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Relationships Among Rhizobia from Native Australian Legumes

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Isolates from 12 legumes at three sites in Victoria showed a wide range of morphological, cultural, symbiotic, and serological properties. Isolates from *Acacia longifolia* var. *sophorae* and *Kennedia prostrata* were fast growing but nodulated ineffectively *Macroptilium atropurpureum* and all native legumes except *Swainsonia lessertiifolia*. Isolates from *S. lessertiifolia* showed anomalous properties intermediate between fast- and slow-growing rhizobia. All isolates from the other two sites were slow-growing "cowpea" rhizobia. Symbiotic effectiveness was usually poor, and there was no relationship between effectiveness and host taxonomy or serological affinities of the isolates. This is the first report of fast-growing rhizobia from temperate Australian woody legumes and the first report of the symbiotic effectiveness of native Australian legumes with indigenous rhizobia.

Rhizobium classification is tentative and controversial since it is based on properties of isolates from less than 10% of known legumes (11, 25). The genus is divided into two major groups (fast and slow growers) on morphological, physiological, symbiotic (cross-inoculation), and serological properties. The classification of strains of *Rhizobium phaseoli* Dangeard, "*Lotus*" rhizobia, and "cowpea" rhizobia is frequently problematical (11, 26), and modern serological techniques have been used to resolve difficulties (17, 25).

Most cowpea rhizobia are slow growing and nonspecific in nodulation and nodulate the test host *Macroptilium atropurpureum* (Benth) Urb cv. Siratro (25). However, isolates from many woody legumes, presumed to be hosts for cowpea rhizobia, do not conform to established properties of this group (6, 8, 10, 22, 23).

Legumes, especially woody species, are important in maintaining ecosystem fertility and are used in soil stabilization and revegetation programs (6, 13). Although early studies (7, 12) showed that most isolates from native Australian legumes were slow-growing cowpea rhizobia on cultural and cross-inoculation properties, there were some problems in classification, modern serological tests were not available, and no reciprocal cross-inoculation tests were performed with native legume hosts and their isolates. It is essential to characterize and rate for effectiveness the rhizobia from native Australian legumes to elucidate reasons for the relatively low levels of nitrogenase activity in the field (13, 14) and to evaluate the worth of artificial inoculation.

This paper reports on investigations to determine some taxonomically useful properties of isolates and the effectiveness of symbioses in 12 wild legumes from three sites in Australia (14).

MATERIALS AND METHODS

Isolation of rhizobia. Rhizobia were isolated from nodules (24) and maintained at 28°C on yeast mannitol agar (YMA) (16). Isolates were from native Australian legumes and sites studied previously (14), as well as *Swainsonia lessertiifolia* DC and the introduced *Melilotus indica* (L) All., both common at Gunnamatta. Abbreviations for isolates are: Mi3, *M. indica*; Sl1, *S. lessertiifolia*; Kp4, *Kennedia prostrata* R. Br.; As1, *Acacia longifolia* var. *sophorae* (Labill) F. Muell; Am1, *Acacia mearnsii* De Wild; Ax2, *Acacia melanoxylon* R. Br.; Ap1, *Acacia paradoxa* DC; Ao1, *Acacia oxycedrus* Sieber ex DC; Ae1, *Aotus ericoides* (Vent) G. Don; Bc1, *Bossiaea cinerea* R. Br.; Ds1, *Dillwynia sericea* A Cunn; and Po2, *Platylobium obtusangulum* Hook. In addition, 10 isolates (Kp5 through Kp14) were made from *K. prostrata* at Cranbourne.

Morphological and cultural characteristics of isolates. Cultures of strains TA1 (*Rhizobium trifolii* Dangeard), U45 (*Rhizobium meliloti* Dangeard), and CB756 (*Rhizobium* sp., cowpea group), gifts from B. Humphrey, were included in all tests. All tests were performed at least twice.

Cultures five days old on YMA at 28°C were examined for motility and Gram staining by light microscopy (24) and for size by electron microscopy (5).

