Analysis of Internal Transcribed Spacer (ITS) Regions + 5.8S Gene of rDNA of *Ganoderma* isolates from Infected Oil Palm and Coconut Stumps

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Introduction

One of the most devastating diseases of oil palm in South-east Asia is the basal stem rot caused by *Ganoderma* species. Felled coconut trunks and stumps colonized by *Ganoderma* species have been implicated to be infection foci from which healthy oil palm becomes infected through root contact with the colonized trunks or stumps. At present, the taxonomic relatedness between *Ganoderma* species from oil palm and coconut is still unresolved as the two groups of *Ganoderma* are indistinguishable based on the macro- and micro-morphology of their basidiomata and cultural characters. Other characteristics, such as molecular characters, should be studied.

Molecular characteristics of fungi are increasingly being used as additional taxonomic criteria in classification or to resolve controversies in taxonomic position of taxa. In recent years, molecular techniques, which can assess genetic variation at DNA level, have been developed rapidly and are widely applied in the study of plant pathology. Restriction analysis of PCR (polymerase chain reaction) amplified rDNA provides a tool to assess the rDNA sequence and has been shown to be a suitable and rapid method for taxonomic studies of fungi. The technique is useful for analyzing closely related or distantly related groups because different regions of the rDNA evolved at different rates (Bruns *et al.*, 1991). The rDNA includes both the highly conserved genes and the internal transcribed spacer (ITS) regions. The ITS region is a non-coding rDNA region and is more variable than the rDNA genes and, thus, can provide more information concerning intraspecific and interspecific genetic variations of any fungal species. The present study was conducted to determine the genetic relatedness between *Ganoderma* isolates from infected oil palm and coconut stumps using restriction analysis and direct sequencing of the ITS regions + 5.8S gene of rDNA.

Materials and Methods

Sixty-eight *Ganoderma* isolates from infected oil palm and coconut stumps were cultured in yeast extract sucrose medium and their genomic DNAs extracted using the phenol-chloroform method described by Raeder and Broda (1985). The primers, ITS1 (5'TCC GTA GGT GAA CCT GCG G 3') and ITS4 (5' TCC TCC GCT TAT TGA TAT GC 3'), were used to amplify the ITS regions + 5.8S gene. PCR reactions and amplification conditions were performed using a modified method of White *et al.* (1990). Seven digestive enzymes, *Hae*III, *Msp*I, *Bam*HI, *Alu*I, *Taq*I, *Eco*RI and *Hind*III, were used to digest the PCR products. The digested products were separated by electrophoresis on 1.75% SeaKem agarose gel and stained with ethidium bromide. The sizes of the PCR products and digested fragments were estimated by comparison with a 100 bp standard marker. The profiles or banding patterns generated by all the different restriction enzymes were scored as presence (1) or absence (0) of band of a particular size to compile a binary matrix which was then subjected to cluster analysis. Direct sequencing of the PCR product and phylogenetic analysis of the nucleotide sequence of the ITS regions + 5.8S gene were also conducted.

Results and Discussion

The PCR product amplified using ITS1 and ITS4 primers was approximately 650 bp for all the *Ganoderma* isolates from infected oil palm and coconut stumps.

Digestion of the ITS regions + 5.8S gene with *Hind*III produced a fragment of 600bp for two coconut isolates. The PCR products of the other *Ganoderma* isolates were undigested. This shows that only two coconut isolates have a *Hind*III recognition site within the ITS regions + 5.8S gene. Digestion of PCR products using *Eco*RI yielded a 320 bp fragment for all the *Ganoderma* isolates from oil palm and coconut, except for three coconut isolates which showed three different fragments of approximately 280 bp, 300 bp and 310 bp. The PCR products of all the *Ganoderma* isolates were undigested using *Bam*HI. This suggests that the ITS regions + 5.8S gene of both groups of *Ganoderma* do not contain a recognition site for the restriction enzyme. Only PCR products of three oil palm isolates and four coconut isolates could be digested using *Msp*I. The three oil palm isolates produced a different pair of fragments each (350 bp and 280 bp; 400 bp and 180 bp; and 450 bp and 299 bp). Of the four coconut isolates, two produced two fragments (450 bp and 200 bp) each, but the other two produced one fragment (600 bp) each. Digestion using *Hae*III produced a single fragment of 600 bp for all the *Ganoderma* isolates, except for two coconut isolates and an oil palm isolate which

