PROAGROBAC

International Incoming Fellowship (IIF)

Determination of novel molecular cross-signaling mechanisms between bacteria and plants leading to enhanced crop production.

This IIF fellowship has enabled Prof. Xiaoguang Liu, a highly experienced senior researcher form Jiangsu University (China) with experience gene regulation in Plant Growth Promoting Rhizobacteria and their interactions with plants to transfer some of this knowledge, whilst undertaking research in the UK, to Professor Miguel Cámara's laboratory at the University of Nottingham. In return Prof. Liu has acquired advanced knowledge on the study of bacterial signalling networks. This has enabled them to increase their research links with a view to establish long-term collaborations between the Universities of Jiangsu and Nottingham in the area of crop sciences with a view to improve agricultural sustainability and adaptation to climate changes.

Main objectives of the project

Using the plant beneficial *Serratia plymuthica* as a model organism, together with state-of-the-art technologies, this IIF project PROAGROBAC aimed at investigating the relationships between bacterial signal-driven regulatory networks such as quorum sensing (QS), the two component regulatory system (TCS) Gac, the posttranscriptional regulatory system Rsm, and the auxin signalling (IAA). The ultimate objective was to study their impact on plant biocontrol and growth promoting traits, as well as characterising the plant responses to bacterial signals with a view to design new environmentally friendly microbial seed inoculants for the improvement of plant growth and stress tolerance by manipulating bacterial regulatory signalling networks. This will contribute to further reducing the risks to human health and the environment by the use of chemical fertilisers and pesticides (Fig. 1).

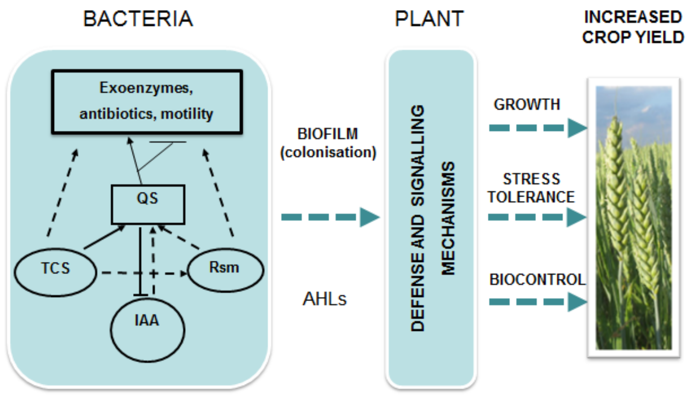

Fig.1 Integrated regulatory networks including QS, TCSs, Rsm and IAA production by *S. plymuthica* and the impact on interactions with host plants. Continuous lines show links backed up by experimental evidence, discontinuous lines show possible link investigated by PROAGROBAC.

Serratia plymuthica

S. plymuthica are ubiquitous bacteria belonging to Gram-negative, rod-shaped & peritrichous member of the Enterobacteriaceae family with varied lifestyle from endophytic to free-living. Both strains G3 (Fig. 2) and HRO-C48 of *S. plymuthica* displayed ability to antagonize a broad spectrum of phytopathogens (Fig. 3) and promote plant growth via production of the antibiotic pyrrolnitrin (PRN), exoenzymes and the plant auxin indole-3-acetic acid (IAA) amongst other traits. Moreover, three copies of QS LuxI/R homologues genes were identified in G3 and involved in the production of high level of *N*-acylhomoserine lactones (AHLs) QS signal molecules regulating pleotropic biocontrol-related phenotypes.

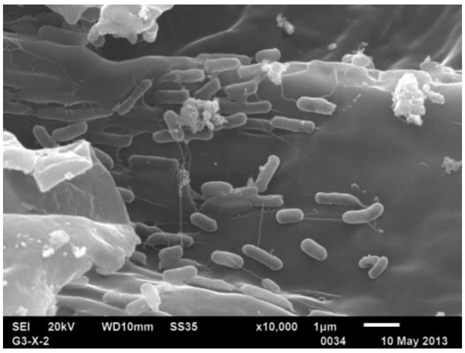

Fig.2 Scanning electron micrograph (SEM) displayed colonisation of *S. plymuthica* G3 on root surface of the wheat.

