University of Glasgow | College of Medical, Veterinary & Life Sciences

Head of College Scholars List Scheme

Summer Studentship

Report Form

This report should be completed by the student with his/her project supervisor. It should summarise the work undertaken during the project and, once completed, should be sent by email to: jill.morrison@glasgow.ac.uk within four weeks of the end of the studentship.

1. Student

   Surname: ***Katkeviciute*** Forename: ***Egle***

   E-mail address: ***2034904K@student.gla.ac.uk***

2. Supervisor

   Surname: ***Cogdell*** Forename: ***Richard***

   E-mail address: ***Richard.Cogdell@glasgow.ac.uk***

3. Research Project Report

   3.1 Project Title (maximum 20 words):

   Investigation of the consequences of puc gene deletions on the spectroscopic form of LH2 produced by Rhodopseudomonas palustris.

   3.2 Project Lay Summary (copied from application):

   The LH2 complexes are the major light harvesting complexes used in purple bacterial photosynthesis. Some species such as Rhodopseudomonas palustris have a multi gene family (the puc genes) that encode the apoproteins, which are used to make the light harvesting 2 (LH2) complexes. Different combinations of puc genes produce different spectroscopic forms of LH2. To really understand the molecular details of how the different apoproteins contribute to the different spectroscopic forms, we have created a set of puc deletion strains. This project will compare the wild type strain to the mutants where the apoprotein composition has been reduced and the structure and function relationships can be clearly investigated.

3.3 Start Date: ***02/06/2014*** Finish Date: ***13/07/2014***

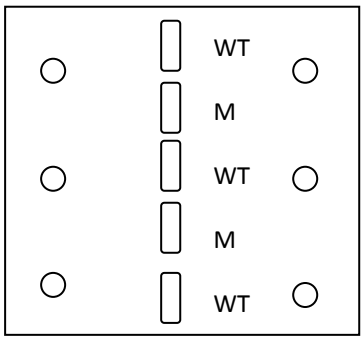
3.4 Original project aims and objectives (100 words max):

The main aim of this project was to determine which spectroscopic forms of LH2 can be made in three puc deletion strains where the possible polypeptide composition of the LH2 complexes has been reduced to the point where the structure/function relationships of the different puc genes is simple enough to be unequivocally determined. However, only one ΔpucBA strain was examined due to the time shortage.

3.5 Methodology: Summarise and include reference to training received in research methods etc. (250 words max):

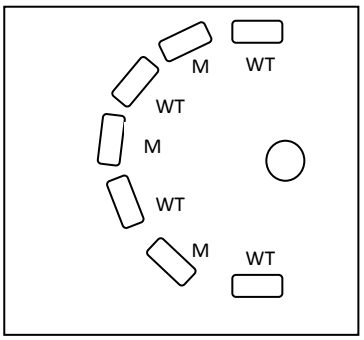
GROWTH

The wild type and mutant strains of Rps. palustris were grown on the C-succinate media under a range of different light intensities (low light – one light bulb (40W) in 30cm distance and high light – 6 light bulbs (100W) in 30cm distance). To equal small differences in intensity bottles were allocated in precise order (WT/M/WT/M, as shown in the figures 1 and 2).



High light

Figure 1



Low light

Figure 2

PREPARATION

The cells grown from both intensities were harvested using centrifuge at 4000RPM at 4°C for 20 min. Cells were resuspended (small pinches of DNAse and magnesium chloride hexahydrate were added) and broken down with a French Press. Absorption spectra of membranes in the fingerprint NIR absorption region (250-950nm) were recorded.

SEPARATION

The membranes then were solubilized with a detergent LDAO (lauryldimethyl amine oxide) and the LH2 and LH1/RC (light harvesting complex 1/reaction centre) complexes separated by sucrose gradient centrifugation. Sucrose gradient was prepared using different sucrose concentrations. In one tube 5ml of 0.8M, 6ml of 0.6M, 6ml of 0.4M and 5ml of 0.2M sucrose solution (1M sucrose was prepared with 20mM Tris and 0.1% LDAO

detergent and then diluted to required dilutions using 20mM Tris) were slowly added, making distinct layers. Then on the top 3ml of sample was added not disturbing the sucrose layers. 8 tubes were centrifuged at 45000RPM at 4°C for 14h. There were two separate bands (see figures 8-11). In each case the absorption spectra of the LH2 and LH1/RC complexes were recorded and conclusions about these complexes expressions in different range of intensities were drawn.

PCR

Bacteria were also grown on agar plates in order to get single colonies, from which DNA could be extracted and amplified using PCR. This then allowed drawing conclusions upon the changes, which were made in bacteria's genome.

