BIOLOGICAL NITROGEN FIXATION

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Summary

Biological Nitrogen Fixation estimate the amount of fixed nitrogen and to select the most effective rhizobial strain x plant genotype combination. The ^{15}N techniques are currently the most accurate method to measure the nitrogen fixed in a given system. The elite strains will be then used for the inoculum production.

However, whatever the elite strains are, the quality control of the inoculum must be performed before any use to maximize the BNF process

1. Introduction

Nitrogen is an essential element for plant growth and development and a key issue of agriculture. Most studies indicate that nitrogen fertilizers contribute to resolving the challenge the world is facing, feeding the human population. The Green Revolution was accompanied by an enormous increase in the application of nitrogen fertilizer. There is however a high heterogeneity of its distribution throughout the world: some areas subjected to pollution whereas others to depleted soil, decreased crop production, and other consequences of inadequate supply.

Biological Nitrogen Fixation (BNF) is known to be a key to sustain agriculture and to reduce soil fertility decline. Research on microorganisms and plants able to fix nitrogen contributes largely to the production of biofertilizers. Thus it is important to ensure that BNF research and development will take into account the needs of farmers in the developing countries mainly.

UNESCO has already addressed this challenge through the Microbial Resources Centre (MIRCEN) initiative. Two BNF MIRCENs were thus established in East (Kenya) and West (Senegal) Africa and focused on the diffusion of rhizobium technology including isolation, identification, collection, maintenance and distribution of rhizobial cultures and inoculants for leguminous crops. Considerable experience in the network approach has been gained through these MIRCENs, which functioned as the bases of projects in BNF technology for East and West Africa.

Below are described briefly some key issues of life supporting system related to basics and applied BNF technology emphasized to rhizobial bacteria (Table 1).

Genres	*Espèces*	*Plantes hôtes*	*Références*
	R. leguminosarum		*Jordan, 1984*
	biovar viciae	*Pisum, vicia, Lathyrus, Lens*	*Jordan, 1984*
	biovar trifolii	Trifolium	*Jordan, 1984*
	biovar phaseoli	Phaseolus vulgaris L.	*Jordan, 1984*
Rhizobium	R. galegae	Galega orientalis	*Lindstršm et al, 1989*
(croissance rapide)	R. tropici	P. vulgaris L., Leucaena	*Martinez-Romero et al, 1991*
	R. etli	Phaseolus vulgaris	*Segovia et al, 1993*
	R. hainanensis	*Lég. des régions arides et salées*	*Chen et al.,1994a;Chen et al.,1997*

	R. gallicum	Phaseolus vulgaris L.	*Amarger et al.,1997 **
	R. giardinii	Phaseolus vulgaris L.	*Amarger et al., 1997 **
	R .mongolense	Medicago ruthenica	*Van Berkum et al., 1998*
	R huautlense	Sesbania herbaceae	*Wang et al., 1998*
	M. loti	Lotus	*Jarvis et al., 1982*
	M. huakuii	Astragalus sinicus	*Chen et al., 1991*
Mesorhizobium	M. ciceri	Cicer arientinum	*Nour et al., 1994*
(croissance	M. hainanensis	Sev. arid reg. pl. sp.	*Chen et al., 1994*
Intermédiaire)	M. tianshanense	13 tropical pl. sp.	*Chen et al., 1995*
	M. mediterraneum	Cicer arientinum	*Nour et al., 1995*
	M. plurifarium	Acacia, Prosopis, Leucaena, Chamaescrista	*de Lajudie et al., 1994*
	M. amorphae	Amorpha fructicosa	*Wang et al., 1999*
	S. meliloti (a, b)	Medicago, Melilotus,	*Jordan, 1984 ; Eardly et al., 1990*
		Trigonella	*de Lajudie et al., 1994*
Sinorhizobium	S. mediceae	Medicago	*Rome et al., 1996*
(croissance rapide)	S. fredii	Glycine max, G. soja	*Chen et al., 1988 ; de Lajudie et al., 1994*
	S. saheli	Sesbania sp.	*de Lajudie et al., 1994*
	S. terangae		
	biovar acaciae	Acacia	*de Lajudie et al., 1994; Lortet et al., 1996*
	biovar sesbania,	Sesbania,	*de Lajudie et al., 1994; Lortet et al., 1996*
	S .xinjiangense	Glycine max, G	*Chen et al., 1988 ; de Lajudie et al., 1994*
	S. kostiense	Acacia, Prosopis	*Nick et al., 1999*
	S. arboris	Acacia, Prosopis	*Nick et al., 1999*
Allorhizobium	undicola	Neptunia natans	*de Lajudie et al., 1998*
(croissance rapide)			
Azorhizobium	A. caulinodans	Sesbania rostrata	*Dreyfus et al., 1988*
(croissance rapide)	A. sp.	Sesbania rostrata	*Rinaudo et al., 1991*
	B. japonicum	Glycine max, G. soja	*Jordan, 1982*
Bradyrhizobium	B. sp.	Vigna, Lupinus, Mimosa, Acacia,	*Jordan, 1982 ; Dupuy, 1994*
(croissance lente)		Aeschynomene	*Alazard, 1985 ; Young, 1991*
	B. elkanii	Glycine max	*Kuykendall et al., 1992*
	B. liaoningensis	Glycine max, G. soja	*Xu et al., 1995*

Table 1: Taxonomic evolution of nitrogen fixing bacteria of the family of *Rhizobiaceae* [from Yattara 2000]

2. Nodulation: From the Infection Process to the Functioning of the Nitrogenase

The production of nitrogen fertilizer by industrial fixation generates large quantities of carbon dioxide, contributing to earth warming. The natural process of BNF offers an economic means of reducing environmental problems and improving the internal resources. It is a process that allows microorganisms to convert atmospheric nitrogen (N_2) to ammonia (NH_3) assimilable by associated plants.

$$N_2 + 6H^+ + ATP \xrightarrow{6e^-} 2NH_3 + ADP + Pi$$

Different types of associations are listed in Table 2.

