An update on research on *Frankia* and actinorhizal plants on the occasion of the 18th meeting of the *Frankia*-actinorhizal plants symbiosis

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Received: 27 May 2016 / Accepted: 30 May 2016 / Published online: 1 July 2016
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A meeting was held from the 24th to the 27th of August 2015 in Montpellier, France, on *Frankia*-actinorhizal plants relations. This meeting was the 18th of the series that began in 1978 at Harvard Forest, USA. The initial meeting was sparked by the first isolation of a microbe from a *Comptonia peregrina* root nodule in pure culture, which had morphological features similar to those of the *Frankia* symbiont in nodules and was capable of forming nodules on its host (Callaham et al. 1978). This effectively boosted research on the symbiosis. The 2015 meeting was the opportunity to have 80 scientists from 17 different countries, present 34 oral presentations and 51 posters. The object was to exchange ideas on a range of subjects, initiate projects and discuss various controversies. The Montpellier meeting was opened by Jean-Marc Chataigner, the IRD deputy managing director who outlined the opportunities and challenges facing scientists working on actinorhizal plants. Then there was an invited presentation by Allan Downie, Emeritus fellow at the John Innes Centre, Norwich, UK, who outlined the positive and negative aspects of the actinorhizal symbiosis research in comparison with the Legumes-rhizobia symbiosis.

Among the various recent developments, that have occurred since the previous meeting held in Shillong, India in 2013, are the cost reduction and ensuing generalization of genome sequencing techniques. Since 2013, there have been 18 genomes published representing all major lineages (Tisa et al. 2016). This mass of data, in turn, permits other “omics” approaches such as transcriptomics (Alloisio et al. 2010; Bickhart and Benson 2011), metabolomics (Brooks and Benson 2016) and proteomics (Mastrorunzio and Benson 2010; Udawary et al. 2011) to analyze various physiological aspects.

The resultant databases permit more precise phylogenetic analyses to be performed.

A study has been completed on the conserved genomic core proteins within actinobacteria and this positioned *Frankia* at the root of aerobic actinobacteria (Sen et al. 2014). The datasets can also be used to generate and characterize mutants (Kakoi et al. 2014), identify cytosine methylations (Kuchō and Kamiharai 2016), or follow the expression of genes involved in a given function such as the *hup* genes coding for hydrogenase (Richau et al. 2013). Metabolic profiling has also been undertaken to discern how PAS domains have evolved (Sarkar et al. 2016) or to compare globally metabolic machineries (Thakur and Sen 2016).

Besides *Frankia*, other bacteria have been isolated from actinorhizal nodules and sequencing them has become a convenient approach to gain knowledge on their function and physiology (Bose et al. 2016; Ghodhbane-Gtari et al. 2014).

A review of stress-responses in *Frankia* has shown the extent of its physiological adaptability (Ngom et al. 2016a). Most *Frankia* lineages have a representative that was isolated many years ago. The exception is cluster2, that resisted numerous isolation attempts and for which a representative genome was determined (Persson et al. 2015). However last year, this obstacle was overcome using an approach combining massive direct phenotypic characterization and growth medium fine tuning (Gtari et al. 2015).

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On the plant-side, the sequencing of the first genomes for the actinorhizal plants, *Casuarina glauca* and *Datisca glomerata*, was announced and will be initiated. Other approaches based on ESTs and transcriptomics were also reported (Hochoer et al. 2011; Demina et al. 2013; Diedhiou et al. 2014). In addition, metabolomics have been used to analyse globally nitrogen and carbon metabolism in (Carro et al. 2016a) and in *Datisca* (Persson et al. 2016).

Signalling between partners in particular was much discussed. Transgenic *C. glauca* expressing a transcriptional fusion between the promoter from the Nodule Inception (*NIN*) gene and the GFP reporter gene was used to develop a bioassay for the purification of the biologically active molecules in the supernatant of *Frankia* sp. CcI3 (Chabaud et al. 2016). This confirmed a previous study on a factor from *Frankia* ACoN24d (Ceremonie et al. 1999). Interestingly, such diffusible active molecules were found to induce calcium spiking in *C. glauca* (Chabaud et al. 2016) and in *A. glutinosa* (Granqvist et al. 2015). The existence of defense peptides in several lineages was also discussed as well as their effects on symbiotic *Frankia* (Carro et al. 2015; Carro et al. 2016b). The role of auxins and auxin transporters was studied (Imanishi et al. 2014) and a gene coding for a chitinase was investigated (Graça et al. 2016).

The phytometabolomic fingerprinting of consumed actinorhizal plants permitted the identification of a large diversity of the compounds present (Kar et al. 2016). Other focused studies also permitted the screen of molecules found in actinorhizal leaves and fruits of *Myrica nagi* that have analgesic, cyclooxygenase inhibiting (Middha et al. 2016b) or anti-inflammatory properties (Middha et al. 2016a).

The ecology of actinorhizal plants was another topic and information was presented on these plants and their associated microbes. Besides the global contribution of filaoes for the rehabilitation of poor sites in various areas of the world such as China and India (Zhang et al. 2016) and of alder for the same purpose in Canada (Callender et al. 2016), a major focus is the search for salinity-resistant casuarina lineages (Ribeiro-Barros et al. 2016). The underlying mechanisms are also being studied (Mansour et al. 2016; Selvakesavan et al. 2016). As pioneer species, actinorhizal plants are exposed to a wide array of stresses (Ngom et al. 2016b) which have been reviewed along with approaches to transform *Casuarina* genetically (Froussart et al. 2016). The ecology and diversity of strains and *Morella* hosts in South Africa (Wilcox and Cowan 2016) or Betulaceae hosts in North America (Samant et al. 2016) and Europe (Cotin-Galvan et al. 2016) have been studied in relation to soils. The impact of climate change on alder has also been examined (Tobita et al. 2016).

The participants at the meeting had the opportunity to visit an Agroforestry site managed by INRA colleagues in Restinclières where trees (mostly high-return walnuts, and black alders) are grown in association with cereal crops (Cardinael et al. 2015). This excursion was followed by a visit to the medieval village of St-Guilhem-le-Désert.

The next meeting dealing with the *Frankia*-actinorhizal plants symbiosis is scheduled for 2017 and will be held in Hammamet, Tunisia.

Acknowledgments We acknowledge the receipt of grants from the Institute of Research for Development (IRD), the Centre National de la Recherche Scientifique (CNRS), the University of Montpellier (UM), the French Ministry of Foreign and European Affairs, the Conseil Régional Languedoc-Roussillon and the Agropolis Foundation for the organization of the 18th *Frankia* and actinorhizal plants meeting.

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