



Submissions for Diagnostic Protocols

I. General information

Submission number	2023-009
Title of Proposal	HTS Identification of regulated bacteria isolated from plants, Annex to ISPM 27 (Diagnostic protocols for regulated pests)
Submitted by (Country or Organization)	IPPC Contracting Party
IPPC Official Contact Point or RPPO	New Zealand
Supported by	

2. Contact information

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3. Summary of proposal

Summary of justification for the proposal	Bacterial plant diseases pose significant risks to global food security, causing important losses across a wide range of food production systems. Diagnostic laboratories across the globe are constantly faced with the challenge of identifying regulated plant pathogenic bacteria isolated from diagnostic samples. The capability to identify these isolates is central to informed biosecurity actions and for efficient plant disease management. With the development of high-throughput sequencing (HTS) technologies, it is now possible to routinely produce cost-effective genomes of isolated bacteria with a fast turnaround time. Genome sequencing allows high-resolution identification and enables determination of biological risk. While HTS technology offers unprecedented resolution as a diagnostic tool, there are currently no standardized protocols for the application of HTS in the identification of regulated bacteria isolated from plants. The lack of such protocols is preventing diagnostic laboratories and NPPOs from fully operationalizing this technology. For example, when assessing identifications
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	performed by a third party it is not possible to assess how the data was generated, its quality, what controls were in place to ensure its integrity, and how it was analysed to produce an identification. In light of CPM recommendation R-08, it is necessary to advance in the development of standardized best practice protocols for the application of HTS in plant health diagnostics. An annex to ISPM27 providing a standardized protocol for HTS identification of regulated bacteria isolated from plants would provide a much-needed diagnostic protocol as well as a step forward in the adoption of the recommendation.
Proposed priority	I (high)
Comments	Reliable identification of regulated plant pathogenic bacteria is key for plant health and biosecurity, reducing the significant impact of these pathogens in food production systems. Developing a HTS protocol for the identification of regulated bacteria would operationalize the latest technology in a harmonized way between contracting partners, maximizing the benefits, and providing assurance on diagnostic quality across the system.

4. Literature review

Literature review	<p>The identification of bacterial isolates to species, sub-species, pathovar, phylogroup or strain level is fundamental for accurate determination of plant health status in a wide range of scenarios. Traditionally, this process has been performed through a series of biochemical assays that are laborious, time consuming, and in some cases do not have enough resolution for low-level bacterial identification. More recently, DNA-DNA hybridization and sanger sequencing of phylogenetic marker genes has been employed for identification of bacteria isolated from plants. These methods provide a reliable way to identify isolates, although they also present limitations such as cost, processing time and low resolution for closely related strains (Barco et al. 2020). The application of HTS technology, has the potential to provide an alternative to traditional methods for the identification of plant pathogenic bacteria, providing high resolution identifications (Richter and Rossello-Mora 2009). The growing interest in the application of HTS technologies for routine diagnostics in plant health laboratories is triggering the development of guidelines on how to prepare laboratories for performing HTS testing (Lebas et al. 2022). CPM recommendation R-08 “Preparing to use high throughput sequencing (HTS) technologies as a diagnostic tool for phytosanitary purposes” encourages contracting parties to “standardise and apply best-practice operational guidelines for HTS, including proper interpretation of results and quality control measures that ensure HTS data outputs are robust and accurate, have biological significance in a phytosanitary context, and are implemented in a harmonized way”. In line with this recommendation, this protocol would address</p>
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	<p>the different processes involved in HTS identification of regulated bacteria to ensure a consistent approach between diagnostic labs. HTS technologies and downstream bioinformatics analysis of the generated datasets represent a complex process including many steps whose reliability must be ensured (Massart et al. 2022). EPPO Standard PM 7/151 “Considerations for the use of high throughput sequencing in plant health diagnostics” provides a general but solid starting point for the considerations needed to develop a HTS diagnostic protocol, and could be used as a reference document. Important components that should be addressed in the protocol are nucleic acid extraction, library preparation, sequencing technology used, bioinformatic analysis, minimum standards, interpretation of the data and reporting of the results (EPPO 2022).</p> <p>-Barco, R. A., Garrity, G. M., Scott, J. J., Amend, J. P., Nealson, K. H., and Emerson, D. 2020. A Genus Definition for Bacteria and Archaea Based on a Standard Genome Relatedness Index. mBio 11.</p> <p>- EPPO. 2022. PM 7/151 Considerations for the use of high throughput sequencing in plant health diagnostics. EPPO Bulletin 52:619-642.</p> <p>-Lebas et al. 2022. Facilitating the adoption of high-throughput sequencing technologies as a plant pest diagnostic test in laboratories: A step-by-step description. EPPO Bulletin 52:394-418.</p> <p>-Massart et al. 2022. Guidelines for the reliable use of high throughput sequencing technologies to detect plant pathogens and pests. Peer Community Journal 2.</p> <p>-Richter, M., and Rossello-Mora, R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci U S A 106:19126-19131.</p>
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5. Criteria for prioritization of Diagnostic Protocols

