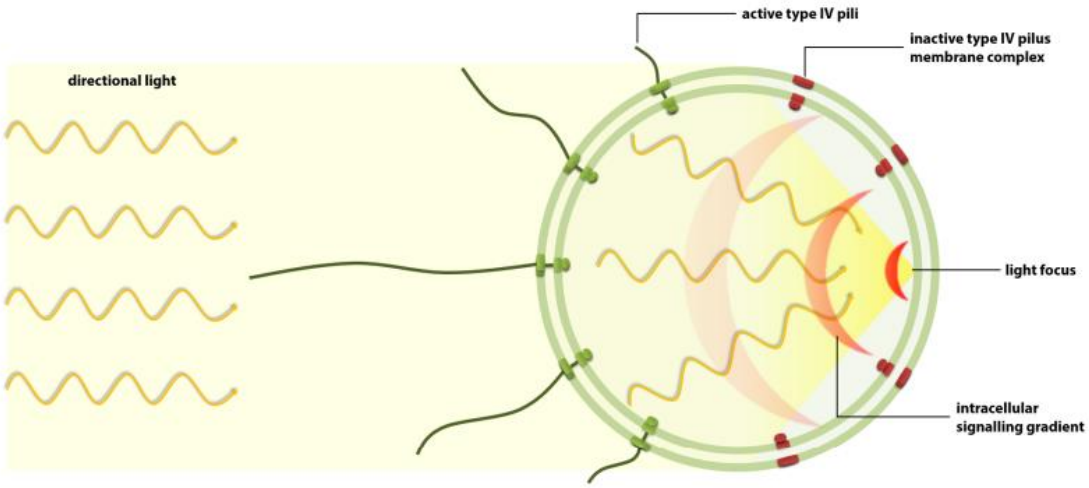

Figure 7 from Schuergers, Mullineaux & Wilde (2017), showing activation and inactivation of the T4P on the surface of *Synechocystis* in response to a light source. It has been shown that T4P are activated on the side of the cell where the light source originates. The T4P on the side away from the light source are inactive.

4.1. Light conditions

T4P have been observed to assist in phototaxis, believed to assist in finding favourable areas of light (Harwood *et al*., 2021). Other filamentous cyanobacterial species such as *Oscillatoria* and *Spirulina* have been observed to move themselves through their microbial mats, using this migration to position themselves based on optimum condition which include light. Results on from *C.fritschii* suggests the same behaviour (Bhaya, 2004). There was an observable change in *pil* gene expression with upregulation of some *pil* genes under UV-B and downregulation under far-red (Fig. 6).

4.1.1. UV-B

UV-B light is damaging to cyanobacteria and survival of the organism would require moving away from light of this wavelength (Conradi, Mullineaux & Wilde, 2020;

Llewellyn *et al*., 2020). Under this light condition, gene expression was similar compared to under white light however did show an overall increased with 42.86% of the genes showing <100% change. As T4P are heavily influential on cell motility, the pili responsible for pilus assembly and twitching motility would be needed to be expressed at a higher rate to assist in the cell movement to find areas of safety within the microbial mat, which has also been observed in the unicellular *Synechocystis* (Conradi, Mullineaux & Wilde, 2020).

The PilMNOQ cluster was shown to be highly upregulated with an increase between 129.72% and 84.79% across these genes. This cluster is linked to pilus assembly, specifically the pilus rod (Schuergers & Wilde, 2015). With the higher rates of expression of the PILMNOQ cluster under UV-B light compared to white light it can be understood that motility of the organism is triggered and needed under these wavelengths of light. PilT and PilB are also found in the T4P complex with PilMNOQ (Schuergers & Wilde, 2015). PilT was observed to increase in expression under UV-B (responsible for retraction of the pilus/pulling movement). As it is understood, the pilus rod is extended and retracted and shown to 'twitch' for motility (Nakane & Nishizaka, 2017). With the main responsibility of PilT linking to twitching motility (Table 2) due to being responsible for this retraction of the pilus rod, specific links to motility can be inferred to be triggered under UV-B (Wilde & Mullineaux, 2015). PilB while being responsible for the extension of the pilus (Wilde & Mullineaux, 2015), was not found within this dataset (Table 2). Comments on any possible relation to the extension of the pilus rod under UV-B cannot be made and are inconclusive.

