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"Biological Nitrogen Fixation" Book Summary

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Abstract

Biological nitrogen fixation is a very valuable alternative to nitrogen fertilizer. This process will be discussed in the "Biological Nitrogen Fixation" book. A wide array of free-living and associative nitrogen fixing organisms (diazotrophs) will be covered. The most extensively studied and applied example of biological nitrogen fixation is the symbiotic interaction between nitrogen fixing "rhizobia" and legume plants. While legumes are important as major food and feed crops, cereals such as wheat, maize and rice are the primary food crops, but do not have this symbiotic nitrogen fixing interaction with rhizobia. It has thus been a "holy grail" to transfer the ability to fix nitrogen to the cereals and this topic will be also addressed in these books.

Keywords

Biological Nitrogen Fixation, Diazotrophs, Nodulation, Nitrogenase, Legumes, Cereals

1. Introduction

Nitrogen is arguably the most important nutrient required by plants, being an essential component of all amino acids and nucleic acids. However, the availability of nitrogen is limited in many soils and although the earth's atmosphere consists of 78.1% nitrogen gas (N_2), plants are unable to use this form of nitrogen [1]. To compensate, modern agriculture has been highly reliant on industrial nitrogen fertilizers to achieve maximum crop productivity. However, a great deal of fossil fuel is required for the production and delivery of nitrogen fertilizer. Indeed industrial nitrogen fixation alone accounts for 50% of fossil fuel used in agriculture. This can be exceedingly expensive. Moreover carbon dioxide (CO_2), which is released during fossil fuel combustion, contributes to the greenhouse effect, as does the decomposition of nitrogen fertilizer, which releases nitrous oxide (NOx), which is a greenhouse gas about 292 times more active than carbon dioxide [2]. In addition, applying chemical fertilizers is a largely inefficient process, as 30% - 50% of applied nitrogen fertilizer is lost to leaching, resulting

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in significant environmental problems, such as the eutrophication of waterways [3]. Thus there is a strong need to reduce our reliance on chemical nitrogen fertilizers and instead optimize alternative nitrogen inputs [1].

1.1 Biological Nitrogen Fixation

Biological nitrogen fixation is one alternative to nitrogen fertilizer. It is carried out by prokaryotes using an enzyme complex called nitrogenase and results in atmospheric N_2 being reduced into a form of nitrogen diazotrophic organisms and plants are able to use (ammonia). It is this process and its major players which will be discussed in this "Biological Nitrogen Fixation" book [4]. The best known and most extensively studied example of biological nitrogen fixation is the symbiotic interaction between nitrogen fixing "rhizobia" and legume plants. Here the rhizobia induce the formation of specialized structures ("nodules") on the roots or sometimes stems of legume plants and fix nitrogen which is directly assimilated by the host plant; in return, the plant provides the required energy source for the energy intensive nitrogen fixation process. It is this symbiotic interaction which will be highlighted in this book [4]. The research in the field of Biological Nitrogen Fixation is very active at the moment, especially in the subfield of symbiotic nitrogen fixation. Although a number of books and proceedings of the International Congresses on Nitrogen Fixation and North American Symbiotic Nitrogen Fixation Conferences have appeared during the last ten and more years, a comprehensive book of the field from biochemistry of nitrogenase, through expression and regulation of nitrogen genes, taxonomy, evolution and comparative genomics of nitrogen fixing organisms, their physiology and metabolism, their life in the rhizosphere and under stress conditions, rhizobial "Omics", plant "Omics", nodulation of legumes and non-legumes, recognition, infection and nodule ontogeny, nitrogen fixation and assimilation, field studies, inoculum preparation and application of Nod factors, endophytic nitrogen fixers, cyanobacteria and nitrogen fixation and cereals does not presently exist and this Book sets aims to fill this void. In this Book, the most recent findings about a variety of free-living, associative or symbiotic diazotrophs will be covered, nitrogenase(s) and their mechanism of action and regulation will be reviewed, and the use of diazotrophs in agriculture will be summarized.

1.2. Biological Nitrogen Fixation and Cereals

While legumes are important as major food and feed crops and are the second group of such crops grown worldwide, the first group (cereals such as wheat, mays and rice) does not fix nitrogen or have this symbiotic nitrogen fixing interaction with rhizobia. It has thus been a focus of a number of studies to transfer the ability to fix nitrogen to cereals and different timely approaches towards this goal are also included in the Book (see below).

