In NEW HORIZONS IN NITROGEN FIXATION
R. PALACIOS et al (ed.)

DREYFUS B.
Doc2

DIVERSITY OF PHOTOSYNTHETIC BRADYRHIZOBIUM STRAINS FROM STEM NODULES OF AESCHYNOMENE SPECIES

J. JOROUIN E. MOLOUBA N. DUPUY S. NDIAYE D. NIANG M. SYLLA

groups have been differentiated (Hollis *et al.*, 1981). Recently, Kuykendall *et al.*(1992) proposed a new species *Bradyrhizobium elkanii* for members of the DNA homolgy group II. *Bradyrhizobium japonicum* is closely related to the purple non-sulfur photosynthetic bacterium *Rhodopseudomonas palustris* (Young *et al.*, 1991) and to *Blastobacter denitrificans* and the genera *Afipia* and *Nitrobacter* (Willems and Collins, 1992 ; Young, 1992). Moreover, the slow-growing strain BTAi1 isolated from stem nodules of *Aeschynomene indica* (group III) was shown

photosynthetic strains were observed according to their culture pigmentation, the light pink strains (LP) similar to BTAi1, the dark pink strains (DP) and the orange strains (O). Forty three strains remained white (W) under light and no carotenoids could be detected after extraction and spectroscopic analysis (Table 1).

The 126 photosynthetic and non-photosynthetic strains were then tested for nodulation and nitrogen fixation on different *Aeschynomene* species belonging to the three groups of *Aeschynomene*. Results are summarized in Table 1 and confirm the division of *Aeschynomene* species into three cross-inoculation groups. Non-photosynthetic strains were found in groups I and II, but were effectively fixing nitrogen in group I species only. Photosynthetic strains are effectively nodulating group II and III, this last group being more specific with a narrow host-range. Photosynthetic strains never formed nodules on *Aeschynomene* species of group I. In group II, photosynthetic strains appeared to be the most effective.

TAXONOMY OF *AESCHYNOMENE* BRADYRHIZOBIA

Rhizobia isolated from *Aeschynomene* were characterized by a comparative study of total proteins using SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoretic separation of whole-cell proteins have been widely used in *Rhizobium*, *Azorhizobium* and *Bradyrhizobium* taxonomic studies (Kersters, 1985 ; Moreira *et al.*, 1992). This discriminative and reproducible technique provides information at the level of species and subspecies. The bacterial protein patterns of some photosynthetic and non-photosynthetic *Aeschynomene* bradyrhizobia were scanned and analysed numerically together with the data of patterns of reference strains available in the data base of our research group at the University of Ghent (Belgium). Preliminar results

Table 1. Cross-inoculation specificities in the genus *Aeschynomene*.

Cross-inoculation pattern	Bacterial strain	Strain color[1]	Bchla[1] and carot.	Test plant[2]			
				Ae.elaphroxylon	*Ae.afraspera*	*Ae.indica*	*Ae.sensitiva*
Host plant				Group I	Group II	Group III	
Group I							
Ae. elaphroxylon	ORS 377	W	-	**E**	I	0	0
	ORS 378	W	-	**E**	I	0	0
Group II							
Ae. afraspera	ORS 313	W	-	I	e	0	0
	ORS 336	W	-	I	e	0	0
Ae. afraspera	ORS 285	LP	+	0	**E**	**E**	**E**
	ORS 362	LP	+	0	**E**	**E**	**E**
Group III							
Ae. sensitiva	ORS 276	LP	+	0	0	**hE**	**hE**
Ae. indica	ORS 399	LP	+	0	0	**E**	**E**
Ae. indica	ORS 344	DP	+	0	0	**E**	**E**
	ORS 371	DP	+	0	0	**E**	**E**
Ae. sensitiva	ORS 278	O	+	0	0	**E**	**hE**
	ORS 279	O	+	0	0	**E**	**hE**

[1] Culture grown in a YM medium under intermittent light.
W, White ; L P, Light pink ; D P, Dark Pink ; O, Orange.
0, no nodules produced.
[2] hE, E, e and I respectively high effective, effective, partially effective and ineffective root nodulation.

