The Early Days of Characterising Ultrasmall Parasitic Bacteria

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Introduction

Saccharibacteria (formerly *TM7*) is present both in humans and in the environment ubiquitously, but was only successfully cultured in 2015 **(1, 2)**. It has a uniquely small size of 200 to 300 nm and a reduced genome which requires a living host, such as *Arachnia propionica*. There is currently insufficient knowledge on the bacteria and the interaction with the host, of which further characterisation is required **(1, 2)**.

Aims and Approaches

- Optimise real-time polymerase chain reaction (qPCR) to quantify *Saccharibacteria* from coculture
- Identify cell surface proteins for host attachment
  - Biotinylate and purify proteins using Streptavidin magnetic beads
- Analyse genomes of *Saccharibacteria* using SignalP to find potential secreted proteins

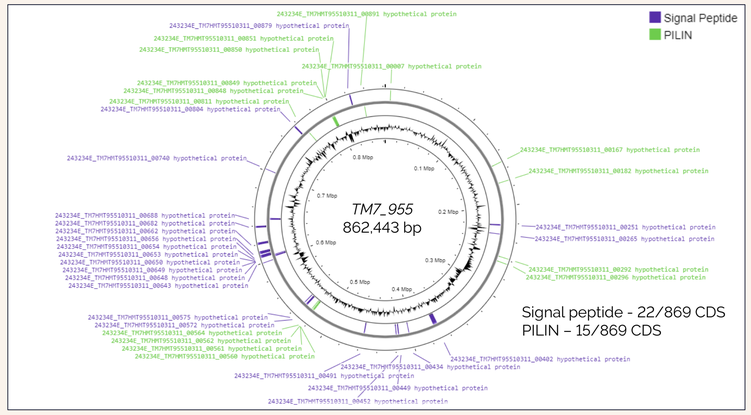

Figure 1. *Saccharibacteria* (*TM7_955*) complete genome construct. Proteins with predicted signal peptide (purple) and pilin-like proteins (green) are annotated.

Results

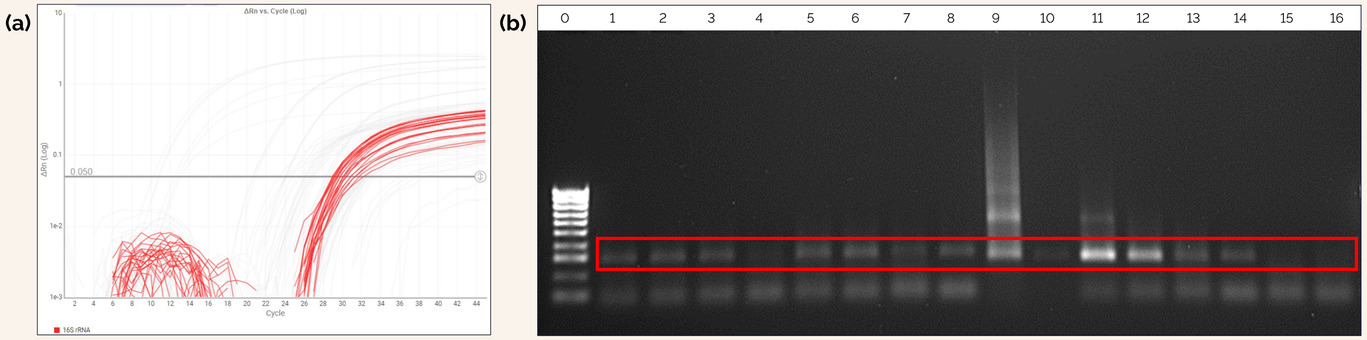

Figure 2. (a) Amplification of *Saccharibacteria* 16S rRNA (*TM7_488 and TM7_955*) from coculture with *A. propionica.* Reaction efficiency was 67.91% with an amplification factor of 1.68. **(b)** Gel electrophoresis was performed to confirm amplicon size of 275 base pairs (in red). Lane 0 (1kb ladder), 1 - 4 (TM7_955 coculture), 5 - 8 (TM7_488 coculture), 9 - 15 (10-fold serial dilutions of TOPO Plasmid), 16 (negative control with water)

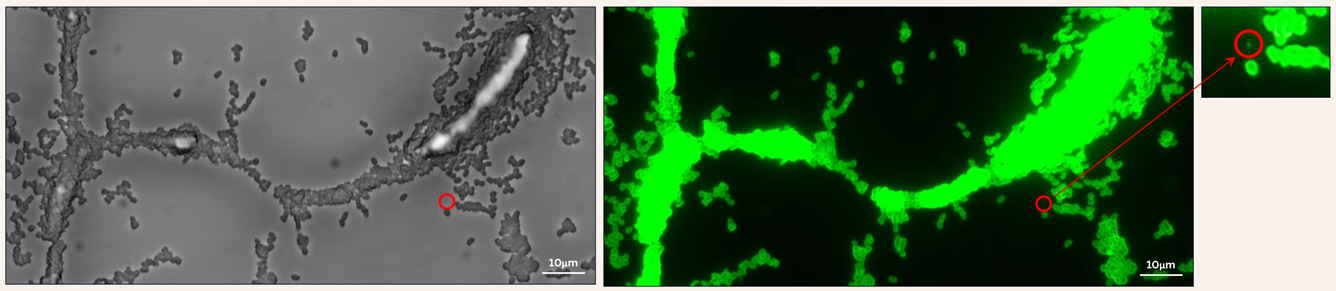

Figure 3. Biotinylated surface proteins labelled with Alexa Fluor™ 488 streptavidin. Coculture was viewed at 100X magnification in bright field (left) and with fluorescent (right) where *Saccharibacteria* can be seen as circled (in red).

Conclusions and Future Work

- *Saccharibacteria* can be stably detected and quantified from coculture, however, reaction efficiency was limited likely due to primer and probe designs
- Biotinylated cell surface proteins were detected using fluorescent microscope, but not detected in western blotting due to low concentration
  - Optimisation of primary and secondary antibodies is also required
- Proteins with signal peptides were identified on *TM7_955* genome

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References

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