OIL PALM MICROBIAL INTEGRATED DATABASE (OPMID) – *Ganoderma* INFORMATION DATABASE (GanoID)

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Over the years, MPOB has identified many microbes that play essential role in the oil palm ecosystem. These microbes namely bacteria, fungi, virus and viroids play pertinent roles in the research field of plant pathology, entomopathology and biodiversity in oil palm. With so much of information available on these microbes, it important to centralise and coordinate the information for in-house and public availability. Additionally, the information gathered should be stored systematically for immediate retrieval by anyone. Having this in mind, a centralised portal was established to organise these valuable information on the microbes, referred to as Oil Palm Microbial Integrated Database (OPMID). The first module focuses on the oil palm basal stem rot pathogen *Ganoderma*. This information database, named GanoID is a subset of microbial information to be integrated under this portal for in house and public use. It is aimed that OPMID will be the central platform integrating information on microbes involved in oil palm research. A white rot fungus, *Ganoderma* species is the causal pathogen of basal stem rot in oil palm. The disease is also prevalent in Southeast Asian countries planting oil palm especially in Malaysia and Indonesia. Based on previous research, the disease was reported to be caused by a number of *Ganoderma* species namely *G. boninense*, *G. zonatum* and *G. miniatocinctum* in descending aggressiveness through pathogenicity test. However, this finding was based on the research carried out almost 20 years ago. With the current advancement in biotechnology tools, updating the systematics of the genus causing the BSR is the next move in filling in the knowledge gap of this pathogen. The research of updating the systematics plays a crucial role in the epidemiology of the pathogen by updating the *Ganoderma* collection through identification of intrinsic morpho-taxonomic features, pathogenicity capacity, molecular and biochemical techniques. Therefore the study was initiated by the sampling of fruiting bodies of the pathogen. The sampling was carried out nationwide including Sabah and Sarawak. To date, a total 20 sampling points have been completed from different geographical locations around Malaysia. The collection of *Ganoderma* fruiting bodies is currently approaching 500 isolates. The huge collection of data on the phenotypic and genotypic of the pathogen prompted the development of a portal. This portal would serve as an information tool on the pathogen that details out the typical phenotypic characteristics and genotypic information of the white rot fungi. The collection is referred as *Ganoderma* Collection and Information Database (GanoID). The portal was developed together with a local bioinformatics company, Codon Genomics Sdn Bhd (CoGen), by utilising the Arkgene™ genomics cloud platform.

NOVELTY OF TECHNOLOGY

The GanoID is generated from the culture collection of *Ganoderma* species from the oil palm field nationwide. The portal serves as a centralised platform that efficiently stores, organises, interlinks, updates and retrieves information of the isolates within and outside MPOB. This portal is also the first portal in Malaysia to display collection, DNA sequences and morphology information on the species to the industry and the rest of the public. We are also offering the depository service of safe keeping the pathogen for those who are interested in preserving their culture for long term. The biological collection also serves as a vital source for genome verification and development of molecular markers that can be used by researchers. The portal provides an essential *Ganoderma* collection for biomarker development for the early detection; biochemical and molecular approach. The collection also provides an important genome source for fundamental studies that looks into pathogenicity related genes, host-pathogen interaction, proteomics and metabolomics.

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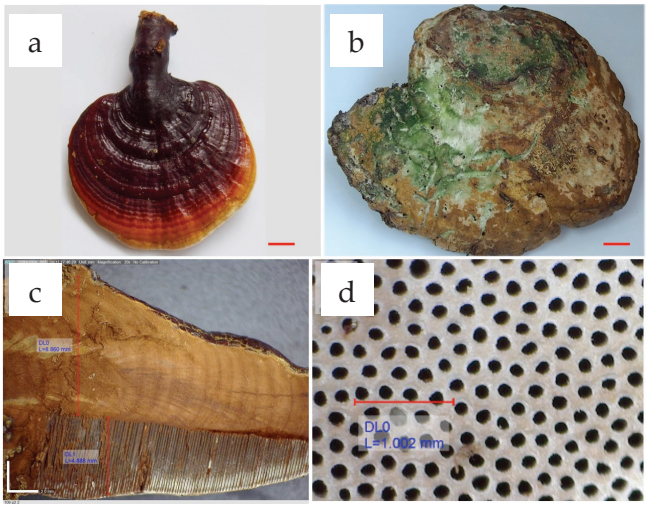

