Development of a molecular tool for the detection of *Ganoderma* on oil palm

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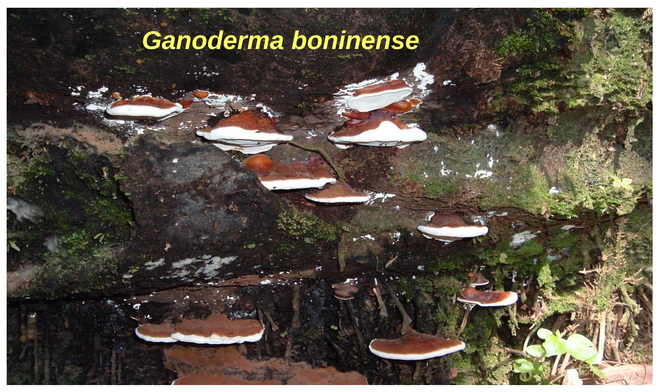
Introduction

Oil palm (*Elaeis guineensis*) is affected by a rot caused by the basidiomycete fungus of the genus Ganoderma. In order for the disease to be confirmed in affected palms. The fungus must be present as a fruiting body or physically isolated from infected stems. The development of a molecular diagnostic tool would enable easier identification as well as a means to study the epidemiology of the fungus in more detail.

The use of PCR technology provides an opportunity to detect the presence of a fungus at very low levels. In addition PCR technology does not require the fungus to be in an active growth phase, and so can be used to detect the presence of spores and other dispersal/resting propagules. Many different DNA regions can be used for the development of diagnostic markers, and the most commonly used region is a section of the ribosomal RNA (rRNA) gene cluster, comprising of 2 internally transcribed spacer (ITS) regions, either side of the 5.8s RNA gene (Figure 1). These spacers are particularly useful, as in many fungi their sequences are largely conserved at the species level, and variable within the genus (See Bridge & Arora, 1998).



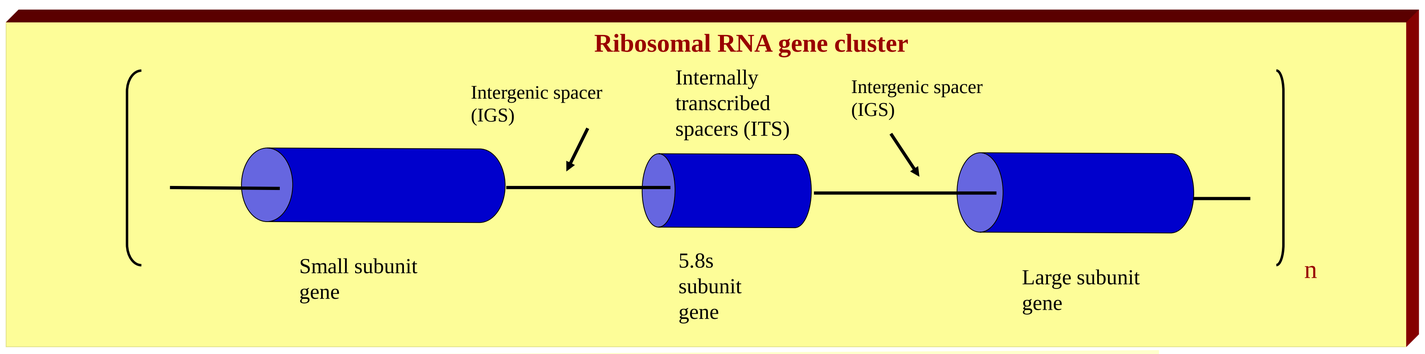


Figure 1. Structure of the ribosomal RNA (rRNA) gene in fungi

Variation in ITS sequences between species can therefore be used to identify short sequences within the ITS regions that are found only in the species of interest. These short sequences (typically between 12 and 30 base pairs) can then be used to design specific primers that will only amplify the ITS region from that fungus.

Considerable work has already been carried out on ITS sequences from fungi, and from *Ganoderma* in particular (Moncalvo *et al*., 1995a; b; c; Moncalvo, 2000). This genus is a particularly good candidate for ITS based detection, as the genus consists of a number of ITS defined groups, and the ITS regions for the basal stem rot pathogen (BSR) *Ganoderma* is distinct among these (Bridge *et al*., 2000).

Methodology

Cultures and basidiocarps were collected from oil palms infected with BSR in Papua New Guinea. Total DNA was extracted from these, and the complete ITS/5.8s (Figure 1) region was amplified in a PCR reaction with universal fungal primers. The amplification products, all single bands of approximately 600 base pairs, were purified and sequenced. The total sequence was compared against all fungal (and other) DNA sequences maintained at the European Molecular Biology Laboratory (EMBL).