Bacterial suspensions were dilution plated to give single colonies at 28°C on agar-solidified media, namely, YMA, with and without 0.0025% bromothymol

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TABLE 1. Morphological and cultural properties of isolates

Character	Data for isolate:											
	Gunnamatta				Lysterfield			Cranbourne				
	Mi3	Sl1	Kp4	As1	Am1	Ax2	Ap1	Ao1	Ae1	Bc1	Ds1	Po2
Cell dimensions												
Length (μm)	1.7	0.8	1.7	2.8	2.7	2.9	2.4	3.5	2.8	2.8	2.1	2.8
Width (μm)	0.7	0.7	0.9	1.0	0.8	0.7	0.6	0.8	0.7	0.7	0.7	0.7
Ratio (length:width)	2.4	1.1	1.9	2.8	3.4	4.1	4.0	4.4	4.0	4.0	3.0	4.0
Colony diam (mm) on YMA												
4 days	0.3	ND[a]	1.5	0.8	ND	ND	ND	ND	ND	ND	ND	ND
8 days	1.0	0.1	2.0	1.5	0.5	0.6	0.2	0.2	0.2	0.1	0.1	0.2
Color of bromothymol blue-YMA (4 days)[b]	Y	Y	Y	Y	Y	B	B	B	B	B	B	B
Litmus milk (28d)[c]												
Reaction	Ac	Alk	Ac	NC	NC	NC	NC	NC	NC	Alk	NC	NC
Serum zone	+	−	+	−	−	−	−	−	−	−	−	−
Precipitation in calcium glycerophosphate (8 weeks)	+	+	+	+	−	−	−	−	−	−	−	−
Utilization of carbon sources (14 days)[d]												
Mannitol	4	2	4	4	4	4	3	3	3	2	2	3
Sorbitol	2	−	3	3	1	2	1	1	1	1	1	1
Fructose	1	1	4	1	1	2	−	1	1	1	−	1
Galactose	3	−	2	3	2	1	2	3	2	1	1	2
Glucose	3	1	2	4	3	2	1	2	1	2	1	2
Xylose	3	−	2	1	−	2	−	1	1	1	−	−
Lactose	3	−	1	2	1	1	2	1	1	1	1	1
Maltose	3	1	3	3	2	1	2	2	2	1	1	1
Sucrose	4	1	3	4	3	2	1	2	2	1	1	1
Raffinose	4	1	1	2	2	1	1	1	1	1	1	1
Total with growth ≥2	9	1	8	8	6	6	4	5	4	2	1	3

[a] ND, Not detectable.
[b] Y, Yellow; B, blue.
[c] Ac, Acid; Alk, alkaline; NC, no change.
[d] Growth scored as absent (−), poor (1), moderate (2), good (3), or abundant (4).

blue (24), calcium glycerophosphate (9), and peptone glucose (24). Colony size, shape, and elevation were scored daily for 14 days.

Utilization of carbon sources was tested with isolated colonies on a basal medium of YMA without mannitol and with only 0.1 g of yeast extract per liter and 10 g of 10 filter-sterilized carbon sources per liter (Table 1). Growth at 28°C was graded after 14 days on a scale of 1 to 4 from poor to abundant.

Tubes of litmus milk at 28°C were inoculated with 10^6 bacteria and scored weekly for four weeks (24).

Production of 3-ketolactose (2) was tested, and the Nile Blue test (18) was performed.

Collection and germination of seeds. Seeds of wild legumes were collected in the field in the year before use. Scarified seeds of *K. prostrata*, *M. atropurpureum* cv. Siratro, and *Medicago sativa* L cv. Hunter River were surface sterilized with 0.1% (wt/vol) acidified $HgCl_2$ (24), rinsed, and germinated on 1.0% (wt/vol) water agar at 22°C. Seeds of all other species were surface sterilized with concentrated H_2SO_4, rinsed, and germinated as above.