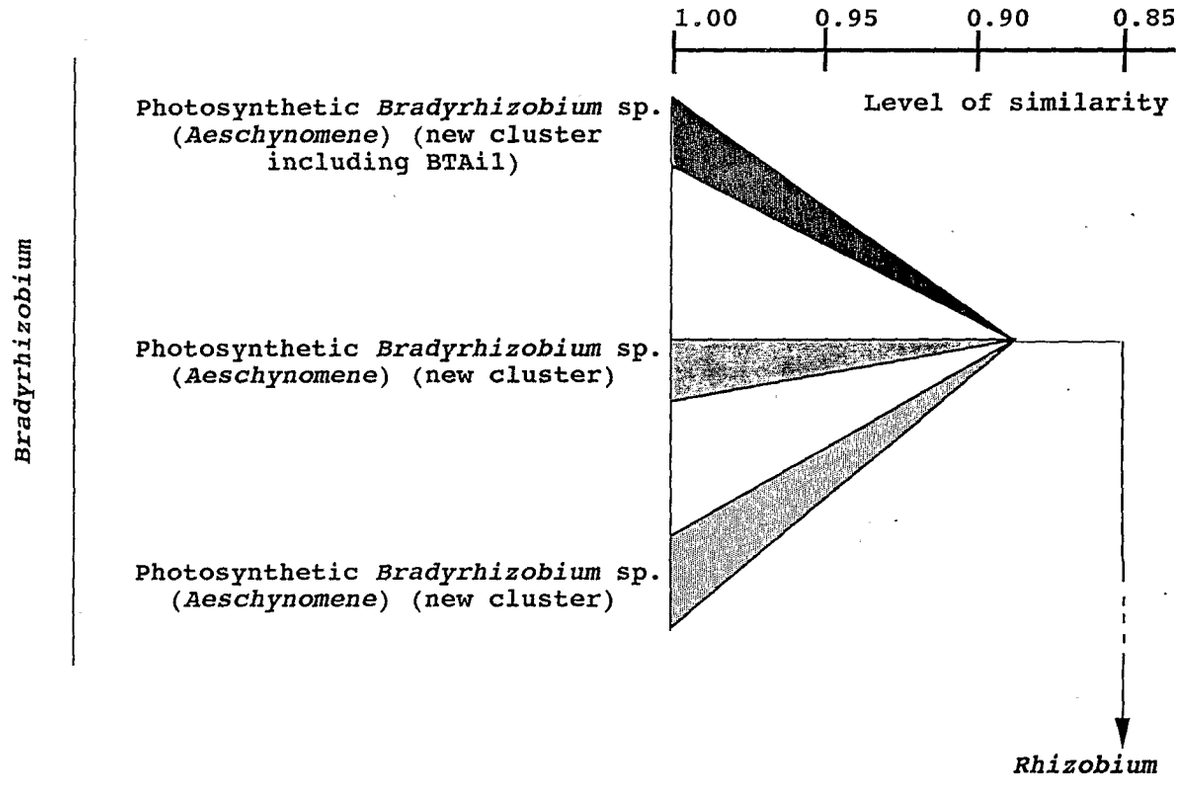

Figure 1. Simplified dendrogram showing the SDS-PAGE relationships among *Bradyrhizobium* sp. nodulating *Aeschynomene* sp., based on *r* values as calculated by the unweighted method using average linkage.

these new clusters correspond respectively to the pink, dark pink and orange coloured strains.

Furthermore, DNA from a few representative strains of *Aeschynomene* were tested for DNA : rRNA hybridization with a labelled rRNA probe from *Bradyrhizobium japonicum* NZP5549^T. The results confirmed that they belong to the *Bradyrhizobium* rRNA cluster.

STEM COLLAR NODULE IN *AESCHYNOMENE SENSITIVA*

As in other *Aeschynomene* species, stem infection by *Bradyrhizobium* strains in *Ae. sensitiva* always occurs at predetermined sites which correspond to root primordia present just beneath the stem epidermal cell layer. Seven days after inoculation by the photosynthetic strain ORS 278 an enlargement of the root primordia is macroscopically observed simultaneously when nitrogen fixation begins.

From the root primordia, infection rapidly seems to progress in the stem cortex. Continuous horizontal stem meristematic activity then forms a collar all around the stem. Later, the collar becomes enlarged and a senescent zone appears in its middle. Finally two collar nodules are formed on each side of the senescent central zone. A cytological study of this new type of nodule is being performed in our laboratory.

CONCLUSIONS

We have shown that the ability to form bacteriochlorophyll a and photosynthetic reaction centers was a common feature to most bradyrhizobial strains isolated from stem-nodulated species of *Aeschynomene*. These photosynthetic strains formed three protein gel electrophoretic clusters, of which the mutual relationships and the relationships with the other *Bradyrhizobium* subclusters has to be unraveled further. Diversity of the strains was confirmed by their carotenoid content.

Furthermore, a new stem collar nodule was described which allows the same light intensity for all infected cells. Perhaps such aerial nodule structure could explain how, during evolution, photosynthetic capability of the rhizobia have been retained in the symbiotic association.

Interestingly, photosynthetic *Bradyrhizobium* strains appeared the most effective for nodulation and nitrogen fixation of the stem-nodulated species such as *Aeschynoneme afraspera* which, like *Sesbania*

rostrata, is widely used as green manure in paddy-fields. Studies under way in our laboratory should determine whether this higher nitrogen fixing activity is due to the close association of photosynthesis and N_2-fixation.

REFERENCES

Alazard D (1985) Appl. Env. Microbiol. 50, 732-734.

Alazard D and Duhoux E (1988) J. Plant Physiol. 132, 123-125.

Dreyfus BL and Dommergues YR (1981) FEMS Microbiol. Lett. 10, 313-317.

Dreyfus B et al (1988) Int. J. Syst. Bacteriol. 38, 89-98.

Evans WR et al (1990) Appl. Environ. Microbiol. 56, 3445-3449.

Hollis AB et al (1981) J. Gen. Microbiol. 123, 215-222.

Kersters K (1985) In Society for General Microbiology, eds, Computer-assisted bacterial systematics.

Kuykendall LD et al (1992) Can. J. Microbiol. 38, 501-505.

Ladha JK et al (1988) In The International Rice research Institute, eds, Green manure in rice