Results

Comparison showed that the ITS sequence from the PNG *Ganoderma* was unique, and most closely related to other species of *Ganoderma* (Figure 2). The sequence was critically compared to those from the most closely related species, and a sequence of 16 base pairs at the 3' terminus of the ITS2 region was identified as being unique to the PNG *Ganoderma* (Figure 3). A downstream primer, GanET, was designed to this sequence (Bridge et al., 2000).

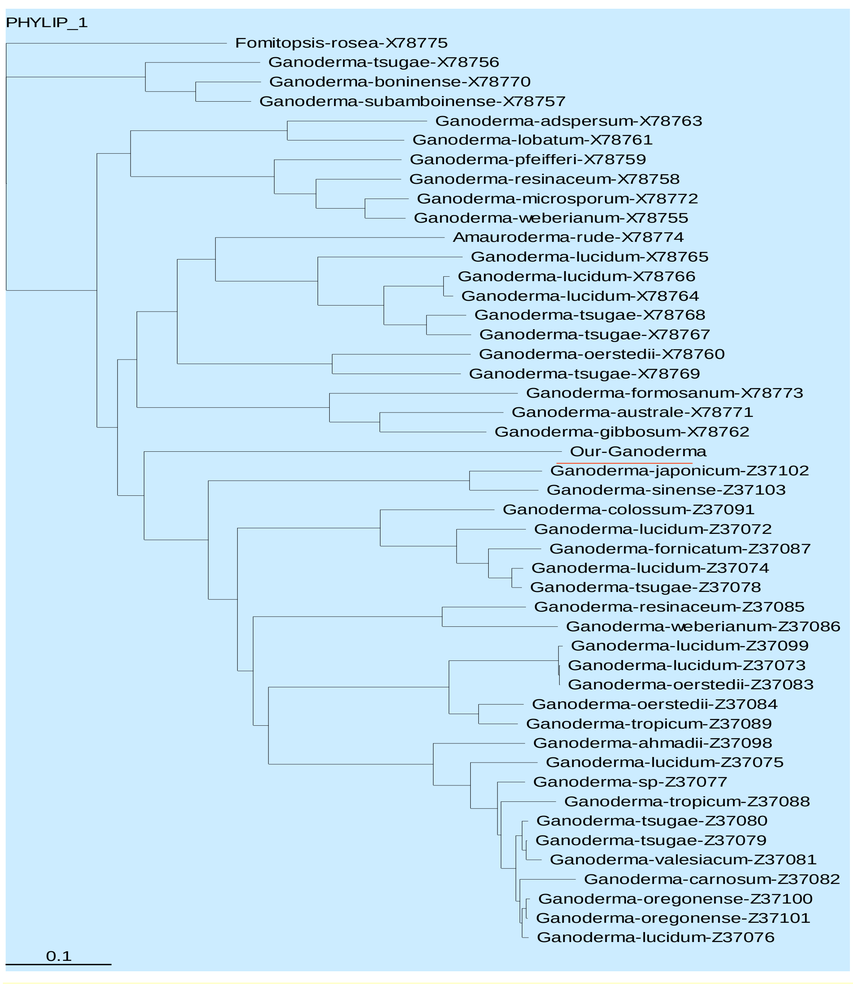


Figure 2. Multiple alignment of ITS2 sequences rooted with *Fomopsis rosea.* The PNG *Ganoderma* is underlined.

AGGATCATTA TCGAGTTTTG ACTGGGTTGT AGCTGGCCTT CCGAGGCATC GTGCACGCCC
TGCTCATCCA CTCTACACCT GTGCACTTAC TGTGGGTTAT AGATCGTGTG GAGCGAGCTC
GTTCGTTTGA CGAGTTTGCG AAGCGCGTCT GTGCCTGCGT TTTATCACAA ACACTATAAA
GTATTAGAAT GTGTATTGCG ATGTAACGCA TCTATATACA ACTTTCAGCA ACGGATCTCT
TGGCTCTCGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT
CAGTGAATCA TCGAATCTTT GAACGCACCT TGCGCTCCTT GGTATTCCGA GGAGCATGCC
TGTTTGAGTG TCATGAAATC TTCAACCTAC AATCTCTTTG CGGTTTTTGT AGGCTTGGAC
TTGGAGGCTT GTCGGTCTTT TATTGATCGG CTCCTCTCAA ATGCATTAGC TTGGTTCCTT
TGCGAGTCGG CTTGTCGGTG TGATAATGTC TACGCCGCGA CCGTGACGCG TTTGGCGAGC
TTCTAACCGT CCCGTTATTG GGACAACTCT TATGACCTCT GACCTCAAAT CAG

Figure 3. The 593 base DNA sequence of the rRNA including the ITS region of PNG *Ganoderma*. Nucleotides in red are conserved regions. The sequence contained in the rectangle is unique to this *Ganoderma* species and was used to construct the GanET primer. The underlined sequence is the site for ITS3.