3.6 Results: Summarise key findings (300 words max). Please include any relevant tables or images as an appendix to this report:

MEMBRANES

All graphs were normalized at the Q_X 580nm absorption band.

In figures 3 and 4 bacteria grown in HL can be seen. In huge difference in spectra is found comparing the mutant to the wild type. In the mutant grown at HL there is no LH2, while the wild type cells show the typical HL LH2 form.

In both 5 and 6 figures similar tendency can be seen in 800-900nm ranges, except the ratio between LH2 and LH1/RC is almost twice as large in mutant than in WT.

Figure 7 shows how bacteria adapted change of light intensity. Bacteria on June 16th were transferred from HL to LL and grown further for a week. The relative amount of LH2 to LH1 is increased.

In the table 1 volumes of membranes got can be seen. All membranes are made up to OD 50.

SUCROSE GRADIENT

LH1/RC and LH2 separations are shown in figures 8-11 (appendix). In the high light (HL) wild type (WT) both LH1/RC and LH2 complexes were expressed. While in pucβAdel only LH1/RC complex showed up. In the low light (LL) both mutant and wild type expressed LH1/RC and LH2 complexes. All amounts of LH1/RC and LH2 produced are shown in table 2, where OD is 100.

LH1/RC AND LH2 COMPLEXES

Figures 12 and 13 shows the differences between mutant and WT LH1/RC and LH2 complexes in HL. Taking graphs and table 3 into account only significant difference is absence of LH2 complex in mutant.

In figures 14 and 15 LH1/RC and LH2 complexes are also compered. Difference can be seen in LH2 separation in the range from 850 to 900 (there LH1 complex is found) (for more detailed difference see table 4). This is due to fault in WT separation, some of LH1/RC complex mixed with LH2, due to huge concentrations and small space between gradients (figure 11).

PCR

PCR checks proved that correct mutations were made. In figure 17 PucA strain was unclear, PucC strains of WT and mutant are clearly seen and using marker size, shown in figure 16, WT size is determined around 1250bp and mutant size is about 900bp, while pipetting error was made with PucD strain, thus results are inconclusive and PRC should be repeated. In figure 18 PucE strain is shown. WT size is around 1000bp and size of mutant is around 1450bp, as expected. Figure 19 is blurred, however WT strain can be seen a bit higher than 1000bp marker and mutant size is around 1600bp. Different PCR programs had to be used for PucB and PucA strains to make them clearer, thus PRC gradient have been done (figure 20 and 21). From the gradient PCR PucA WT size can be determined around 1000bp, while mutant size is about 800bp. WT and recombinant strains' sizes can be found in table 5.

Discussion (500 words max):

The light harvesting system in typical purple photosynthetic bacteria is composed of LH1/RC and LH2 complexes. These antenna complexes absorb solar radiation and transfer this energy to reaction centers, where it is used to start photosynthetic electron transport. (Cogdell, 2006)

Rhodopseudomonas palustris is from the group of purple bacteria, which produces LH2 complexes with unusually high absorption peak comparing with LH1/RC complexes, while grown in the low light intensity. This leads to the conclusion that concentration of proteins composing LH2 complex is higher then LH1/RC. (Brotosudarmo, 2009) In this project wild type and mutant bacteria, which were grown in the low light, proves this statement. Moreover, spectroscopy of WT, grown in low light, shows lower LH2 peak than mutant and very broad LH1/RC peak in 850-920nm range. While in mutant spectroscopy high LH2 peak shows up and only small and narrow peak is found in LH1/RC area. This shows that pucβA mutation is involved in higher LH2 expression and suppression of LH1 complex in the low light.

To add more, volumes of LH1/RC and LH2 complexes' proteins are quite different in bacteria grown in low light and in high light. This is due to the fact that low light

bacteria were grown longer and it requires much more apoproteins in the light harvesting system in order to harvest similar amount of solar radiation. (See table 2)

Furthermore, this deletion also influences the LH2 complex production in the high light. The difference can be clearly seen from the spectroscopies of WT and mutant membranes (figures 3 and 4) and from lack of LH2 complex in sucrose gradient (figure 8). This leads to conclusion that pucβA gene is responsible for LH2 complex expression in HL. It might be the case of fault in some apoproteins production or some other protein, which works as a switch for LH2 complex construction. In order to find out the reasons, further and more detailed experiments have to be carried out.

Finally, using PCR I was able to prove that the desired deletion had been made (reference to results section, figures 16-21 and table 5), however some additional experiments have to be made in order to overcome pipetting mistakes.