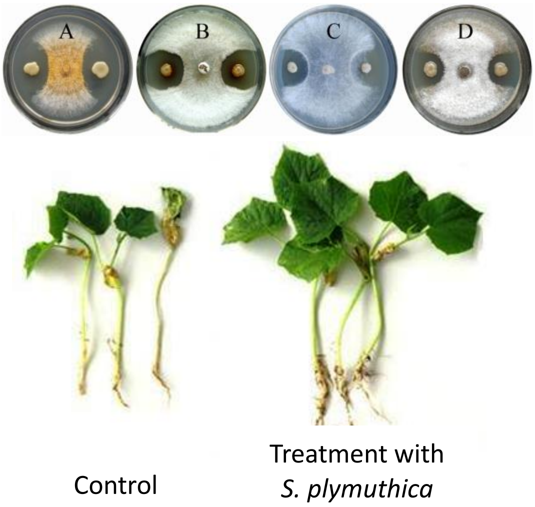

Fig. 3 Antifungal activities on plates (top panel) and suppression of *Pythium*-damping off of cucumber seedlings (lower panel) by *S. Plymuthica*

A: *Cryphonectria parasitica*; B: *Valsa mali*; C: *Botrytis cinerea*; D: *Colletotrichum capsici*

Representative results obtained from PROAGROBACT

1) Characterisation of the integration of regulatory networks in *S. plymuthica* such as the QS systems, the stationary phase sigma factor RpoS, LysR-type transcriptional regulators, as well as the post-transcriptional Rsm system to control key metabolic pathways involved in the interactions with plants such as that required for IAA biosynthesis. A brief illustration of the results obtained are: (i) mutation of some AHL QS signalling pathways resulted in the upregulation of IAA production compared to the wild type G3; (ii) the post-transcriptional Rsm system showed a significant impact on the production of the AHL signals through analysis by TLC (Fig. 4) & LC-MSMS, suggesting that the Rsm system can control directly or indirectly QS-mediated responses in *S. plymuthica* G3.

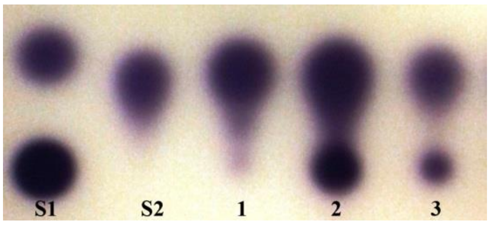

Fig. 4 AHLs detection in solvent extracted culture supernatants of G3 by TLC overlaid with the AHL biosensor *Chromobacterium violaceum* CV026. S1: Standard C4-HSL/C6-HSL; S2: Standard 3-oxo-C6-HSL; 1: G3 overexpressing *rsmA* from pUCP26; 2: G3 with the backbone vector pUCP26; 3: A G3 mutant in the *rsmB* small RNA which negatively regulates RsmA with the control plasmid pUCP26.

2) Characterisation of the impact of the *S. plymuthica* regulatory networks on the interactions of this bacterium with host plants including the fine-tuning of the biosynthesis of both the antibiotic PRN and the plant auxin IAA, bacterial motility, biofilm formation and plant colonisation. The results obtained from this part of the project demonstrated that mutants deficient in plant auxin IAA production were also impaired in biofilm formation, as well as colonisation of wheat seedlings in comparison to the parent G3.

3) The sensing of QS signal molecules by a number of plants was also investigated. These studies revealed that plants can perceive QS signals and respond to them by modulating a variety of plant physiological processes such as spore germination, alteration of root architecture, growth and stress tolerance. These results supported the idea that some AHL-producing bacteria can be exploited to develop new inoculants which can be used to increase crop production.