Work Plan for Short-Term Scientific Mission

COST Action EuroXanth (CA 16107)

STSM title: Characterization of new type VI effectors in *Xanthomonas*

Prospective host: Dr. Ralf Koebnik
Host institution: Institut de Recherche pour le Développement (IRD), Laboratory for Plant-Microbe-Environment Interactions (IPME), Research Team on Genomics and Transcriptomics of the Interactions between Plants and Prokaryotes (GTIPP), 911 avenue Agropolis, 34394 Montpellier – France.
Collaboration partner: Dr. Eran Bosis, ORT Braude College in Karmi'el (Israël)
Planned dates and length of stay:
3 months (60 working days), summer / fall 2020 (depending on COVID-19 dynamics).

Description of the proposed work plan of the STSM

1. Aim and motivation

Type VI secretion systems (T6SS) have been discovered in *Xanthomonas* only 10 years ago, with very few studies since then (Sarris et al. 2012). Initially thought to deliver type VI effectors into eukaryotic cells of host organisms (Pukatzki et al. 2006), several studies suggest that the xanthomonads' T6SS do not contribute to pathogenicity on their host plants (Abendroth et al. 2017; Ceseti et al. 2019; Zhu et al. 2020). Instead, it was shown that the T6SS from *Xanthomonas citri* contributes to resistance of the bacteria against predatory amoeba (Bayer-Santos et al. 2018).

However, since the initial discovery of a role of the T6SS in the interaction with eukaryotic cells it became more and more evident, that in most cases the T6SS may rather act against other microbes and thus shapes the ecological niches in which the bacteria persist. Since in this case the effector proteins act inside prokaryotic cells, producing cells need an immune mechanism to avoid self-killing. Indeed, in many cases, effector proteins are encoded next to an immunity gene, the presence of which can be taken as evidence for an antibacterial activity of such effector proteins.

As an important component in the ecology of xanthomonads, including species that are regulated as quarantine organism by the European and Mediterranean Plant Protection Organization (EPPO) (e.g. *X. citri*, *Xanthomonas fragariae*, *Xanthomonas oryzae*, *Xanthomonas translucens*), we therefore wish to identify and confirm new type VI effectors and initiate their functional characterization. The realization of the planned STSM will thus enhance the knowledge about hitherto understudied bacterial factors with importance for the life cycle of the pathogen.

2. Contribution to the scientific objectives of the EuroXanth COST Action

Since T6SSs are likely to play an important role in the ecology of the pathogen, we consider this project as highly relevant for the EuroXanth COST Action. This project will focus on the quarantine pathogen *X. translucens* pv. *translucens* and its sister pathovar *X. translucens* pv. *undulosa*. These two pathovars are the causal agents of bacterial leaf streak on barley and wheat, which are the economically most important diseases of small-grain cereals caused by bacteria.

Comparative and functional genomics has allowed identifying new families of type VI effectors in members of the widespread family of aquatic bacteria belonging to the *Vibrionaceae* (Dar et al. 2018; Friedman et al. 2020; Jana et al 2019). In this project we will collaborate with Dr. Eran Bosis, who is the Vice Leader of the Working Group 2 of the EuroXanth COST Action and who was implicated in the above-mentioned work on *Vibrionaceae*. Therefore, this collaboration between the host institute, the ORT Braude College in Karmi'el (Israël), and the STSM fellow from a third country will have a structuring effect on the research landscape in the participating COST countries.

3. Strategy and techniques

The work plan includes:

- Bioinformatic prediction of type VI effectors and immunity proteins in *X. translucens* based on comparative genomics and presence of conserved T6SS markers: MIX and FIX domains (Dar et al. 2018; Jana et al. 2019).
- Molecular cloning by Gibson assembly and heterologous expression of effector and immunity genes in *Escherichia coli*.
- Bioassays to demonstrate effector activity in the cytoplasm or periplasm of bacterial cells (Fridman et al. 2020).

4. References

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