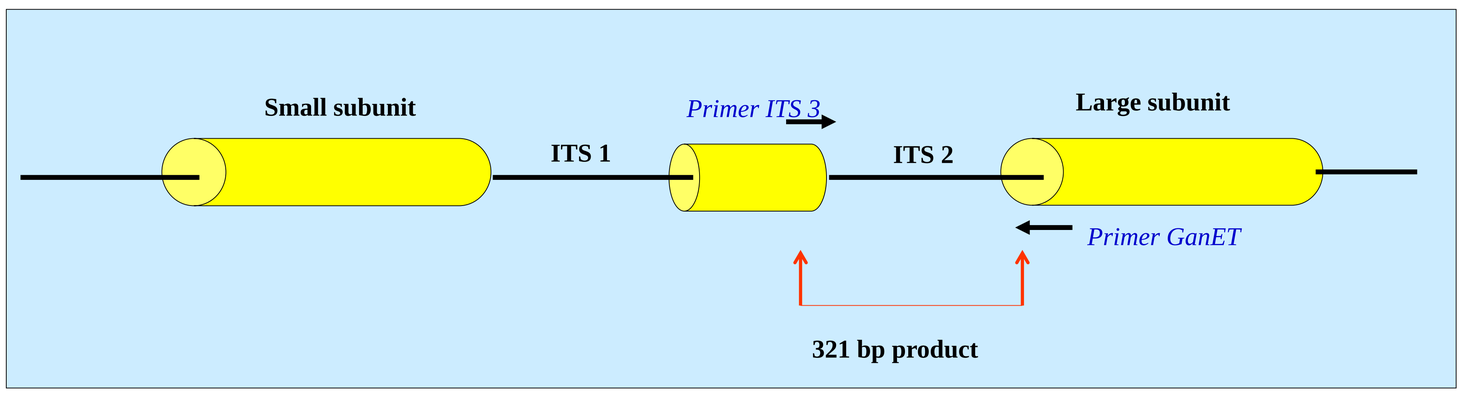


Figure 4. The amplification product of 321base pairs that is diagnostic for PNG *Ganoderma* on oil palm

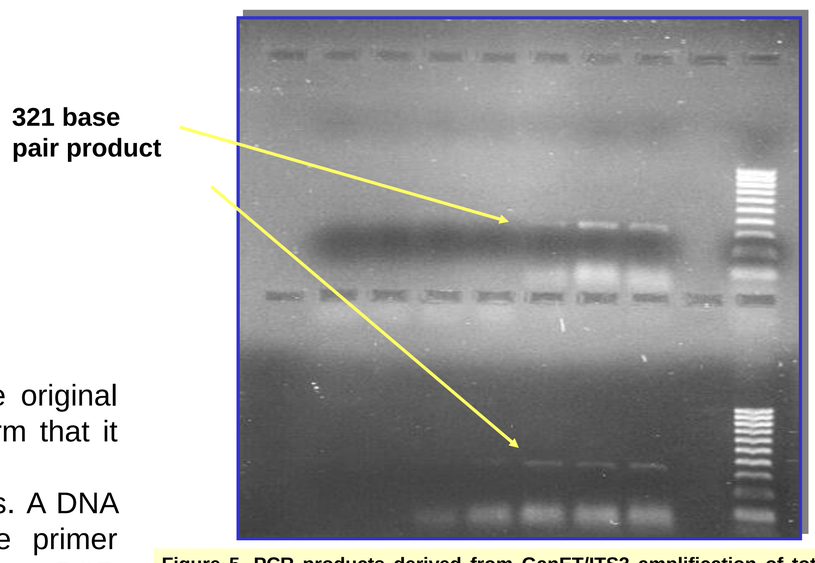


Figure 5. PCR products derived from GanET/ITS3 amplification of total DNA from frond bases of young palm in West New Britain. Upper samples 1/10 dilution DNA, lower samples concentrated DNA.

Conclusion

The GanET primer was used with an upstream universal primer, ITS3 (White *et al*., 1990), to amplify DNA from the original samples. These all produced a single PCR product of the 321 base pairs. This product was then sequenced to confirm that it consisted of the *G. boninense* ITS2 region.

Palm tissue contains a wide range of chemical components which may interfere with DNA extractions and amplifications. A DNA extraction method was developed based on *cetyl*-trimethyl ammonium bromide and polyvinylpolypyrrolidone. The primer combination was then tested against a wide range of palm associated fungi and uninfected palm tissue. In all instances no PCR product was detected. The primer pair was then tested with further known *G. boninense* cultures, brackets and infected palm material, where the PCR product was consistently detected.

References

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