2. Book Outline

The flow of the Chapters is as follows: The first Chapter is an Introduction to the Book by the Editor, giving the background to the field, describing the flow of Sections and Chapters and highlighting Sections, individual Contributions and some future trends. The **first Section** contains a number Focus Chapters (reviews) introducing the main topics of the Book, including nitrogenases and how they work, evolution and taxonomy of nitrogen fixing organisms, the evolution of *Rhizobium* nodulation and bioengineering nitrogen acquisition in rice (see also below). The **second Section** covers selected recent advances in the biochemistry of nitrogenases, including the biosynthesis of the FeMoCo subunit of nitrogenase, and conserved amino acid sequence features in MoFe, VFe and FeFe nitrogenases.

Section 3 covers the regulation of nitrogen fixation genes and nitrogenase itself. The Chapters in this section will describe regulatory aspects of several different nitrogen fixing systems, such as *Azotobacter vinelandii*, *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, *Pseudomonas stutzeri* and *Rhizobium etli*. **Section 4** covers taxonomic and evolutionary features of nitrogen fixing organisms, including Chapters on taxonomy, including the origin and diversity of *Burkolderia* and other beta-rhizobia, the phylogeny of nodulation and nitrogen-fixation genes in *Bradyrhizobium*, and a global census of nitrogenases and nitrogen fixation genes. **Section 5** covers the genomics of selected nitrogen fixing organisms and comparative analysis of their genomes. Also included are a Chapter on the transfer of the Symbiotic Island of *Mesorhizobium loti* and a software program Chapter for pan-genomic analysis.

Section 6 covers aspects of the physiology and metabolism of nitrogen fixing organisms and a chapter on the need for photosynthesis for efficient nitrogen fixation in a rhizobial strain, as well as Chapters on cytochrome oxidases, the role of BacA in rhizobia, and the analysis of flagellins in *Rhizobium leguminosarum*. **Section 7** contains a number of Chapters on rhizobial life in the rhizosphere of plants, including the effect of plant root exudates, role of quorum sensing and quenching, exopolyssacharides, flavonoids, luminochrome and the response to various stresses. **Section 8** deals with the physiology and regulation of nodulation. Chapters include the root hair as a single cell model for systems biology, two chapters on the conserved genetic program among arbuscular mycorrhizal, actinorhizal and legume-rhizobial symbiosis, the molecular determinants of nodulation in the *Frankia/Discaria* symbiosis and the physiology of nitrogen assimilation in the *Datisca-Frankia* root nodule symbiosis, as well as Chapters on the Nod—independent symbiosis in *Aeschynomene*, the role of phosphorus efficiency, the regulation of nodule development by auxin transport, and the *NOOT* mutant of *M. truncatula*. **Section 9** then initiates a series of Sections on nodulation. The recognition in nodulation Section 9 will cover the very early events in nodulation, including putative Nod factor receptors and signal transduction, early signalling in *Frankia*, the role of ectoapyrases and cellulose $CelC_2$ in nodulation, and Calcium Spiking.

Section 10 addresses the infection and nodule ontogeny topics. A multitude of aspects are covered in the Section, including Chapters on topics such as Ca^{2+} signalling and infection thread formation, the role of hormones in nodulation, the role of a transporter in integrating nutrient and hormone signalling with lateral root growth and nodule development, and the role of genes encoding MYB coiled coil and ERF transcription factors, the dissection of the roles in outer and inner root cell layers of plant genes that control rhizobial infection and nodule ontogeny, the multifaceted role of nitric oxide in nodulation, the role of pectate lyase in root infection, the identification of novel *M. truncatula* genes required for rhizobial invasion and bacteroid differentiation, as well as novel approaches such as RNA-seq, and cortical auxin modelling for nodulation.

Section 11 covers the "next" stage in nodule biology, namely the development of bacteroids required for nitrogen fixation and the proteomic profile of the soybean symbiosome membrane. **Section 12** addresses briefly N-assimilation (ammonium transport) in nodules and nodule senescence. In **Section 13** several "Omics" applications in rhizobia and *Frankia* (metagenomics, transcriptomics, proteomics, genomics) are discussed, such as the metagenomic analysis of microsymbiont selection by the legume host plant, proteomic profiling of *Rhizobium tropici*, the *Frankia alni* symbiotic transcriptome, a comprehensive survey of the Rhizobiales using high throughput DNA sequencing and gene targeted metagenomics of diazotrophs in coastal saline soil. **Section 14** does the same with (host) plant genomics, proteomics and transcriptomics, including Chapters on the *M. truncatula* genome, retrotransposon *Tnt*1 mutagenesis, leveraging large scale approaches to dissect legume genomics and the *Rhizobium*-legume symbiosis, databases, and functional genomics of symbiotic nitrogen fixation in legumes.