Figure 1. Ganoderma *fruiting body characteristics and microscopic visualisation. (a) Laccate and reddish brown; (b) Non laccate and light brown of the pileus (surface). (c) Hymenium and context of the crust. (d) The pore size of the fruiting body. Red scale bar of a and b indicate 10 mm. Measurement of* Figure c *and* d *are specified in the respective images.*

SELECTION OF FIELDS AND SAMPLING *Ganoderma* HERBARIA

A typical process of sampling for *Ganoderma* fruiting body starts from the selection of suitable plantation. The plantation was selected based on the BSR infection and a census will follow suit. The census will determine the selection of palms for fruiting body samplings. Similar standard operating procedure (SOP) takes place for all the sampling process. To date, *Ganoderma* fruiting body on the base of oil palm trunk was collected from the oil palm plantations throughout Malaysia. As mentioned earlier, BSR prevalence sensing at the stipulated locations was carried out beforehand to identify specific location heavily infected and the infected palms bearing fruiting body were tagged. The GPS location, palm condition, age, soil type and other relevant parameters were recorded as part of the recording purpose. The fruiting bodies were carefully removed from the infected trees to ensure that the structure remains intact for further investigation.

Ganoderma fruiting bodies collected from Sabah and Sarawak require specific quarantine procedures starting from sampling until the disposal process to enable the transportation to GanoDROP laboratory. All samples collected were checked by DOA to obtain the phytosanitary certificate. Samples arrived in Peninsular Malaysia were inspected by MAQIS located at the respective airport for biosecurity examination. Then, Post Entry Quarantine (PEQ) Division in Serdang screened the samples where all samples were re-packed in new plastics for biosafety and biosecurity purposes. These samples will eventually go through similar data recordings as the rest of the samples collected in Peninsular Malaysia.

Figure 2. Spore characteristics of Ganoderma *isolates.*

PHENOTYPIC AND GENOTYPIC CHARACTERISTICS

Mycologist has been continuously describing *Ganoderma* genus for the past 120 years since it was first discovered in 1881 by Karsten. It was then updated by numerous taxonomist with basic description based on the host specificity, geographical distribution and macro-morphological features of the fruiting body including the context colour and shape of the margin pileus and finally the stipitate and sessile nature of the fruiting body. However, with the recent development in clastidics methods to reconstruct natural classifications and the application of these methods to both traditional and new molecular data, the potential of understanding the taxa becomes more practical (Seo and Kirk, 2000). It was proposed that high phenotypic plasticity contributes to the complication of the systematics. The only characteristic that remains similar is the pore size of the fruiting body while size and colour show significant difference. Another characteristics that very much influences the identification to genus level is the laccate and non laccate nature of the pileus (surface) (*Figure 1a* and *1b*). This remains as useful aid to the identification of *Ganoderma* genus. Even the substrate was used to grow the pileus artificially would influence the phenotypic characters of the fruiting bodies (Shin and Seo, 1988).

