Cross Inoculation with Coronilla and Other Rhizobium, Bradyrhizobium Strains Among Crownvetch and Different Legumes

B. BIRÓ, S. TIMÁRI and M. KECSKÉS

Department of Microbiology, University of Agricultural Sciences, Gödöllő /HUNGARY/

Based on early investigations of Rhizobium-legume symbiosis which focused on agriculturally important crops in temperate climates, the concept of cross-inoculation groups was introduced /FRED et al., 1932/. So the Rhizobium classification became controversial because it is based on properties of isolates from less than 10% of known legumes /JORDAN and ALLEN, 1974; VINCENT, 1974/. The genus was divided into two major groups /fast and slow growers/ according to morphological, physiological, symbiotic and serological properties and six species were generally considered to be sufficently unique.

The classification of strains of Coronilla varia L. rhizobia is frequently problematical, and modern serological techniques have been used to resolve difficulties /PANKHURST, 1979; VINCENT, 1974/. VAN SCHREVEN /1972/ states that from 8 Rhizobium strains symbionts of different leguminous plants, cross-inoculation was only found in Onobrychis viciaefolia /sainfoin/, JARVIS et al. /1982/ reported that Rhizobium loti should be placed in a seventh species and could form a symbiosis with crownvetch /and sainfoin too/, but many nodule-forming bacteria still have not been studied or grouped

This paper presents results of investigations carried out to determine some taxonomically useful properties of Coronilla Rhizobium strains and the possibility of cross-inoculation among some Hungarian legumes, because it is a necessity to classify the genus Rhizobium on the basis of isolates from a wider range of naturally occurring symbioses.

Materials and methods

Light chamber experiments

Leguminous plant - Seeds of the crownvetch /Coronilla varia L./ var. Kompolti were surface sterilized with 0.1% /wt/vol/ acidified $HgCl_2$ /VINCENT, 1970/, rinsed and germinated on 1.0% /wt/vol/ water agar at room temperature.

Bacterial strains - Rhizobia belonging to the early 6 species were maintained at 28 °C on yeast mannitol agar /YMA/ /NUTMAN, 1946/. Some available information on the strains is given in Table 1.

Table 1

Available information on Rhizobium strains inoculated to Coronilla varia plants

Rhizobium species	lab. sign.	Place of origin	Plant
R. leguminosarum	1012	Rothamsted	Pisum sativum
	baltacim 3	GATE	Onobrychis viciaefolia
	bükköny 75/4	GATE	Vicia sativa
	lóbab Z	GATE	V. faba
R. meliloti	2012	Rothamsted	Medicago sativa
	Lu K	GATE	M. sativa
R. phaseoli	bab 5/3	GATE	Phaseolus vulgaris
	NA 575	Melbourne	P. vulgaris
R. trifolii	ló 73/3	GATE	Trifolium pratense
	TA 1	Melbourne	T. subterraneum
R. lupini	Csf 75/1	GATE	Lupinus albus
	3203	Rothamsted	L. luteus
R. japonicum	NA 611	Melbourne	Glycine max
	g/3	GATE	G. max

Place of origin:

Rothamsted Experimental Station; GATE = Agricultural University, Gödöllő; University of Melbourne

Plant culture - The plants were cultured under bacteriologically controlled conditions growing entirely within test tubes /180 mm x 20 mm/ on a minus nitrogen seedling agar slope /GIBSON, 1967/. After one week they were inoculated with 10^6 to 10^7 bacteria. Uninoculated tubes were included with and without 0.05% KNO_3 in the agar. All experiments were performed with 6 replications in light chamber.

Greenhouse experiments

Leguminous plants - Anthyllis vulneraria, Astragalus glyciphyllos, A. cicer, Coronilla varia, C. emerus, Cicer arietinum, Doronicum germanicum, Glycine max, Lathyrus niger, L. tuberosus, L. sativus, Lotus corniculatus, Lupinus albus, L. luteus, L. polyphillos, Medicago sativa, M.falcata, M. lupulina, Melilotus albus, M. officinalis, Ononis spinosa, Onobrychis viciaefolia, Phaseolus vulgaris, Pisum sativum, Tetragonolobus siliquosus, Trifolium repens, T. hybridum, T. incarnatum, T. pratense, T. arvense, Vicia faba, V. cracca, V. sativa, V. sepium, V. pannonica.