PilH and PilI are also part of the MCP-like complex and these both showed upregulation, however pilH1 was non-differentially upregulated (Schuergers, Mullineaux & Wilde, 2017). One form of PilJ2 showed a decrease, however it fell within the Log2FC bounds of -1.3<1.3 showing non-differential expression, while the others (PilJ1 and PilJ3) showed an increase that was differentially upregulated as shown in figure 6A. The lack of significant change suggests that these are expressed at a similar rate to being exposed to white light. These are CheW-like proteins responsible for methyl-binding and are involved in signal transduction mechanisms. Whilst being a part

of the MCP-like complex, it is still shown to be important for responding to T4P formation and function such as motility as it is linked to the production of thick pili (Pisareva *et al*., 2007). In a study conducted by Yoshihara, Geng & Ikeuchi (2002), it was shown under white light that when using mutant strains of *Synechocystis* with inactivated PilJ there was a decrease in the production of thick pili and they were seen to be non-motile. This disruption shows how PilJ is important when the organism needs motility (Yoshihara, Geng & Ikeuchi, 2002).

Other than the PilMNOP cluster, PilI and PilT6, the remaining Pil proteins did not show a significant change in expression. PilA2 and PilD only showed a Log2FC of ~0.9 which was the lowest change in the UV-B dataset. This would suggest that the pilus is formed at the same rate under UV-B as it does under white light. These proteins are responsible for the formation of the pilus rod in the T4P, with PilD being responsible for processing PilA subunits to form the T4P pilus rod (Schuergers & Wilde, 2015).

As mentioned, the *pil* genes observed to increase in expression were responsible for the function of the pilus such as phototactic motility, possibly indicating that the difference between responses to UV-B light compared to white light may be relating to the function of the pilus rather than the formation (Conradi, Mullineaux & Wilde, 2020).

Enomoto *et al* (2014), it is noted that UV light can trigger negative phototaxis due to the damaging effect it has on these organisms, however cell aggregation was also observed within *Synechocystis*. Aggregation could occur with an increased expression of T4P components. This aggregation and clumping can reduce damage caused as the cells at the surface shades those underneath, protecting other cells from damage that could lead to photoinhibition (Enomoto *et al*., 2014). Conradi *et al* (2019) found that when working with *Synechocystis*, PilB mutants showed no cell aggregation, which may be due to a lack of ability for pilus extension. PilT mutants would show cell aggregation, at higher rates than the wild type results. Their findings suggest the importance for pilus extension and T4P expression for cell aggregation, without pilus retraction being required. Figure 5 shows an increase in expression of the T4P components when under UV-B light compared to white light, however as there are no results available for PilB expression, it

is uncertain if the pili function is for cell aggregation or motility in this instance. Future testing focused on directional movement could differentiate what the dominant function of a higher expression of pili components is when placed under UV-B light.

The pilus apparatus is also responsible for the uptake of extracellular DNA for natural competence (Yoshihara *et al*., 2001). *Synechococcus* and *Synechocystis* species have been observed to uptake and recombine extracellular DNA into their genome. As UV-B light can be damaging to cyanobacterial cells, natural competence may occur under these conditions to repair any damage. (Cassier-Chauvat, Veaudor & Chauvat, 2016). Cryptochrome photoreceptors detect UV wavelengths that are responsible for DNA repair and inhibit motility (Wilde & Mullineaux, 2017). Examination of the gene expression of these photoreceptors and their response regulators would assist in explaining the causes of the upregulation observed in figure 6A.