The internal structure of the pileus crust and cortex are some the useful characters in the taxonomy of *Ganoderma*. The crust is divided into hymenium and context (*Figure 1c* and *1d*) and the colour of the sectioning differs in different specimens of a single species and may show various structural forms (Stayaert, 1980). Basidia and basidiospores are also considered as one of the most important characters for species identification in basidiomycetes. These spores in basidiomycota have a unique double walled and usually ovoid or ellipsoid-ovoid (*Figure* 2). The surface is usually smooth or wrinkled with numerous small and shallow holes. All the above characteristics were carefully selected and itemised in custom made forms to further elucidate the systematics of species in Malaysia. The characteristics were examined, grown and finally preserved for molecular and future investigation. Actively growing cultures of suspected *Ganoderma* species were then subjected to molecular identification. A set of primers for isolation of internal transcribed spacer (ITS) region, TW81 (5'-GTTTCCGTAGGTGAACCTGC -3') and AB28 (5'-ATATGCTTAAGTTCAGCGGGT -3') (Howlett *et al.*, 1992) was expected to amplify approximately 650 bp products. Sequences retrieved were analysed using Basic Local Alignment Search Tool (BLAST) for similarity hits in the National Centre for Biotechnology Information (NCBI) GenBank database for identification of each *Ganoderma* strain.

The huge information on each of the isolates was found to be highly valuable considering there was never a study on the updating of the species. The collection, morphology and DNA sequence information was traditionally recorded and stored in different Excel sheets or FASTA files, which has a limitation to link them together, inefficient data management and share among research group members. Hence, GanoID was proposed by utilising the Codon Genomics Barcoding System developed via Arkgene™ cloud platform, to modernise and digitise the data structure and management of *Ganoderma* information, thus facilitating data retrieval, data acesss, data analytics and data sharing among researchers both internally and externally.

GanoID PORTAL SYSTEM

Database Planning, System Definition and Database Management System

The GanoID was set up on a cloud platform (*i.e.* Arkgene™ genomics cloud). All the standard security protocols were included in the platforms such as Secure Socket Layer (SSL), Geo Redundant Storage (GRS), AES 256 Bit Encryptions and Firewall technologies to ensure secure connectivity between application and user, data backup and redundancy, data encryption and privacy, and protection from intrusion, respectively. Next, the data entry, data management, data control and access control were controlled by MPOB through advance user management system. MPOB's admin user will review and give approval in data sharing from the registered public user. The summary of the process is summarised and shown in *Figure* 3.

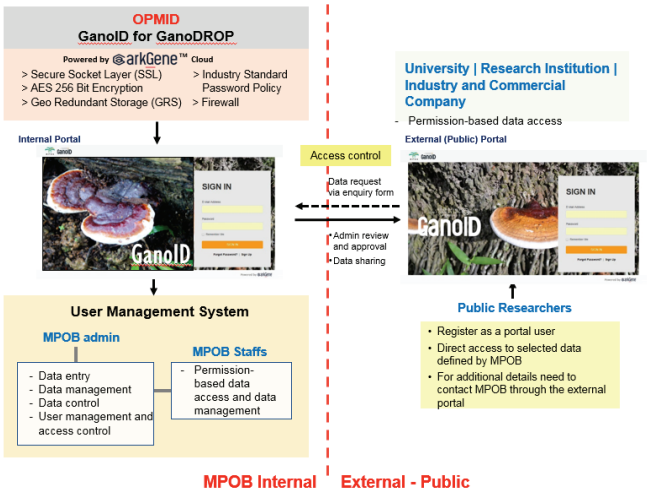

Figure 3. Summary of GanoID system planning and management.

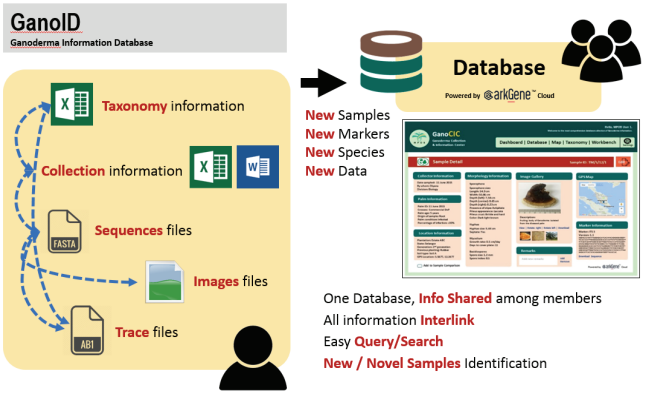

Figure 4. Summary of types of data input that were uploaded into GanoID.