Reflection by the student on the experience andβ value of the studentship (300 words max):

I am very grateful for the opportunity to spend my summer in prof. R. Cogdell's lab. It was invaluable, interesting, challenging and very useful experience. At the beginning of last academic year, I could not even imagine that I will have a summer placement and gain so much practice, knowledge and confidence before starting my third year in university. I am sure that this project gave me very useful skills for PCR, Spectroscopy and using a French Press, running the DNA gels and simply doing stock solutions. This will make my transition from very broad second year subjects to third year biochemistry studies so much easier.

Moreover, this small 6 weeks practice let me realize that I want to work in the lab in the future. Thus, it made my decisions for future easier and let me realize why I have to study even harder.

Finally, I am very grateful for this scholarship, which allowed me to concentrate only on my project. I also want to thank prof. R. Cogdell, Ms J. Southall and other people who helped me and thought me so much. Their long-year experience is not only very useful and educational, but also very inspiring to never give up and always keep trying again and again.

4. Dissemination: (note any presentations/publications submitted/planned from the work):

This work was a part of a larger effort, where the different combination of deletion will be carried out.

6. Signatures:

Supervisor	Date	Student	Date
PROF. RICHARD COGDELL	31/07/2014	EGLE KATKEVICIUTE	31/07/2014

APPENDIX

Membranes

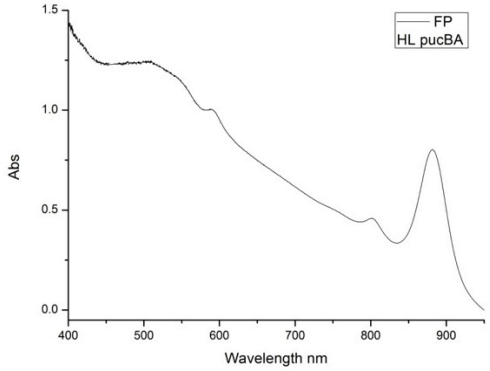


Figure 3 Spectroscopy of HL pucβAdel

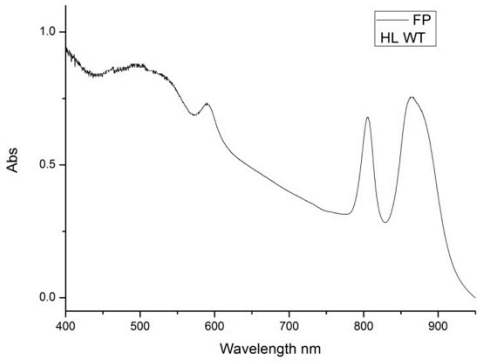


Figure 4 Spectroscopy of HL WT

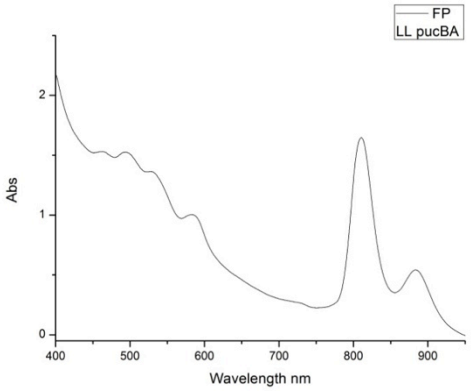


Figure 5 Spectroscopy of LL pucβAdel

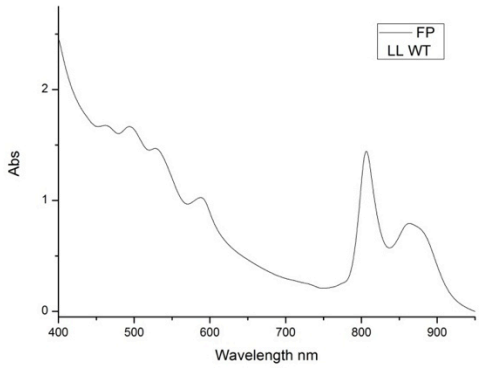


Figure 6 Spectroscopy of LL WT

	Volumes (ml) OD50
HL WT	***47***
HL pucβA	***33***
LL WT	***161***
LL pucβA	***64***

Table 1 Volumes of membranes

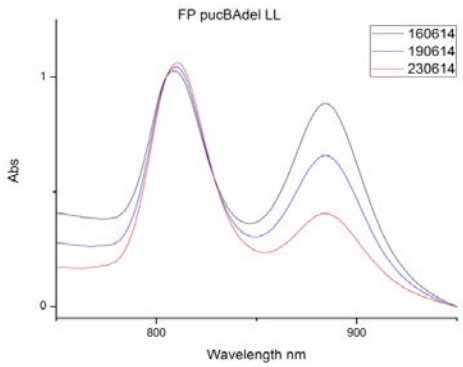
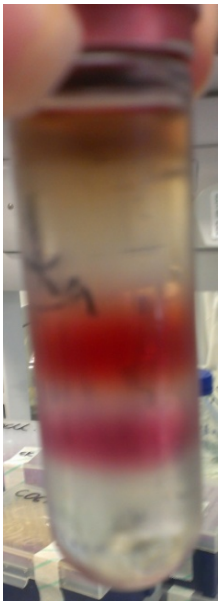