Seeds of these wild legumes were collected in the field a year before use and were surface sterilized /VINCENT, 1970/.

Bacterial strains - Two strains of Coronilla Rhizobia /K_{59} and K_{111}/ were isolated from the nodules of crownvetch grown in a chernozem brown forest soil at Kompolt. Five-day-old pure culture on YMA at 28 °C were examined for motility and Gram staining by light microscopy /VINCENT, 1970/.

Plant culture - The plants were grown in a glasshouse for 8 weeks after inoculation in washed quartz sand at 10-23 °C. They were supplied with nitrogen-free Bond-Chrone nutrient solution /MANNINGER and BAKONDI-ZÁMORY, 1970/. Uninoculated nitrogen controls received nitrogen as ammonium nitrate. Each treatment was carried out with 3 replications.

Results and discussion

Morphological and cultural properties of Coronilla varia isolates

In pure culture we have and maintain 15 Coronilla Rhizobium strains, isolated from the nodules of crownvetch grown on a chernozem brown forest or sandy soil with different nitrogen content at Kompolt on a calcareous chernozem brown forest soil at Gödöllő and on a brown forest soil at Szajla.

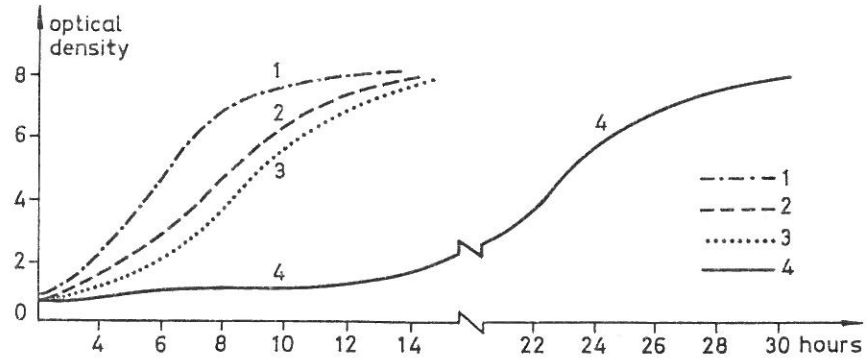

Fig. 1
Biophotometric examination on the growing of Coronilla rhizobia and a Bradyrhizobium japonicum strain. Strains: 1. K_{59}; 2. Sz_1; 3. G_1 Coronilla 4. Bradyrhizobium japonicum 157

All these isolates were motile, gram-negative rods, arranged singly on YMA. Isolates grew fast /Fig. 1/. Colonies were white to cream, circular, convex and had entire edges.

This behavior /and some biochemical characteristics/ also show that the Coronilla Rhizobia belongs to the Rhizobium genus, distinguishing it from the member of slow-growing Bradyrhizobium genus /JORDAN, 1982/.

Nodulation of different Rhizobia on Coronilla varia plants

Among the twelve tested Rhizobium strains nodulation occurred not only with sainfoin Rhizobia /R. leguminosarum baltacim 3 strain/ but the R. meliloti 2012 strain too, which had been isolated from lucerne.

"Baltacim 3" strain was isolated from sainfoin /Onobrychis viciaefolia/, thus in conformity with VAN SCHREVEN /1972/, we found that there may be cross-inoculation between crownvetch and sainfoin. We tested two Rhizobium meliloti strains, but only one, strain 2012 /originating from the Rothamsted Experimental Station/ could form a symbiosis on crownvetch. MÁTÉ /1984/ also found nodules on Coronilla a year after lucerne was cultivated.

Nodulation of Coronilla Rhizobium strains on different legumes

Table 2 shows, that from the 37 leguminous plants Coronilla Rhizobium strains could inoculate the Lotus corniculatus, Medicago sativa, M.falcata, Melilotus albus, Onobrychis viciaefolia and Phaseolus vulgaris plants.