Requirement Collection and Analysis and Application Design

Figure 4 shows the details of the *Ganoderma* fruiting bodies collected and being arranged accordingly to the specific column. The unique ID for each fruiting bodies were assigned, together with taxonomy and collection information recorded in Microsoft Excel or Word, were imported into GanoID database via individual or batch upload function. Additionally, images of corresponding morphological characters and DNA sequence

files were uploaded as well. The latter includes the chromatogram (ABI) and DNA sequencing (FASTA) files. However, information on the sequences (*i.e.* ABI and FASTA files) will not be released to public and will only be furnished upon request through the GanoID portal and due payment. Finally, after all the data being migrated to the database accordingly to the specific subject, the graphical user interface of the GanoID database was displayed as in *Figure 5*. *Figure 5* is the representation of the sample details that will be viewed by registered public user through the GanoID portal.

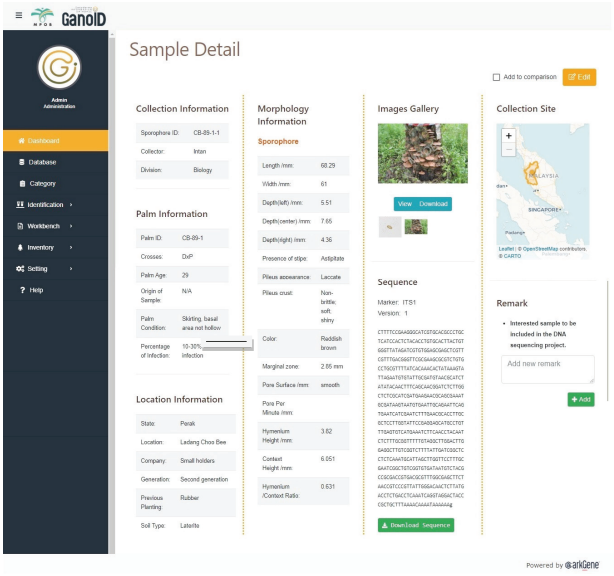

Figure 5. Graphical user interface of GanoID showing the Ganoderma *fruiting bodies, the* Ganoderma *sequences and the location for the collection.*

POTENTIAL USERS

The portal can be assessed with a specific objective to discover and educate oneself on the white rot fungi. It also includes the service of culture depository for long-term safekeeping. The technology is suitable for all research departments, universities and industry with laboratory facilities that actively carry out research on *Ganoderma* with interest in the epidemiology of the pathogen.

BENEFITS TO THE INDUSTRY

The service of OPMID through the first module, GanoID would definitely benefit those who are actively carrying out research on *Ganoderma*, specifically on the species identification and retrieval of its associated environmental and morphological data. This is the first database under OPMID that compiles valuable information on *Ganoderma* and would serve many researches linked to fundamental study. The purpose of the *Ganoderma* portal is to ensure the data collected from field and all the morphotaxonomy (macro and micro) of *Ganoderma* fruiting bodies recorded in the portal and made available to public. The GanoID is an excellent platform that will be able to assist many researchers to carry out fundamental research in *Ganoderma*. This will also mean that MPOB will be providing information on *Ganoderma* that may bring in future collaboration with research institutions. This portal also opens up opportunity for future oil palm disease epidemiology study via *Ganoderma* case reporting using mobile apps as well as traceability of disease prevalence through a unified database. With cutting edge technology such as big data analytics and artificial intelligence in place, this portal could serves as foundation toward future establishment of geographical data-based large-scale disease prediction model for better oil palm early disease prevention. The technology also offers live culture preservation at a price that will be determined later.

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