Figure 7 Changes in LH1/RC complex after light intensity modification

Sucrose gradient

LH1/RC

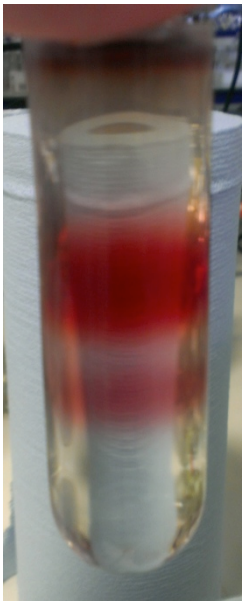
Figure 8 PucβAdel HL



LH2

LH1/RC

Figure 9 WT HL



LH2

LH1/RC

Figure 10 PucβAdel LL

LH2

LH1/RC

Figure 11 WT LL

OD 100	*Volume of LH1/RC (ml)*	*Volume of LH2 (ml)*
HL pucβAdel	*76.5*	*-*
HL WT	*11*	*40.25*
LL pucβAdel	*13.49*	*163.9*
LL WT	*22.18*	*102.23*

Table 2 Volumes of LH1/RC and LH2 complexes

LH1/RC and LH2 complexes

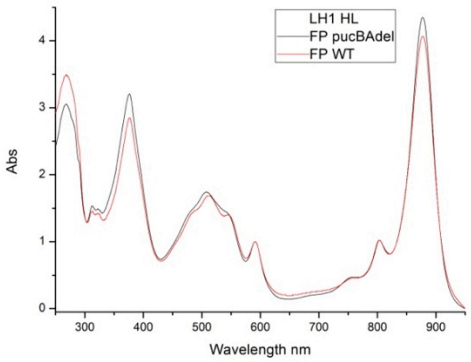


Figure 12 Comparison of LH1 complexes in HL

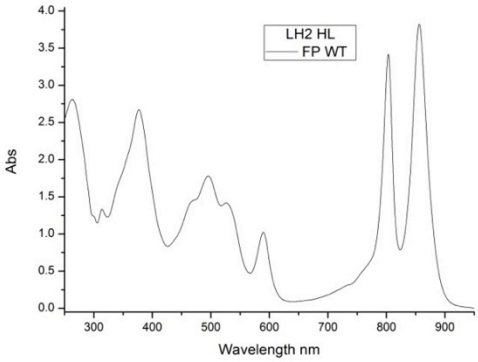


Figure 13 Graph of WT LH2 complex in HL

	LH1		LH2	
	Wavelength (nm)	Absorption	Wavelength (nm)	Absorption
PucBA (LH1)	877.00	4.35227	803.40	1.02273
WT (LH1)	877.20	4.06522	803.60	1.02174
WT (LH2)	856.00	3.82328	803.40	3.4181

Table 3 Data of wavelengths and absorptions in HL

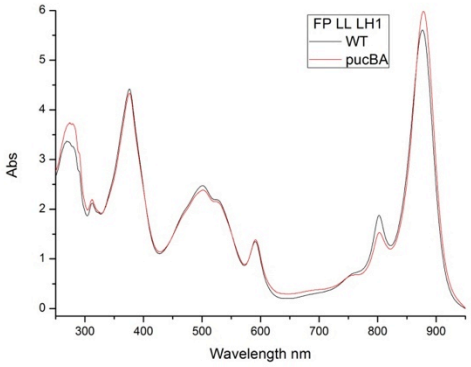


Figure 14 Comparison of LH1 complexes in LL

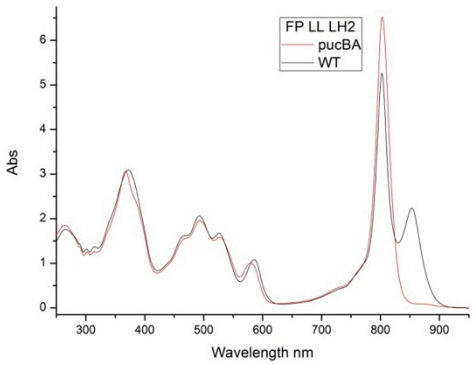


Figure 15 Comparison of LH2 complexes in LL

	LH1		LH2	
	Wavelength (nm)	Absorption	Wavelength (nm)	Absorption
PucBA (LH1)	878.20	5.98319	802.80	1.52941
WT (LH1)	876.60	5.6051	802.60	1.87898
PucBA (LH2)	854.40	0.10294	803.20	6.51471
WT (LH2)	853.20	2.23429	802.60	5.26286