Cross-inoculation experiments. All cross-inoculation experiments were performed twice, with 10 plants per treatment. Seedlings were planted on minus nitrogen seedling agar (22) in glass tubes (25 by 150 mm) with cotton wool bungs and were inoculated with 10^6 to 10^7 bacteria. Uninoculated tubes were included, with and without 0.05% KNO_3 in the agar. Preliminary experiments with different phosphorus levels showed no evidence of phosphorus toxicity (Lawrie and Walker, unpublished observations).

Plants were harvested after 12 weeks (7 weeks for *M. indica*) in a glass house at 15 to 25°C, and the following were measured: total nitrogen (Kjeldahl analysis), dry weight, nodule fresh weight, nodule number per plant, and acetylene-reducing activity (14). Statistical analysis was performed by the Student-Newman-Keuls test (19) after appropriate transformation. Isolates were characterized for symbiotic

effectiveness by the equation effectiveness (%) = [(A − C)/(B − C)] × 100, where A is the mean total nitrogen per plant for inoculated treatment, B is the mean total nitrogen per plant for plus nitrogen controls, and C is the mean total nitrogen per plant for minus nitrogen controls (see Tables 2 and 4). Effectiveness was graded by a slight modification of the method of 'tMannetje (21).

The identity of nodule endophytes was checked by immunodiffusion tests on reisolates from two nodules for each treatment (see below).

Serological affinities. Bacteria were grown for 7 days on a defined medium (1) at 28°C and were suspended (10^9 to 10^{10} cells ml^{-1}) in 0.85% (wt/vol) NaCl. Antisera were prepared in albino rabbits by eight weekly intramuscular infections of 10^9 bacteria of three isolates: As1 (Gunnamatta site), Ax2 (Lysterfield site), and Ao1 (Cranbourne site). Antisera were collected at eight weekly intervals by bleeding from the marginal ear vein. Agglutination tests were performed at 52°C (24) with steamed and unsteamed whole cells. Immunodiffusion tests were performed with broken (sonicated) cells, and results were recorded after 2 weeks (24). Antisera to strains TA1, U45, and CB756 were gifts from B. Humphrey.

RESULTS

Morphological and cultural properties of isolates. All isolates were motile gram-negative rods, arranged singly, measuring 1.7 to 3.5 by 0.7 to 1.0 μm (Table 1). The length:width ratio of isolates from Gunnamatta was less than 2.9. Isolate Sl1 was unusually small and almost coccoid.

On YMA (Table 1), isolates Mi3, Kp4, and As1 grew much faster than other isolates. Colonies were white to cream, circular, convex, and having entire edges. Isolates Kp4 and As1 had more opaque areas set in a milky gum, but all others were evenly and densely opaque.

Acid production was shown in YMA with bromothymol blue (Table 1) by Gunnamatta isolates and Am1, but in litmus milk it was shown only by Mi3 and Kp4 (which also produced clear serum zones).

All Gunnamatta isolates formed precipitates in calcium glycerophosphate agar, whereas others showed no growth even after 8 weeks (Table 1).

Isolates Mi3, Kp4, and As1 gave moderate to abundant growth with 80% or more of the carbon sources tested, whereas growth was poor for Sl1 and all Lysterfield and Cranbourne isolates with most carbon sources other than mannitol (Table 1).

Tests for contaminating agrobacteria were negative for all isolates: there was no production of 3-ketolactose, reduction of Nile Blue, or growth within 3 days on peptone glucose agar.