Types of association	Microorganisms	Host plants
Symbiotic	Bacteria (ex. *Rhizobium*)[a] Actinomycetes (ex. *Frankia*) Cyanobacteria (ex. *Anabaena azollaea*)	Legumes Actinorhiza Fern
Non symbiotic	Bacteria (ex. *Azotobacter*, *Azospirillum*)	Cereals
Free living systems	Bacteria (ex. *Thiobacillus*, *Clostridium*)	

Table 2. Different types of nitrogen fixing systems [a] : *Rhizobium*-leguminous plants is the most studied symbiotic association. There are six main genus of rhizobia : *Allorhizobium* (fast growing), *Azorhizobium* (fast growing) *Bradyrhizobium* (slow growing), *Mesorhizobium* (intermediate growing), *Rhizobium* (fast growing), *Sinorhizobium* (fast growing).

2.1. Nodule Formation

Leguminous plants and rhizobia communicate through the gene expression by reciprocally transmitting signals for the activation of the symbiotic genes in two partners. A type of phenolic called flavonoids, are released by host roots plants into the rhizosphere. Flavonoids act as a chemo-attractant for the bacteria to the plant roots, and eventually colonies of rhizobia attach to the root hairs. Flavonoid signal activates expression of nodulation (nod) genes.

In the rhizobial strains there are numerous nodulation genes including the nod genes nodABC and nodD (Figure 1). On the surface of the rhizobial bacteria the flavonoids are recognized by a expressed nodD protein. Then nodD binds to a promoter DNA sequence, and thereby activates transcription of nod genes of the operons. A group of nod genes encode enzymes synthesise the rhizobial nodulation signal, Nod factor (Figure 2), which triggers development of the root nodules by the plant. The plant roots

recognise Nod factor, through binding to to a surface protein receptor at the sub apical root tip. Perception of Nod factor induces a development inside the root, producing pronounced curling of the root hairs entrapping the rhizobia which establish infection of root. Bacteria gain access to plants cell membrane. The plasma membrane invaginates to form novel infection structure known as the infection thread, a tubular structure that extends from the root hair tip to the lower cells of the root cortex.

Rhizobia enter the infection threads in which they actively multiply. At the same time the underlying root cortex cells are quickly proliferated to constitute the nodule primordia. The infection threads branch out into cells of the nodule primordia. The rhizobia are finally released into the nodule cell and enveloped in a membrane derived from the host cell plasma membrane. At this stage, rhizobial bacteria become bacteroids able to fix nitrogen.

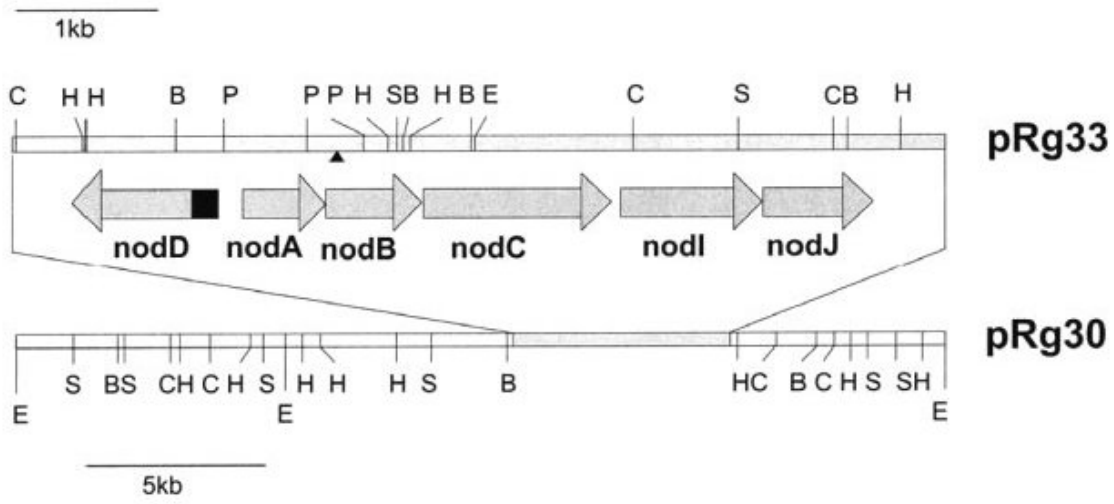

Figure 1**.** Restriction map of the common *nod* gene region of *Rhizobium galegae* HAMBI 1174. The cosmid clone pRg30 carries the six open reading frames homologous to *nodDABCIJ* genes subcloned in pRg33. ▲indicates the site of the Tn5 insertion in pRg33. The black square indicates the *nod*-box sequence. Restriction enzymes used were as follows:
E = *Eco*RI; B = *Bam*HI; C = *Cla*I; H = *Hin*dIII; P = *Pst*I; S = *Sal*I.

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Biographical Sketches

Mamadou Gueye was born in 1950 in Dakar, Senegal, studied microbiology at the University of Lyon, France [1975-1982]. He studied for his doctorate at University of Lyon [1979-1982] on microbial ecology. After receiving his doctorate, he worked in the scope of biological nitrogen fixation (BNF) and

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