In **Section 15** the intricacies of nodule formation and functioning are left behind and the focus is on nitrogen fixing cyanobacteria, with studies in the open ocean, requirement of cell wall remodelling and cell differentiation in a cyanobacterium of the order Nostocales, and nitrogen fixation in the oxygenic phototrophic prokaryotes (cyanobacteria): the fight against oxygen. **Section 16** deals with diazotrophic plant growth promoting rhizobacteria (PGPR) and non legumes. The Section begins with a historical overview of PGPR and non-legumes. Beneficial plant associated *Burkolderia* species, agronomic applications of *Azospirillum* inoculants and molecular characterization of the diazotrophic bacterial community in sugarcane are discussed. Moreover the role of auxin signalling in plant-microbe interactions is presented. Lastly a Chapter on how fertilization affects the selection of PGPR by the plant is included, as well as the genetic and functional characterization of *Paenibacillus riograndensis*, *Herbaspirillum* attachment to maize, and isolation of novel diazotrophs from sugar cane plants.

Section 17 covers field studies, inoculum preparation, and quality and response to stress, such as dessication, evaluation of elite soybean varieties in the field, phase variation in *Azospirillum* and the application of LCO's to legume and non-legume seeds. **Section 18** is special and deals with the opportunities for nitrogen fixation in rice and other cereals (See above). In the first Chapter the history of the quest for biological nitrogen fixation in cereals is reviewed and associative (endophytic) diazotrophy with grasses and transfer of the legume nodulation and nitrogen fixation traits to cereals are discussed (See below). In the second Chapter the environmental and economic impacts of biological N_2 fixing cereal crops are discussed. In this Chapter the question is raised "how novel are nodules?"The third Chapter of **Section 18** deals with the conservation of the symbiotic signalling pathway (CSSP or SYM; see below) between legumes and rice and related functional cross complementation studies. The fourth Chapter in **Section 18** describes the ecophysiology of the natural endophytic Rhizobium-Rice association and the translational assessment of its biofertilization performance in the Nile delta. **Section 19**

contains a first Chapter on nitrogen fixation and nitrogen recycling in insects. In the second Chapter a protocol for the rapid identification of nodule bacteria with MALDI-TOF mass spectrometry is presented. The last Chapter of the Book presents a comprehensive review on endophytes in plants.

3. Transferring the Ability to Fix Nitrogen to Cereals

This has been a "holy grail" for a long time. Two major approaches are discussed again in the Book: Developing the root nodule symbiosis in cereals and transferring the nitrogenase genes into cereal plants and finding a proper location for their expression in terms of low free O_2 concentration and sufficient energy supply. These two options and associative and endophytic nitrogen fixation were already discussed in previous meetings such as those of the Working Group of the IRRI frontier project on assessing Opportunities for Nitrogen Fixation in rice (Opportunities for Biological Nitrogen Fixation in Rice and other Non-Legumes [5]. as well as The Quest for Nitrogen Fixation in Rice [6]. However, we are in a much more informed state now. Recently, the Bill and Melinda Gates Foundation convened a small Meeting on the subject and several Projects were funded, including the transfer of the nitrogen fixation (nitrogenase) genes into cereals, and the transfer of the ability to form nitrogen fixing nodules to cereals. The BBRC in Norwich, UK and NSF in the USA have followed suit in providing funding for analogous projects. This has greatly stimulated research in these areas.

3.1. The Common Symbiotic Signalling Pathway (CSSP or SYM)

In the case of rice transformation, it is now routine and since the genome has been sequenced and extensive genetics is available, it should serve as a "model species", while for other cereals (grasses) a recently developed *Brachypodium* model system has been established [7].

The discovery of a "common symbiotic pathway" in arbuscular mycorrhizal, rhizobial, and actinorhizal symbioses and the identification of conserved pathway genes (CSSP or SYM pathway) in legumes and monocots (including rice) has made the extension of the ability to fix nitrogen in nodular associations with cereals a more realistic endeavour [8] [9].