TABLE 2. Symbiotic effectiveness of cross-inoculation

Host	Variance ratio among isolates[a]	Symbiotic effectiveness (%)[b]											
		Gunnamatta				Lysterfield			Cranbourne				
		Mi3	Sl1	Kp4	As1	Am1	Ax2	Ap1	Ao1	Ae1	Bc1	Ds1	Po2
M. indica	245.46***	54^{e}	–	–	–	–	–	–	–	–	–	–	–
S. lessertiifolia	152.11***	–	92^{E}	–	–	–	–	–	–	–	–	–	–
K. prostrata	0.95^{NS}	–	–	13^{I}	26^{i}	69^{i}	12^{I}	57^{i}	87^{i}	42^{i}	30^{i}	46^{i}	61^{i}
A. longifolia var. *sophorae*	3.70*	–	–	92^{i}	9^{I}	117^{E}	86^{i}	132^{E}	105^{E}	92^{i}	34^{I}	8^{I}	0^{I}
A. mearnsii	2.04^{NS}	–	–	35^{I}	39^{I}	37^{I}	22^{I}	30^{I}	24^{I}	22^{I}	8^{I}	8^{I}	11^{I}
A. melanoxylon	3.50*	–	–	0^{I}	10^{I}	39^{i}	59^{i}	76^{i}	74^{i}	50^{i}	109^{E}	25^{i}	141^{E}
A. paradoxa	2.70^{NS}	–	–	0^{I}	12^{I}	31^{I}	46^{I}	29^{I}	4^{I}	4^{I}	23^{I}	20^{I}	9^{I}
A. oxycedrus	1.39^{NS}	–	–	5^{I}	9^{I}	22^{I}	5^{I}	19^{I}	7^{I}	28^{I}	9^{I}	51^{I}	0^{I}
Aotus ericoides	2.26^{NS}	–	–	9^{I}	9^{I}	17^{I}	19^{I}	11^{I}	9^{I}	9^{I}	21^{I}	17^{I}	11^{I}
B. cinerea	0.83^{NS}	–	–	–	–	12^{I}	0^{I}	–	–	0^{I}	–	–	14^{I}
D. sericea	0.00^{NS}	–	–	0^{I}	–	–	–	0^{I}	–	0^{I}	–	–	–
P. obtusangulum	0.86^{NS}	–	–	2^{I}	–	0^{I}	–	11^{I}	–	–	–	–	0^{I}
M. atropurpureum	65.94***	–	–	0^{I}	0^{I}	130^{E}	173^{E}	66^{i}	19^{I}	37^{I}	12^{I}	0^{I}	146^{E}

[a] Calculated on symbiotic effectiveness data after angular transformation. ***, $P \leq 0.001$; **, $0.001 < P \leq 0.01$; *, $0.01 < P \leq 0.05$; NS, $P > 0.05$.

[b] Calculated as detailed in the text. Abbreviations: –, no nodulation; 0, nodulation but ≤0% effectiveness. Superscript symbols were calculated on nitrogen content (in milligrams) per plant after logarithmic transformation. Level of probability used was $P \leq 0.05$. Symbols: ^{E}, effective (not significantly different from plus nitrogen controls and significantly different from minus nitrogen controls); ^{I}, ineffective (significantly different from plus nitrogen controls and not significantly different from minus nitrogen controls); ^{e}, partially effective (significantly different from both plus and minus nitrogen controls); ^{i}, indeterminate (not significantly different from either plus or minus nitrogen controls).

TABLE 3. Correlation of nitrogen content per plant with other parameters

Host	Correlation coefficient of nitrogen content (mg per plant) with[a]:				
	Plant dry wt (mg)	% Nitrogen (dry plants)	Nodules per plant		C_2H_2 reduction[b]
			Fresh wt	No.	
M. indica	0.99***	−0.44^{NS}	0.98***	0.98***	0.98***
S. lessertiifolia	0.98***	−0.53^{NS}	0.96***	0.96***	0.92***
K. prostrata	0.80***	0.79***	0.28^{NS}	0.49^{NS}	0.55^{NS}
A. longifolia var. *sophorae*	0.72**	0.88***	0.29^{NS}	0.51^{NS}	0.34^{NS}
A. mearnsii	0.74**	0.47^{NS}	0.43^{NS}	−0.06^{NS}	0.52^{NS}
A. melanoxylon	0.85***	0.94***	0.76*	0.67*	0.70*
A. paradoxa	0.94***	0.35^{NS}	0.72*	0.74*	0.62*
A. oxycedrus	0.86***	0.53^{NS}	0.03^{NS}	0.28^{NS}	0.48^{NS}
Aotus ericoides	0.99***	0.46^{NS}	0.51^{NS}	−0.07^{NS}	−0.36^{NS}
B. cinerea	0.99***	0.64*	0.63^{NS}	0.50^{NS}	ND[c]
D. sericea	0.19^{NS}	0.85***	−0.09^{NS}	−0.13^{NS}	ND
P. obtusangulum	0.93***	0.03^{NS}	0.95*	−0.35^{NS}	0.93***
M. atropurpureum	0.87***	0.94***	0.92***	0.63*	0.05^{NS}

