A step further on *Frankia* biology

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Three decades have passed since a few pioneers envisioned the need to concert efforts and foster research of nitrogen-fixing root-nodule symbioses between *Frankia* and a wide taxonomic range of dicotyledonous plants (Torrey and Tjepkema 1979). In those days, the excitement was high: the first *Frankia* isolates were established in pure culture; ultramicroscopic studies of root nodules and *Frankia* isolates revealed the colonial and pleomorphic organization of this actinobacteria; nitrogen-fixation efficiency was measured and compared in both free-living and symbiotic frankiae; host specificity was addressed by cross-inoculation experiments; importance of actinorhizal symbioses for land reclamation was discussed and their implications for the silvicultural and agricultural sustainability in diverse ecosystems were debated (Gordon et al. 1979). Nowadays, new questions arose but the enthusiasm remains the same. In fact, while some challenging issues have been addressed, such as the innovative protection of nitrogenase to oxygen damage by a hopanoid lipidic multilayer wrapping *Frankia* vesicles, and the matchless plasticity of frankiae

genomes, other fundamental queries are yet to be answered, namely the molecular signals that modulate actinorhizal infection and *Frankia*—plant hosts specificity and the implementation of a reliable and efficient system for genetic manipulation of *Frankia*.

The 16th International Meeting on *Frankia* and Actinorhizal plants, held from the 5th to the 8th of September of 2010 in Porto, Portugal, gathered frankiologists from four continents. Longtime friends and colleagues have met and some of them brought new people, eager to learn, to discuss their data and to peek new opportunities. Frankiologists have been working side by side with plant biologists to improve our understanding of actinorhizal symbioses. Therefore, besides the studies focused on *Frankia*, this meeting brought up relevant progresses on several aspects of actinorhizal plant biology, out of which a series of manuscripts have been written and compiled on a special double issue of Functional Plant Biology (2011, vol 38, issues 8 and 9). Concerning the microbial symbiont, the recent advances and the availability of several *Frankia* genomes completed or on their way, prompted comparative genomic analyses which complemented the proteomic and transcriptomic studies, leading to an overall optimistic view of the coming years.

In this issue of Archives of Microbiology, forefront research in *Frankia* is illustrated by several papers. Starting with a retrospective of nitrogen fixation in Actinobacteria, an important trait once thought to be exclusive of *Frankia* spp. within this phylum, Gtari et al. review the sparse physiological and biochemical studies made these last 50 years and emphasize that nitrogen fixation within actinobacteria is unlikely to be restricted to frankiae. Although most of these reports need to be confirmed and direct evidence for nitrogenase activity is missing, they certainly do constitute a starting point for further studies.

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Moreover, the presence and phylogenetic distribution of *nifH*- and *nif*-like gene homologs in several actinobacteria, further suggest that nitrogen fixation in actinobacteria is not a circumstantial event and pose alternative hypotheses for nitrogen fixation, worth to be pursued in the future.

Within this context, an original paper from Oh et al. highlights the conserved organization of a *nif* gene cluster. This cluster includes the *nif*-associated genes, believed to be needed for the biosynthesis and maturation of the nitrogenase complex in several *Frankia* strains. Proteomic analysis and expression studies of these genes should help to ascertain their function, particularly because the conventional approach to construct knockout mutants and access the function of particular genes has not yet been achieved in *Frankia*. In fact, and despite all the efforts made, the failure for genetic manipulation of frankiae is still a tremendous drawback for functional gene analysis. One of the reasons that is hindering this molecular approach is the difficulty in handling *Frankia* cultures. Notwithstanding that over three decades have passed since the isolation and maintenance of *Frankia* in pure culture was achieved, the rich culture media, slow colonial growth and inadequacy to routinely use antibiotics to keep contaminants away, are the main reasons why *Frankia* cultures are not routine procedures. In this regard, Furnholm et al. propose a semi-high throughput growth assay that aims to assess multiple properties of *Frankia* and other filamentous species. By using 24-well plates, the described method allows the rapid evaluation of the effects caused by multiple variables on *Frankia* growth trends starting with a small amount of biomass standardized by its OD₆₀₀. Additionally, Huang and Benson brought new insights on hyphal growth, contributing to better define the exponential growth phase in these mycelial bacteria, a knowledge that has promising applications in the improvement of *Frankia* growth conditions.

Despite the current impossibility to genetically manipulate *Frankia*, the availability of fully sequenced genomes makes possible comprehensive functional genomic studies and should allow infer the patrimony of tRNA genes and ensure that codon usage is not a constraint factor to construct stable heterologous mutants. Interestingly, Sen et al.

studied the prevalence of TTA codons in numerous *Frankia* genes and found that TTA-containing genes are mostly associated with basic metabolic functions, contrary to what has been described for *Streptomyces coelicolor*, where this rare codon has a regulatory role in secondary metabolism and morphological development. Furthermore, *Frankia* has the highest prevalence of genes containing TTA codons among actinobacteria, most of them vertically inherited, while maintaining their translation efficiency. Altogether, these data urge for further studies of codon usage and function in frankiae, which may be important, for instance, to devise *Frankia* customized vectors for genetic manipulation.

Another classical yet unanswered question regarding *Frankia* biology has to do with the nodulation determinants and the dynamics of plant-microsymbiont communication and symbiosis establishment. In this regard, Pujic et al. characterized a *Frankia* ACN14a gene (FRAAL0616) encoding for a sugar-binding protein thought to adhere to both root hairs and hyphae. This lectin was associated with a higher density of nodules in *Alnus glutinosa*, which according to the authors may facilitate nodulation under conditions of low *Frankia* density. Interestingly, no homologs to this lectin were found in *Frankia* strains EAN1pec and CcI3, suggesting that these molecules may also act as determinants of plant host specificity.

In conclusion, beyond the independent contributions provided by these reports, it is noteworthy the complementarity found among them, which underline the community-driven research effort to address new and long-standing questions. Ultimately, the forum raised by these and all the other inputs gained during those 4 days in Porto has set the pace for forthcoming studies.

References

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