4.1.2. Far-red

Under far-red, downregulation of many genes was observed as shown in figure 6B. Within microbial mats, far-red can permeate to lower levels due to its wavelength, so it can still be detected the further down the mat and deeper within the water (Ohkubo & Miyashita, 2017). What was potentially expected within the results was higher formation of pili under far-red light due to the need to move and seek out areas of white light. However, what was observed was a lower gene expression of *pil* genes under far-red compared to white light. Figure 7 taken from Schuergers, Mullineaux & Wilde (2017) shows how in *Synechocystis*, movement from pili in the T4P apparatus is triggered by a phototactic response from the cyanobacterium. This similar trend was observed within the results through the downregulation of the *pil* genes, showing pili formation may be a result of moving towards the presence of detected white light, rather than initiating motility to find areas when they currently reside in an area that lacks white light. It was not shown that there was a complete inactivation of the pili under far-red light, ~50% showed a decrease in gene expression of ≥ 75% (Fig. 6B). This may be explained by the

fact that phototaxis is not the only response that pili are involved in (Schuergers, Mullineaux & Wilde, 2017).

The PilMNOQ cluster responsible for pilus assembly decreased by over 90%. As these pili surround the pilus rod and are responsible for pili assembly, there may be indication of inactivity or decreased activity from the pilus rod under far-red compared to white light (Schuergers & Wilde, 2015; Fig. 6B). Following this, both observed forms of PilA decreased in gene expression under far-red (Fig. 5B&C). As PilA are needed to form the pilus rod, is may be suggested that the pilus rod either decreases in production or that there is a lack of thick pili formed. This trend was seen in Conradi, Mullineaux & Wilde (2020) with the deletion of PilA leading to the mutant variants of the bacterium *Pseudomonas aeruginosa* that lack thick pili. PilC which is responsible for anchoring this pilus rod showed a downregulation under far-red of Log2FC -3.94 ± 0.23 for *C. fritschii*. With a decrease in possible production of the pilus rod due to the reduction of PilA, it would make sense for PilC to decrease in production due the lack of pilus for it to anchor, as this is a main role within the T4P (Schuergers & Wilde, 2015).

PilT5 and PilB showed differential downregulation. All other forms of PilT were not significant or were non-differentially expressed. PilT and PilB are important for motility as they are responsible for the extension and retraction of the pilus rod (Wilde & Mullineaux, 2015). These show to decrease in gene expression under far-red light which suggests the lack of motility under this light condition (Fig. 6B).

The PilJIHG cluster while being observed to decrease, only decreased by -80.91%<-73.28%. The PilJIHG cluster is not found in the T4P membrane complex, but the MCP-like complex. This region is usually influenced by the chemical environment, but this suggests a response to light with an interaction with the T4P complex to form the pilus (Schuergers, Mullineaux & Wilde, 2017; Wiltbank & Kehoe, 2018).

PilW is more commonly linked to natural competence and there have been links to light conditions and a circadian rhythm in *Synechococcus elongatus* PCC 7942 (Taton *et al.*, 2020). Here it was observed that PilW decreased in expression under far-red compared to white light by 74% but was not identified in the UV-B experiment. However, this

gene was found in Taton *et al* (2020) to be higher expressed at dusk as light levels decrease, with the main function being for natural competence. Similar results were also found in Schirmacher *et al*, (2020), with the upregulation of competence protein around dusk, with others needed for natural competence (rntA and dprA) being induced by darkness. Here it was observed that PilW decreased in expression under far-red compared to white light by 74% but was not identified in the UV-B experiment. Further investigation with consideration for the competence proteins would help to identify if this process was taking place. PilW has been found to possibly have the secondary function of pilus stabilisation in the bacterium *N. meningitidis* and if this function translates over to *C.fritschii* this decrease would be explainable as similar to what was suggested about PilC; with the decrease in PilA expression leading to the lack of or a thinner pilus there would not be need for assistance in pilus stability (Koo *et al*., 2008).

