Introduction and aims.

Bacterial microcompartments (BMCs) are selectively permeable protein shells which encapsulate an enzymatic core (Hagen et al., 2018). Their formation provides a competitive advantage as it allows for the separation of toxic intermediates formed during catabolic reactions from the cytosol, contributing to a larger metabolic versatility (Planamente & Frank, 2019). It is therefore unsurprising that operons encoding for BMCs are found amongst a myriad of bacterial species (Kerfeld et al., 2018), including members of the gut microbiome. A 1,2-propanediol-utilization (PDU) BMC encoding operon has been identified in *Ruminococcus gnavus*. The PDU genes within the BMC operon are vitamin B12 independent, however the PDU CDE genes elsewhere in the genome are predicted to be vitamin B12 dependant through bioinformatic analysis. This project aimed to establish the association of *R. gnavus* PDU CDE genes with the BMC and determine whether their action is vitamin B12 dependent.

Methods.

Anaerobic growth assay and propionate production

An anaerobic growth assay was set up in order to measure and compare the growth of *R. gnavus* ATCC29149 in the presence of glucose, fucose, rhamnose and 1,2-propanediol in YCFA media. In order to analyse the vitamin B12 dependency of *R. gnavus* growth, three different YCFA medias were used, which consisted of: normal YCFA; YCFA with vitamin B12 removed through BTUG2 protein affinity chromatography; YCFA with vitamin B12 added. The optical density was measured every 30 minutes over a 24-hour period, and the data was analysed on Microsoft excel, where growth curves were generated. This was repeated with samples taken at 0, 3 and 8 hours, for analysis of propionate production.

Heterologous expression of *R. gnavus* genes

Several *R. gnavus* genes encoding for PDU and BMC proteins were cloned and transformed into *E. coli* BL21 cells. Target genes were cloned through infusion cloning, utilising primers which were designed using SnapGene. The target inserts were amplified through a PCR reaction, before subsequent transformation into *E. coli* BL21 competent cells through heat shock.

Following incubation at 37º for 1 hour, 100 µl of cells were spread on LB plates containing 50 µg/ml kanamycin and incubated at 37º overnight. Six of the resulting colonies from each transformation were analysed through colony PCR and DNA agarose gel electrophoresis, to confirm the correct plasmid was present.

In total, we expressed 9 different PHISTEV plasmids which consisted of: the PDU CDE genes 189, 190 and 191, each with and without the BMC proteins; a cargo protein with the BMC proteins; just the BMC proteins; and a cluster containing putative shell proteins aside from the main PDU BMC genes.

Protein expression and purification

Following the successful transformation of *R. gnavus* genes into *E. coli* BL21 cells, the proteins were expressed in autoinduction media (AIM). A 1% innocula of AIM containing 50 µg/ml kanamycin was

prepared for each transformation. These were incubated at 37º for 5 hours, before the temperature was lowered to 22º and incubated for 72 hours.

The proteins were subsequently extracted from each AIM. The cell lysates which were thought to contain the PDU CDE genes without the BMC proteins, were purified through nickel affinity chromatography on an ÄKTA Pure protein purification system. As we were unable to isolate any protein from our samples, SDS-PAGE analysis of the insoluble cell pellet revealed that the PDU CDE proteins 189, 190 and 191 were insoluble.

The cell lysates extracted from the cells consisting of plasmids containing the BMC proteins were subjected to ultracentrifugation in a 35-65% sucrose density gradient at 130,000 G for 16 hours. Following this 1 ml fractions were taken from each of the samples for SDS-PAGE analysis.

Results and outcomes of studentship.

Analysis of *R. gnavus* anaerobic growth assay data indicated the utilisation of glucose, rhamnose and fucose, however not 1,2- propanediol. The addition of vitamin B12 to YCFA did not significantly improve growth.

