The Detection of Termite Group One Methanogens in Termite Guts Using Intergenic Spacer Analysis.

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Figure 1. Zootermopsis angusticollis, a wood feeding termite.

Introduction

Termites are fundamental components of the tropical soil community, their consumption and digestion of plant material has a significant effect on the structure and nutrient content of soil. Termites are split into two groupings: the lower and the higher termites. The lower termites all feed on wood or grass and have simple gut structures that contain flagellated protists. The higher termites include 70% of all known termite species and feed on a wide range of plant material. Digestion in termites is closely related to gut structure, the physiochemical conditions in different gut regions and symbiotic microbiota found in their guts. Methanogens are archaea that produce methane as a metabolic bi-product, the hydrogen consumption involved in this process is expected to promote anaerobic cellulose decomposition in the hind gut of termites. The presence of methanogens in termites is fascinating this is especially true as some wood feeding termites emit no methane whilst in soil feeders methanogenisis can represent a large percentage of the termites respiratory effort. It is also apparent that at least one group of methanogens (termite archael group one) is only found in termite guts. My project focused on identifying this particular group of methanogens in a termite gut sample by looking at the length of a particular region of their genome, the ribosomal RNA intergenic spacer region.

The Intergenic Spacer Region (Figure 3)

The ribosomal intergenic spacer region, or ISR, is a region of non-coding DNA situated between the small (16S) and large (23S) ribosomal subunit genes. While the 16S and 23S genes are ideal for construction of the phylogenetic relationship of species, they is generally little variation within a species. The ISR, on the other hand, provides sufficient variability to be able to resolve strains within a species. Using primers that are located in the conserved 16S and 23S regions to amplify DNA across the ISR means that various strains, as well as species of methanogen could theoretically be identified.

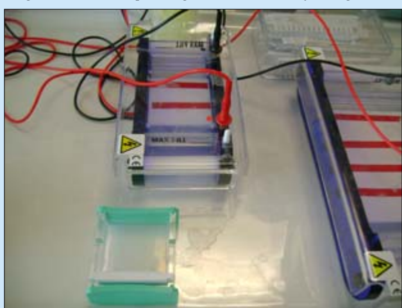


Figure 2. A photograph showing the gel electrophoresis rigs used to separate products of PCR.

Methods

In order to isolate and amplify the ISR regions of any methanogens that were in the sample I used PCR with primers specific to archaea (see below). I then went on to clone these ISR fragments so each cell line contained a single insert in a plasmid. I analysed the clones by amplfying the individual cloned ISR regions using the same primers and comparing the length. In order to confirm that these ISR's belonged to methanogens, in particular termite group one methanogens, I also used the DNA from the clones with methanogen specific primers in a separate set of PCR's.

Cloning

With a complex microbial community cloning PCR products helps separate the fragments from individual components this allows identification of the organisms in a community. I used cloning to separate ISR fragments of various lengths that appeared as a 'smear' on the aragose gel. This meant that each ISR region could be analysed individually.

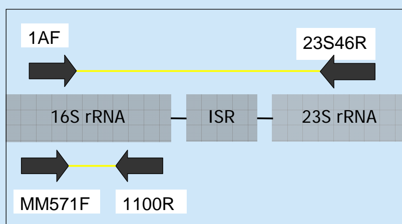


Figure 3. A diagram showing the ISR and the primers used to isolate the group one methanogens.

PCR (Figure 7)

The polymerase chain reaction is a biological technique used to amplify regions of DNA many millions of times in a short period of time. Samples are heated to 96°C to denature the DNA. The temperature is then lowered to 50-60°C to allow oligonucleotide primers to anneal to the DNA strands. The temperature is then raised to 72° C, the optimum temperature for a heat stable DNA polymerase (taq) that extends the primers and synthesises a new DNA strand complementary to the existing one. This cycle is repeated many times with the number of target DNA copies increasing exponentially. The products are then stained and separated by gel electrophoresis and can be seen under UV light. For PCR I used 2 sets of primers (Figure 3); the first amplified across the ISR as the forward primer (1AF) annealed in the 16S rRNA and the reverse primer (23S46R) annealed in the 23S. These two primers were archael specific, but not specific for methanogen DNA, so an additional set was used. MM571F is a forward primer that anneals in the 16S rRNA and is specific for group one methanogens. 1100R is a reverse primer that also anneals in the 16S and is thought to be group one specific. The two sets of primers were used to identify various lengths of the ISR and also to confirm that the ISR being analysed belonged to termite archael group one methanogens.

Figure 4. The gel showing same size inserts proving the organisms to be termite archael group one methanogens.

Results

Figure 5 shows the various sized DNA fragments isolated by the primers that amplified across the ISR. Figure 4 shows the same clones treated with the archael group one specific primers. Clone three shows I have successfully isolated the ISR region of at least one termite archael group one methanogen and there is strong evidence to suggest the presence of more than one although I could not say with 100% certainty. Further work would involve cleaning up the PCR products from the clones to identify more termite archael group 1 ISR's from the sample and sending these off for sequencing and to increase the size of the clone library.

Figure 5. The gel that shows different lengths of the ISR from all clones.

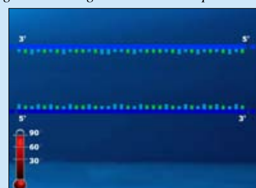
The URSS Experience

Working in the Biological Sciences with Dr. Purdy and his research team was both enjoyable and interesting. The time I spent in the lab allowed me to have an insight into the world of scientific research and experience all of the positive and negative aspects of this career path.I thoroughly enjoyed being part of a research group and attending research seminars and lab meetings. At times I found the lack of results frustrating however I came to learn that this feeling is common throughout long research projects and I only had six weeks! I feel the most useful thing I gained from URSS was mastering the biological techniques vital to modern molecular biology, most of which I was completely unaware of before starting the project.

Figure 6. The PCR machine

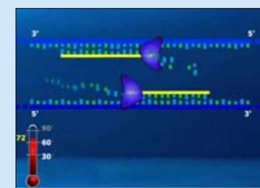
Figure 7. A diagrammatical interpretation of the stages of the polymerase chain reaction

1. PCR reaction tubes are set up.

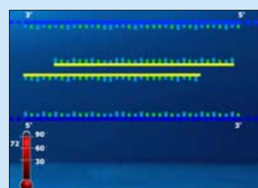


2. DNA strands denature at 92°C

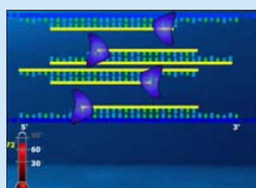
3. Primers anneal to DNA strand (50-60°C)



4. Taq polymerase extends the primers (72°C)

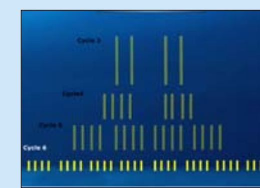


5. 92°C ensures DNA does not hybridise.



6. Taq extends annealed primers.

7. Primers now anneal to all new strands.



8. Target DNA increases exponentially