4.1.3. Further research

While the results suggest that white light and UV-B light is a trigger for the formation of pili with far-red light showing the opposite, the purpose for the changes in expression could not be deduced. The results could only suggest the presence or absence of pili, however it could not show any directional movement or if the intended function of the pili was for something other than motility (natural competence or cell aggregation). As previously mentioned, downregulation of the genes responsible for pil proteins under far-red light may suggest a lack of motility, while the expression and upregulation under white light and UV-B suggests possible motility, as understood within their behaviour in their natural environment (Fig. 6; Wilde & Mullineaux, 2017). Biddanda *et al* (2015) investigated the phototaxis of *Oscillatoria* through using a light source and foil cutouts over a petri dish of sample water. Through using a time lapse of images, motility was observed. An experiment similar to this using separate samples under white light, UV-B, and far-red would be able to show any directional motility and whether the light wavelengths trigger a positive, negative, or a lack of phototactic response. This would also show any possible cell clumping for protective adhesion or natural competence.

After a set amount of time, cells would be extracted for RNA sequencing preparation and analysis.

Within samples if there were lone filaments showing motility and clumps that had aggregated, these could be analysed separately to check for differences in gene expression for other genes such as competence proteins (for example, comEA and comF), DNA processing protein (DprA) and repair protein (RecA) which are required for natural competence (Nies *et al*, 2022).

5. Conclusion

Pili are appendages that continue to be further understood in filamentous cyanobacteria. Through analysing gene expression counts from sequencing that identified genes coding for various Pil proteins, it was found that under UV-B light there was upregulation of some genes, with the PilMNOQ cluster showing major upregulation compared to others. However, there were many *pil* genes shown to not change in expression rates. The PilMNOQ cluster was shown to generally be highly expressed showing vital importance with pili formation. Downregulation was also shown under far-red light overall, with this PilMNOQ cluster as well as the PilACTB showing major downregulation in comparison to the other observed Pil proteins.

These results indicated that under UV-B the rate of pili formation is similar to the formation under white light, while it was observed to decrease under far-red specifically for the formation and function of the T4P. The presence of a sources of light that is located near the surface of a microbial mat is a trigger for T4P activation. The inclusion of Pil-proteins from the MCP-like complex may indicate inclusivity of chemotactic responses when it comes to pili formation.

Understanding how this formation is influenced by light of different wavelengths found in their environment may further the insight of how filamentous species interact within microbial mats, taking direction of movement into account to deduce the specific influence of UV-B on cyanobacteria to possibly reflect how they move differently to influence from white light.

Glossary

Chemotaxis – movement in response to detected chemicals.

Depolymerisation – the breaking down of a polymer into a smaller polymer or monomer.

Downregulation – decrease of gene products in response to a stimulus.

Filamentous cyanobacteria – cyanobacteria that grow in string or ‘filaments’ of cells.

Phototaxis – directional movement in response to a light source.

Prokaryote –an organism that is comprised of cells that do not have a distinct nucleus.

Signal transduction – transmission of a chemical signal through the cell.

Trichome – appendage or structure (usually as a filament) that grows from the cell.

Upregulation – increase of gene products in response to a stimulus.

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Appendix

Datasets:

UVB

accession number	protein	cwUVB	UVB	fcUVB	UVBpv	SE
WP_016872478.1	PilO1	203.1430925	127.8416951	-1.063383805	7.704187551	0.141458171
WP_016873320.1	PilT2	53.30650455	99.08076081	1.118385406	4.1963331	0.172700358
WP_016874078.1	PilQ	35.7994087	79.37655686	2.126691855	9.493470963	0.202032035
WP_016874079.1	PilO2	22.94360595	52.70642972	2.870497774	8.681644734	0.232733008
WP_016874080.1	PilN	20.19804495	43.7093767	3.630038702	9.95259797	0.231820903
WP_016874081.1	PilM	44.93126854	83.02929485	3.196402366	15.05024109	0.194407287
WP_016874123.1	PilD	144.4807741	233.4027215	0.913169789	5.232839338	0.167513703
WP_016875341.1	PilT6	9.356140931	18.87899364	1.572083616	1.860115739	0.209617023
WP_016876361.1	PilH1	27.92471466	55.92289587	1.26403627	3.24117611	0.20972371
WP_016876363.1	PilJ1	100.5496228	176.8995014	1.257470017	7.458475989	0.200956121
WP_016879164.1	PilJ2	503.2964427	323.3362	-1.006754216	2.488832308	0.15161683
WP_016879165.1	PilI	5.562108306	14.96696332	1.791179625	1.59118671	0.205287258
WP_016879293.1	PilA2	51.93928224	63.72843562	0.931375717	2.709474058	0.312038986

WP_016879562.1	PilJ3	70.28352343	97.24513138	1.222707385	5.06587626	0.155588059

Far-red

accession number	protein	cwFR	FR	fcFR	FRpv	SE
WP_016872478.1	PilO1	401.1766333	714.3375667	0.822778866	8.220948417	0.141458171
WP_016872930.1	PilT1	9.717318333	5.060248333	0.253669561	0.639357322	0.21106789
WP_016873320.1	PilT2	861.7068667	392.5605333	-1.122637964	10.09660783	0.172700358
WP_016873630.1	PilT3	109.4024567	130.3889667	0.263351366	0.667844529	0.212326679
WP_016874001.1	PilT4	548.1573667	412.3994333	-0.39876252	1.625981491	0.176240645
WP_016874078.1	PilQ	9240.675	692.3907333	-3.680184892	73.41272017	0.202032035
WP_016874079.1	PilO2	3221.595	157.1587333	-4.265738113	74.3127017	0.232733008
WP_016874080.1	PilN	3273.974	169.3887	-4.18291247	72.05363771	0.231820903
WP_016874081.1	PilM	4663.155667	408.6085667	-3.462342591	70.22629765	0.194407287
WP_016874123.1	PilD	756.0644667	613.2065333	-0.293690913	1.099296217	0.167513703

WP_016874200.1	PilB	3437.624667	223.5297333	-3.874947018	73.87826771	0.212042439
WP_016874201.1	PilT5	2192.229	128.7532	-3.991588767	60.53160126	0.241718718
WP_016874202.1	PilC	1252.117033	76.61879667	-3.944400076	66.7989663	0.227180241
WP_016874203.1	PilA1	371.258	31.52807	-3.468062135	36.34583271	0.272621077
WP_016875341.1	PilT6	373.7575333	178.3179333	-1.044727616	6.205591078	0.209617023
WP_016875933.1	PilT7	258.9816667	183.6308	-0.486145799	2.598310306	0.160936639
WP_016876360.1	PilG1	388.5589333	192.8192333	-0.993854655	5.603355738	0.211066034
WP_016876361.1	PilH1	1135.2059	96.59752	-3.490013162	61.45386456	0.20972371
WP_016876363.1	PilJ1	5110.805	440.8587667	-3.481275538	66.50526814	0.200956121
WP_016877190.1	PilT8	139.8959	124.2018333	-0.156821103	0.347101947	0.207446804
WP_016878569.1	PilT9	134.2109	60.24168667	-1.089651668	3.804705437	0.288261952

WP_016878612.1	PilW	303.9655667	78.92282667	-1.906252707	13.57679366	0.250349289
WP_016878846.1	PilT10	20.82919667	22.08723333	0.123109053	0.136710409	0.356631178
WP_016879164.1	PilJ2	2239.020333	598.3039333	-1.890195725	34.94651315	0.151616835
WP_016879165.1	PilI	283.1874333	60.34668	-2.183719058	25.69970948	0.205287258
WP_016879166.1	PilH2	301.0285667	66.75647333	-2.123053752	26.47542478	0.196531965
WP_016879167.1	PilG2	776.7017333	148.2811333	-2.371804152	42.11023117	0.172878143
WP_016879293.1	PilA2	8734.5	610.0091	-3.698464518	31.68050665	0.312038986
WP_016879352.1	PilF1	1346.423333	1640.527	0.28062648	1.226738237	0.148814037
WP_016879562.1	PilJ3	551.2953333	704.4369667	0.34945065	1.607231635	0.155588059
WP_016879735.1	PilF2	597.7218667	541.1345667	-0.141485612	0.555272706	0.130986652

