QBOL: Development of a new diagnostic tool using DNA barcoding to identify quarantine organisms in support of plant health

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Development of accurate identification tools for plant pathogens and pests is vital to support European Plant Health Policies. For this project Council Directive 2000/29/EC is important, listing some 275 organisms for which protective measures against introduction into and their spread within the Community needs to be taken. Those threats are now greater than ever because of the increases in the volumes, commodity types and origins of trade, the introduction of new crops, the continued expansion of the EU and the impact of climate change.
Currently identifying pathogens (in particular new emerging diseases) requires a staff with specialised skills in all disciplines (mycology, bacteriology, etc.); which is only possible within big centralised laboratory facilities. Taxonomy, phytopathology and other fields which are vital for sustaining sound public policy on phytosanitary issues are threatened with extinction.
Modern molecular identification/detection techniques may tackle the decline in skills since they often require much less specialist skills to perform, are more amenable for routine purposes and can be used for a whole range of different target organisms. Recently DNA barcoding has arisen as a robust and standardised approach to species identification. The new FP7 EU project QBOL wants now to make DNA barcoding available for plant health diagnostics and to focus on strengthening the link between traditional and molecular taxonomy as a sustainable diagnostic resource. Within QBOL collections harbouring plantpathogenic Q-organisms will be made available. Informative genes from selected species on the EU Directive and EPPO lists will be DNA barcoded from vouchered specimens. The sequences, together with taxonomic features, will be included in a new internet-based database system. A validation procedure on developed protocols and the database will be undertaken across worldwide partners to ensure robustness of procedures for use in a distributed network of laboratories across Europe.
More information can be found on: http://www.pri.wur.nl/UK/research/projects/qbol/

Quantitative multiplex pathogen detection using proximity dependent DNA ligation

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At present, many diagnostic assays are based on ELISA or real time PCR. Whereas the latter is very sensitive and suitable for high throughput, many assays rely on detection of proteins. In the case of plant viruses, more than 95% of the virus genomes are ssRNA coded, which leads to higher sequence variability than pathogens with dsDNA. Consequently, molecular detection for viruses are not always reliable, and antigen detection is required.
A new technique that provides the specificity of an immunoassay with the sensitivity and manageability of PCR is proximity ligation. Proximity ligation uses antibodies or other binding reagents, coupled to oligonucleotides for proximity-dependent ligation reactions that depend on dual recognition of target molecules. The two oligonucleotide extensions hybridize with a free oligonucleotide, followed by ligation after which the new chimeric DNA strand can be amplified by PCR. The sensitivity of such assays exceeds that of ELISA's. The improbability of ligation in the absence of the specific target ensures a low background and thus high assay specificity. This combination of high sensitivity and specificity offers opportunities for designing multiplex assays that target antigens and/or antibodies in a wide variety of samples.
Quantitative multiplex proximity ligation for the detection different plant viruses will be demonstrated.

Developing tools for Mycosphaerella fijiensis studies

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Mycosphaerella fijiensis is the causal agent of the black leaf streak disease, or Black Sigatoka, the most devasting fungal disease of banana around the world. Together with *M. fijiensis*, *M. musicola* and *M. eumusae* constitute the Sigatoka complex that causes significant effects on photosynthesis resulting in premature ripening of the fruit and consequent yield loss. The only solution has been costly and environmentally threatening fungicide control. *M. fijiensis* has a high diversity among subpopulations around the world and outcompetes *M. musicola* that is now mainly confined to higher altitudes. Meanwhile, *M. eumusae* is present on Pacific Islands and spreads into Asia and Africa. Despite the importance of these pathogens, their biology is largely unknown. We are developing genomic and molecular tools to improve the understanding of these pathogens. Protoplast isolation of Sigatoka complex pathogens enables the determination of electrophoretic karyotypes to study chromosomal variation among isolates from these pathogens. *M. fijiensis* isolate CIRAD086 (Cameroon) was sequenced and initial analyses indicate a genome size of 73.4 Mb, which is almost twice the genome size of the closely related *M. graminicola* pathogen of wheat. The genome sequence has a coverage of approximately 7.11X with 10,327 predicted open reading frames distributed over 395 scaffolds. In addition, three cDNA libraries from different *in vitro* conditions were developed and sequenced resulting in 32,392 ESTs. Altogether, this set of ESTs encompasses approximately 22.5 Mb of high quality sequence, with a 53.25% CG content and represent over 6000 unigenes. A set of 12 genes with a high expression profile in the different cDNA libraries as well as orthologs with known function in other pathogens were selected for further expression studies under *in vitro* and *in vivo* conditions.
This transcriptome database is an important resource for whole genome assembly and gene discovery in *M. fijiensis*.

Development of a detection method for Tropical Race 4 of Fusarium oxysporum f. sp. cubense

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Fusarium oxysporum f. sp. *cubense* (Foc) is the causal agent of Panama disease, the devastating threat that ruined the Gros Michel-based banana production in the early 1900s. The occurrence of a Foc *n*ew variant in South-East Asia that overcomes the resistance in Cavendish clones such as 'Gran Naine' is a major concern to current banana production worldwide. The threat imposed by this Foc variant, called Tropical Race 4 (TR4), could be counteracted by the introduction of