Table 2

Cross-inoculation data between Coronilla Rhizobia and different leguminous plants

Leguminous plants	inoculated with Coronilla rhizobia strains	
	K_{59}	K_{111}
Lotus corniculatus	+++	++
Medicago sativa	++	+
M. falcata	+	+
Melilotus albus	++	++
Onobrychis viciaefolia	+++	+++
Phaseolus vulgaris	++	++

Only those plants are listed where nodulation occurred

\+ nodules in one pot, ++ nodules in two pots, +++ nodules in three pots

VAN SCHREVEN /1972/ also tested Medicago sativa and Vicia sativa plants, but he didn't find any nodules on lucerne, only GREENWOOD /1970/ reported them on Lotus corniculatus. He and JARVIS et al. /1982/ also state that Coronilla Rhizobium may be put into a new Rhizobium loti species, but it is still needed to examine more Rhizobia and leguminous plants.

In legume-rhizobium symbiosis the problem is the symbiotic effectivity /and not the nodulation/. In nature, strains have the ability to nodulate more than one legume species. As we have seen in our present study, leguminous species were often well nodulated in these tests, nodules were often ineffective, and the effectivity may be variable with the host species. This demonstrates the usefulness of internal antigens in the classification of fast- and slow-growing isolates, and the necessity to select rhizobia for inocula on the basis of their effectiveness with the proposed host plant.

References

FRED, E.B., BALDWIN, L.L and McCOY, E., 1932. Root nodule bacteria and leguminous plants. University of Wisconsin, Studies in Science No.5.

GIBSON, A.H., 1967. Carbon dioxide limitations to plant growth in tube culture, with special reference to legume nodulation studies. Aust. J. Biol. Sci. 20. 837-842.

GREENWOOD, R.H., 1970. Host Rhizobium associations and possible evolutionary relationships. Rhizobium Newsletter /Australia/ 15. 208-214.

JARVIS, B.D.W., PANKHURST, D.E. and PATEL, J.J., 1982. Rhizobium loti, a new species legume root nodule bacteria. Internat. J. System. Bacteriol. 32. 378-380.

JORDAN, D.C., 1982. Transfer of Rhizobium japonicum Buchanan 1980 to Bradyrhizobium gen. nov., a genus of slow-growing, root-nodule bacteria from leguminous plants. Internat. J. System. Bacteriol. 32. 136-139.

JORDAN, D.C. and ALLEN, O.N., 1974. Family III. Rhizobiaceae Conn 1938. In: Bergey's manual of determinative bacteriology. /Eds.: BUCHANAN,R.E., and GIBBONS, N.E./. 8th ed. 261-267. The Williams and Wilkins Co., Baltimore.

MANNINGER, E. and BAKONDI-ZÁMORY, É., 1970. Pillangósvirágú növények rhizobium oltása. Agrokémia és Talajtan Kiskönyvtára. 2. Research Inst. Soil Sci. Agric. Chem. of the Hung. Acad. Sci., Budapest.

MÁTÉ, A., 1984. A tarka koronafürt /Coronilla varia L./ termését meghatározó tényezők vizsgálata. Kandidátusi értekezés, Gödöllő.

NUTMAN, P.S., 1946. Variation within strains of clover nodule bacteria in the size of the nodule produced and in the "effectivity" of the symbiosis. J. Bacteriol. 51. 411-432.

PANKHURST, C.E., 1979. Some antigenic properties of cultured cell and bacteroid forms of fast- and slow-growing strains of Lotus rhizobia. Microbios. 24. 19-28.

VAN SCHREVEN, D.A., 1972. Note on the specificity of the Rhizobia of crownvetch and sainfoin. Pl. Soil, 36. 325-330.

VINCENT, J.M., 1970. A manual for the practical study of root-nodule bacteria. Blackwell Scientific Publications. Oxford.

VINCENT, J.M., 1974. Root-nodule symbioses with Rhizobium. The biology of nitrogen fixation /Ed. QUISPEL, A./ 226-341. North-Holland Elsevier, Amsterdam.