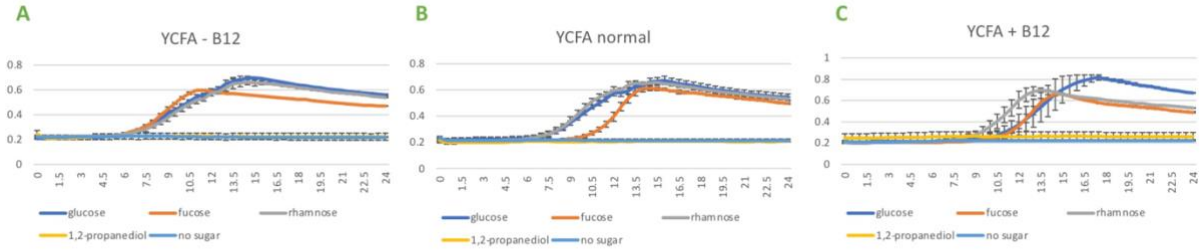

Figure 1: **Growth curves from anaerobic growth assay on *R. Gnavus.*** *Ruminococcus gnavus* was grown in four different sugars, and no sugar as a control, with three different YCFA media's, which differed in vitamin B12 content. **A** was YCFA with vitamin B12 removed through BTUG2 protein affinity chromatography. **B** was normal YCFA. **C** was YCFA with vitamin B12 added.

Heterologous expression of *R. gnavus* genes into *E.coli* BL21 cells was successful and the resulting colonies were verified through colony PCR.

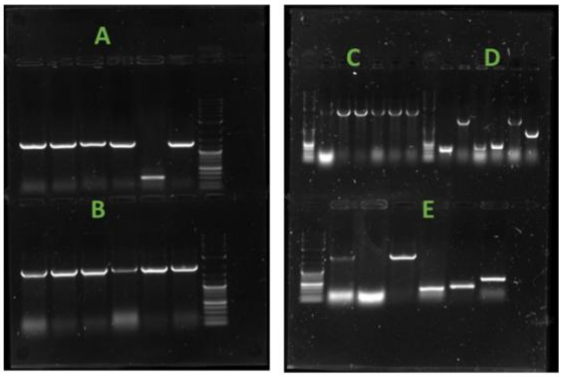

Figure 2: **DNA gel electrophoresis following colony PCR.** Six colonies were selected for colony PCR following transformation. **A** shows the plasmid containing the cargo protein with the BMC. **B** shows the putative shell proteins. **C, D and E** show the PDU CDE 189, 190 and 191 genes without the BMC.

Future directions.

Future goals include the heterologous expression of *R. gnavus* bacterial microcompartments in *E. coli.* This will be performed in presence and absence of known cargo proteins and putative cargo proteins, to determine the full extent of BMC utilisation in *R. gnavus.* Furthermore, the impact of expression of this BMC will be investigated in relation to the production of SCFAs, in particular propionate.

Departures from original project plan.

Due to the difficulties in purifying the proteins, we were unable to do vitamin B12 binding assays or bioimaging of BMCs. Samples taken during the anaerobic growth assay on *R. gnavus* will be analysed at a later date for propionate production, to determine if addition of vitamin B12 had an effect. As the PDU CDE genes are insoluble they will require solubilisation through denaturing, followed by refolding and subsequent purification through chromatographic techniques.

Value of studentship to the student.

Woking in the Juge lab has allowed me to develop a myriad of lab and interpersonal skills which will be invaluable to me when completing a PhD in a closely related area of research. I was able to gain experience in microbiological techniques such as cloning and subsequent culturing and heterologous expression, as well as the use of the anaerobic chamber. I was able to extract plasmids for analysis through DNA agarose gel electrophoresis and verify proteins through SDS-PAGE. Through shadowing of my supervisor, I was able to gain some experience in the use of the AKTA pure protein purification system and the ultracentrifuge. In addition to lab skills, I was able to expand a range of interpersonal skills such as my oral presentation skills by presenting the progress I had made at fortnightly lab meetings. Within the Norwich research park there are several weekly seminars which I was able to attend, allowing me to expand my scientific curiosity and knowledge in other areas of research.

Value of studentship to the research group.

The studentship gave valuable teaching and supervisory experience to a PDRA which they can take forward in future roles. The student provided the group with valuable support in pushing forward the research project, successfully cloning multiple constructs, and testing expression of different constructs. Furthermore, the student performed numerous growth curves which progressed the project and generated data which will form a basis of future publications, determining the roles on BMCs in *R. gnavus.*

Bibliography

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