3.2. *Nif* Gene Transfer to Cereals

The direct transfer of nitrogen fixation (*nif*) genes into non-legumes has also become more feasible especially since it has been shown that six out of the numerous *nif* genes are absolutely required for FeMo-co biosynthesis and nitrogenase activity, both *in vitro* and *in vivo*. It is likely that the products of some genes that are required for FeMo-co biosynthesis *in vivo* could be replaced by the activities of plant counterparts [10]. Several synthetic biology approaches modifying *nif* clusters for transfer into plants [11] [12] are described in Chapter 108 [9].

4. Conclusion

Thus we are entering a very promising period of research on BNF, both in more conventional systems as well as non-legumes such as rice, based on rapidly advancing basic studies on the chemistry, biochemistry, genetics, physiology, regulation, taxonomy, genomics and metagenomics, and metabolism of nitrogen-fixing organisms (and their hosts). This is the topic of this book, which should be a major resource for scientists in the field, and those wanting to enter it, as well as teachers and agricultural specialists wanting to apply the technology.

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References

[1] Ferguson, B.J., Indrasumar, A., Hayashi, S., Lin, Y.-R., Lin, Y.H., Reid, D.E. and Gresshoff, P.M. (2010) Molecular Analysis of Legume Nodule Development and Autoregulation. *Journal of Integrative Plant Biology*, **52**, 61-76. http://dx.doi.org/10.1111/j.1744-7909.2010.00899.x

[2] Crutzen, P., Mosier, A.R., Smith, K.A. and Winiwarter, W. (2007) N_2O Release from Agro-Fuel Production Negates

Global Warming Reduction by Replacing Fossil Fuel. *Atmosphere Chemistry Physics Discussion*, **7**, 11191-11120. http://dx.doi.org/10.5194/acpd-7-11191-2007

[3] Graham, P.H. and Vance, C.P. (2003) Legume Importance and Constraints to Greater Use. *Plant Physiology*, **131**, 872-877. http://dx.doi.org/10.1104/pp.017004

[4] de Bruijn, F.J. (2015) Biological Nitrogen Fixation. Wiley-Blackwell Publishers, Hoboken, 1-1196. http://dx.doi.org/10.1002/9781119053095.ch1

[5] Ladha, J.K., de Bruijn, F.J. and Malik, K.A. (1997) Opportunities for Biological Nitrogen Fixation in Rice and Other Non-Legumes. Kluwer Academic Publishers, Dordrecht, 1-216.

[6] Ladha, J.K. and Reddy, P.M. (2000) The Quest for Nitrogen Fixation in Rice. International Rice Research Institute Press, Los Banos, 1-354.

[7] Bevan, M.W., Garvin, D.F. and Vogel, J.P. (2010) *Brachypodium distachyon* Genomics for Sustainable Food and Fuel Production. *Current Opinion in Biotechnology*, **21**, 211-217. http://dx.doi.org/10.1016/j.copbio.2010.03.006

[8] Venkateswaran, M., Volkening, J.D., Sussman, M.R. and Ane, J.-M. (2013) Symbiosis and the Social Network of Higher Plants. *Current Opinion in Plant Biology*, **16**, 1-10. http://dx.doi.org/10.1016/j.pbi.2012.11.007

[9] de Bruijn, F.J. (2015) The Quest for Biological Nitrogen Fixation in Cereals: A Perspective and Prospective. In: de Bruijn, F.J., Ed., *Biological Nitrogen Fixation*, Wiley-Blackwell Publishers, Hoboken, 1089-1101. http://dx.doi.org/10.1002/9781119053095.ch108

[10] Jimenez-Vicente, E., Hernandez, J.A., Echavarri-Erasun, C. and Rubio, L.M. (2015) Biosynthesis of the Iron-Molybdenum Cofactor of Nitrogenase. In: de Bruijn, F.J., Ed., *Biological Nitrogen Fixation*, Wiley-Blackwell Publishers, Hoboken, 75-86. http://dx.doi.org/10.1002/9781119053095.ch7

[11] Wang, X., Yang, J.-G., Wang, J.-L., Cheng, Q., Dixon, R. and Wang, Y.-P. (2013) Using Synthetic Biology to Distinguish and Overcome Regulatory and Functional Barriers Related to Nitrogen Fixation. *PLOS ONE*, **8**, e68677. http://dx.doi.org/10.1371/journal.pone.0068677

[12] Temme, K., Zhao, D. and Voigt, C.A. (2012) Refactoring the Nitrogen Fixation Cluster from *Klebsiella oxytoca*. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 7085-7090. http://dx.doi.org/10.1073/pnas.1120788109