R code for figures:

```
# load in data & setups #

library(readxl)

piliUVB <- read_excel("piliUVB.xlsx") # saved in documents #

piliFR <- read_excel("piliFR.xlsx") # saved in documents #


########## UVB BARCHARTS – figure 5 ##########

# UV-B control white #

par(mar = c(10, 10, 5, 2))

barplot(piliUVB$cwUVB, names.arg = piliUVB$`protein`, cex.names = 1.5, las = 2,
ylim = c(0, 500), main = "A)", adj = 0,  cex.main = 3, cex.axis = 1.25)

mtext("Pil-protein", side=1, line=6, cex = 2)

mtext("Gene expression

(read count)", side=2, line = 4, cex = 2)

# UV-B #

par(mar = c(10, 10, 5, 2))

barplot(piliUVB$UVB, names.arg = piliUVB$`protein`, cex.names = 1.5, las = 2, ylim =
c(0, 500), main = "B)", adj = 0,  cex.main = 3, cex.axis = 1.25)
```

```
mtext("Pil-protein", side=1, line=6, cex = 2)

mtext("Gene expression

(read count)", side=2, line = 4, cex = 2)

########## FAR-RED BAR CHARTS ##########

# far-red control white #

par(mar = c(10, 10, 5, 2))

barplot(piliFR$cwFR, names.arg = piliFR$`protein`, cex.names = 1.25, las = 2, ylim = c(0, 10000), main = "C)", adj = 0,  cex.main = 3, cex.axis = 1.25)

mtext("Pil-protein", side=1, line=5.5, cex = 2)

mtext("Gene expression

(read count)", side=2, line = 4, cex = 2)

# far-red #

par(mar = c(10, 10, 5, 2))

barplot(piliFR$FR, names.arg = piliFR$`protein`,cex.names = 1.25, las = 2, ylim = c(0, 10000), main = "D)", adj = 0,  cex.main = 3, cex.axis = 1.25)

mtext("Pil-protein", side=1, line=5.5, cex = 2)

mtext("Gene expression

(read count)", side=2, line = 4, cex = 2)

### all 4 barplots were edited into one image using powerpoint and exported to a jpeg image ###

########## Volcano plots – figure 6 ########

# far-red volcano plot #

par(mar = c(4.5, 10, 3, 10))
```

```
plot(piliFR$fcFR, piliFR$FRpv, xlab = "Log2FoldChange", ylab = "-Log10(p-value)", xlim = c(-5, 5), ylim = c(0, 80), cex.lab = 2, cex.axis = 1.5)

mtext("B)", side=1, cex = 3, line = -22.5, adj = 0)

abline(h = 1.3, col = 'red')

abline(v = 0)

abline(v = -1.3, col = 'red')

abline(v = 1.3, col = 'red')

# UVB volcano plot

par(mar = c(4.5, 10, 3, 10))

plot(piliUVB$fcUVB, piliUVB$UVBpv, xlab = "Log2FoldChange", ylab = "-Log10(p-value)", xlim = c(-4, 4), ylim = c(0, 15), cex.lab = 2, cex.axis = 1.5)

mtext("A)", side=1, cex = 3, line = -22.5, adj = 0)

abline(h = 1.3, col = 'red')

abline(v = 0)

abline(v = -1.3, col = 'red')

abline(v = 1.3, col = 'red')

### both volcano plots were edited into a single figure using powerpoint and exported into a jpeg image ###
```