Criteria	Information provided by Submitter
1. Need for international harmonization of the diagnostic techniques for the pest (e.g. due to difficulties in diagnosis or disputes on methodology)	The identification of bacteria is challenging due to the intrinsic nature of these organisms and often convoluted taxonomy. HTS has the potential to provide high-resolution diagnostics to all plant health laboratories and it is currently being operationalized by a growing number of NPPO reference plant diagnostic laboratories. However, harmonized standards for the application of this new technology are required to ensure sound diagnostic results that are valid across jurisdictions.
2. The relevance of the diagnosis to the protection of plants including measures to limit the impact of the pest.	Accurate identification of regulated bacteria isolated from plants is essential for safe international trade, border protection and disease management. In case of biosecurity incursions, high-resolution identification methods are central in preventing the spread of unwanted bacterial organisms. Given the significant number of plants that are host to pathogenic bacteria, the relevance of this diagnostic protocol is very high.
3. Importance of the plants protected on the global	Bacterial plant diseases account for an important proportion of the USD 220 billion that plant diseases cost yearly to the global

level (e.g. relevant to many countries or of major importance to a few countries).	economy (FAO, 2019). Numerous bacterial diseases affect crop production in many countries and regions. These include fire blight in fruit trees, bacterial wilt in banana, bacterial blight in rice and crown gall in many perennial plants. The epidemics caused by bacteria can cause significant economic burden, disproportionately affecting countries with low resources.
4. Volume / importance of trade of the commodity that is subjected to the diagnostic procedures (e.g. relevant to many countries or of major importance to a few countries).	Potentially all commodity plants that are subjected to trade are susceptible to plant pathogenic bacteria that can be considered regulated depending on the jurisdiction. This affects all countries, and it is considered a global problem.
5. Other criteria for topics as determined by CPM that are relevant to determining priorities	This diagnostic protocol would contribute towards addressing CPM recommendation R-08 “Preparing to use high throughput sequencing (HTS) technologies as a diagnostic tool for phytosanitary purposes” which encourages contracting parties to “standardise and apply best-practice operational guidelines for HTS, including proper interpretation of results and quality control measures that ensure HTS data outputs are robust and accurate, have biological significance in a phytosanitary context, and are implemented in a harmonized way”.
6. The balance between pests of importance in different climatic zones (temperate, tropics etc) and commodity classes.	Bacterial pathogens cause disease in host plants in all climatic zones that allow agriculture. It is expected that climatic change will increase outbreak risks by altering bacterial evolution and host-pathogen interactions while facilitating the emergence of new strains.
7. Number of labs undertaking the diagnosis.	Many quarantine and research laboratories are involved in HTS identification of bacteria e.g., James Hutton Institute (Scotland), FERA (United Kingdom), USDA (USA), CFIA (Canada), ANSES (France), NIWA (Netherlands), AgriBio (Australia), MPI (New Zealand), etc.
8. Feasibility of production of a protocol, including availability of knowledge and expertise.	A protocol is feasible as there are several publications available that cover key aspects of HTS identification of bacteria, that include nucleic acid extraction, library preparation, sequencing technology platform options and bioinformatic analysis. There are many experts available throughout Asia, Australasia, UK, Europe, and the Americas.