Table 4 Data of wavelengths and absorptions in LL

PCR

Checks

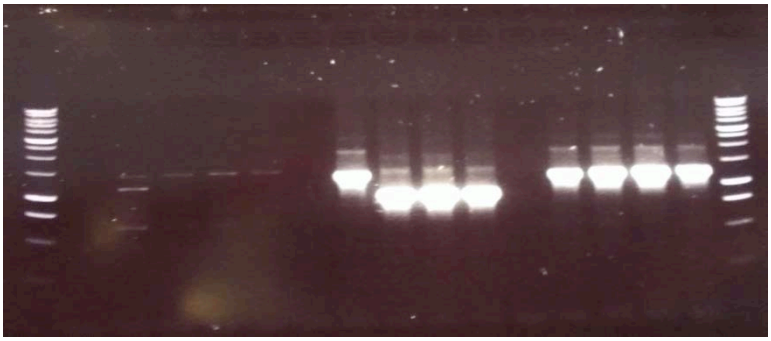
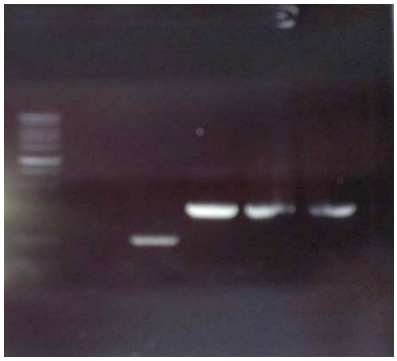


Figure 16 Marker size ***PucA*** ***PucC WT Mutants*** ***PucD***

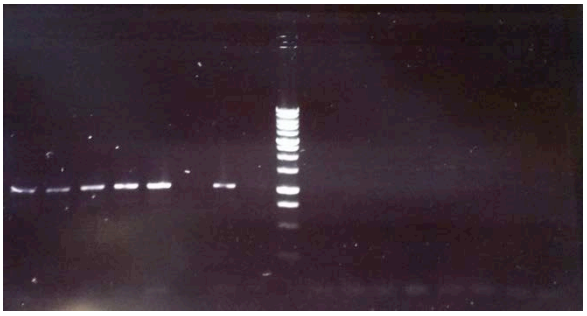
Figure 17 PCR checks of PucA, PucC and PucD respectively



PucE WT Mutants ***PucB WT*** ***Mutant***

Figure 18 PCR check of PucE ***Figure 19 PCR check of PucB***

Gradients



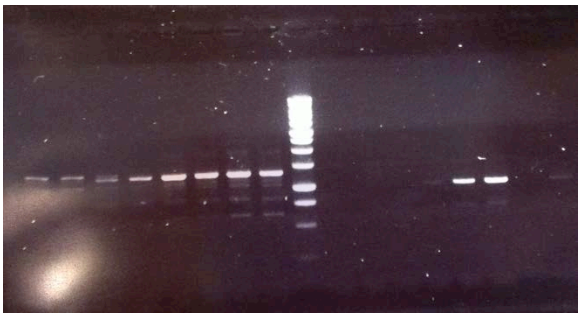


Figure 20 PCR gradient of PucB ***PucA WT*** ***Mutants***

Figure 21 PCR gradient of PucA

	Wild type (bp)	Recombinant (bp)
PucA	1170	850
PucB	1047	1661
PucC	1280	930
PucD	1246	978
PucE	1014	1488

Table 5 Sizes of WT and recombinant Puc strains

REFERECE:

Brotosudarmo, T.H., Kunz, R., Böhm, P., Gardiner, A.T., Moulisová, V., Cogdell, J.R. and Köhler, J., 2009. Single-molecule spectroscopy reveals that individual low-light LH2 complexes from Rhodopseudomonas palustris 2.1.6. have a heterogeneous polypeptide composition. **Biophysical Journal,** ***[online]. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19720038> [Accessed 31 July 2014].***

Cogdell, J.R., Gall, A. and Köhler, J., 2006. The architecture and function of the light-harvesting apparatus of purple bacteria: from single molecules to in vivo membranes. **Cambridge journals,** ***[online]. Available at: <http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=552712&fileId=S0033583506004434>[Accessed 31 July 2014].***