showed a 500 bp fragment, an oil palm isolate which produced two fragments of 400 bp and 600 bp, and another oil palm isolate which produced two fragments of 500 bp and 600 bp. Digestion using *Taq*I showed that most isolates of both groups of *Ganoderma* produced two fragments of approximately 250 bp and 350 bp, but two oil palm isolates produced four fragments of 250 bp, 300 bp, 350 bp and 180 bp or 100 bp, and a coconut isolate produced three fragments of 100 bp, 150 bp and 180 bp. Digestion of PCR products using *Alu*I produced a single fragment of approximately 350 bp for most of the *Ganoderma* isolates from infected oil palm and coconut stumps. However, four oil palm isolates and one coconut isolate produced a 320 bp fragment, two coconut isolates produced a 300 bp fragment and two oil palm isolates produced a different pair of fragments each i.e. 600 bp and 320 bp, and 350 bp and 420 bp. The results showed that it was not possible to distinguish *Ganoderma* from infected oil palm and coconut stumps based on the restriction patterns of the ITS regions + 5.8S gene of rDNA produced using *Hind*III, *Eco*RI, *Bam*HI, *Msp*I, *Hae*III, *Alu*I and *Taq*I as variations occurred within and between both groups of *Ganoderma*.

From the similarity matrix based on Simple Matching Coefficient, the similarity values obtained for *Ganoderma* isolates from infected oil palm and coconut stumps ranged from 0.700 – 1.000 i.e. 70% to 100% similarities, which indicated high levels of similarity. A dendrogram based on UPGMA cluster analysis of PCR-RFLP bands of the ITS regions + 5.8S gene showed that *Ganoderma* isolates from oil palm and coconut did not cluster separately. All the isolates formed tight and overlapping clusters which indicated a close relationship.

From direct sequencing of the PCR products, only PCR products from six oil palm isolates and six coconut isolates produced sequences that could be aligned and showed satisfactory homology with sequences of *G. lucidum* and *G. adspersum* from the GenBank database. From the sequence alignment, the position of ITS1 was approximately 21-353 bp; ITS2, 601-900 bp; and 5.8S gene, 354-600 bp. None of the isolates produced identical sequences. From the sequence alignment of the isolates, variations were observed not only within the ITS regions (ITS1 and ITS2) but also within the 5.8S gene. Variations in the 5.8S gene of *G. boninense* isolates could be a unique feature as the 5.8S gene of most fungi is conserved. In the analysis of the ITS regions and 5.8S gene sequences with either the distance or parsimony method, the *Ganoderma* isolates from infected oil palm and coconut stumps were clustered together, showing close relationship.

Conclusions

The results from the restriction analysis and direct sequencing of PCR products of the ITS regions and 5.8S gene revealed that *G. boninense* isolates from infected oil palm and coconut stumps at the population level are highly variable and genetically heterogenous. Cluster analysis of the restriction patterns and phylogenetic analysis of *Ganoderma* isolates from infected oil palm and coconut stumps showed that the two groups of *Ganoderma* are very closely related and probably belong to the same species. This supports the supposition that *Ganoderma* from dead coconut stumps or trunks could act as an inoculum to healthy oil palm and plays an important role in the development of the disease.

Benefits from the study

The realization that the *Ganoderma* species colonizing felled coconut trunks or stumps is probably the same species as the one infecting oil palm will have an important bearing on the formulation of disease control measures and replanting procedures, especially in oil palm plantings where the previous crop is coconut.

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Latiffah, Z., Ho, Y. W., Tan, S. G., Tan, S. H., Abdullah, F. and Harikrishna, K. 1999. PCR-RFLP analysis of internal transcribed spacer region (ITS) of *Ganoderma* from oil palm and coconut. Proceedings of the MCB-MAPPS Plant Protection Conference, pp. 112-115.

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Expertise Development

Name of Graduate	Degree Awarded	Field of Expertise	Research Topic	Graduation Year
Latiffah Zakaria	PhD	Plant Pathology	Comparative studies on *Ganoderma* (Karst.) from infected oil palm and coconut stumps with special reference to their morphological, molecular and isozyme characteristics	2002

Graduate Research or Expertise Development

Research Topic: Comparative studies on *Ganoderma* (Karst.) from infected oil palm and coconut stumps with special reference to their morphological, molecular and isozyme characteristics

Patent(s), if applicable:

None.

Stage of Commercialization, if applicable:

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