[a] Levels of probability are shown as described in Table 2, footnote *a*. All data analyzed after appropriate transformation.

[b] Measured as micromoles of C_2H_2 reduced per gram of nodules (fresh weight) per hour.

[c] ND, Not determined; no C_2H_2 reduction found.

Isolates Kp5, Kp6, and Kp9 resembled Kp4 in morphology and cultural behavior, whereas isolates Kp7, Kp8, and Kp10 through Kp14 resembled Ax2 (data not shown).

Cross-inoculation experiments. (i) Wild legumes. Isolates Mi3 and Sl1 and their hosts showed strict host-isolate specificity and effective nodulation (Table 2). Isolate Mi3 also nodulated *M. sativa*.

Most other hosts and isolates showed reciprocal nodulation (Table 2). Symbiotic effectiveness was generally low; most (73%) of the symbioses were graded as ineffective, 21% as partially effective or indeterminate, and only 6% as effective. Effective nodulation was restricted to *A. longifolia* var. *sophorae* and *A. melanoxylon*, which were the only hosts to show significant differences in symbiotic effectiveness among isolates (Table 2). All combinations of hosts with their own isolates were ineffective, except for *A. melanoxylon* with Ax2, which was indeterminate. The symbiotic effectiveness of host-isolate combinations was not reciprocal among hosts and isolates, and no isolate showed outstanding effectiveness on all hosts.

All hosts except *D. sericea* showed significant correlation coefficients between nitrogen content per plant and dry weight per plant (Table 3), but not between nitrogen content per plant and other measurements, except for *A. melanoxylon*.

(ii) Siratro. All isolates except Mi3 and Sl1

TABLE 4. Symbiotic effectiveness of cross-inoculation on *M. atropurpureum* and *K. prostata*

Isolate used as inoculum	Plant dry wt (mg)[a]	
	M. atropurpureum	*K. prostrata*
Kp4	29.1^{I}	50.4^{I}
Kp5	29.3^{I}	45.8^{I}
Kp6	32.8^{I}	53.8^{I}
Kp7	67.6^{E}	39.7^{I}
Kp8	70.5^{E}	43.1^{I}
Kp9	22.1^{I}	44.5^{I}
Kp10	73.2^{E}	51.7^{I}
Kp11	74.9^{E}	55.1^{E}
Kp12	66.7^{E}	49.1^{I}
Kp13	72.8^{E}	40.9^{I}
Kp14	68.3^{E}	62.5^{E}
Variance ratio among isolates[b]	17.69***	21.2^{NS}
Correlation coefficient of plant dry weight (in milligrams) with:		
Nodule fresh wt	0.88***	−0.01^{NS}
Nodule no.	0.85***	−0.83***

[a] Superscript symbols: ^{E}, effective (significantly different from minus nitrogen controls); ^{I}, ineffective (not significantly different from minus nitrogen controls). Effectiveness was calculated on dry weight (in milligrams) per plant; $P \leq 0.05$.

[b] Calculated on dry weight (in milligrams) per plant after logarithmic transformation. Levels of probability are shown as described in Table 2, footnote *a*.

nodulated *M. atropurpureum* (Table 2), but nodulation was ineffective except with Am1, Ax2, Ap1, and Po2. Nitrogen content per plant was significantly correlated with other measurements, except acetylene reduction (Table 3).

Table 4 shows that isolates Kp5-14 showed significant variation in effectiveness on *M. atropurpureum*, but not on *K. prostrata*, and that effectiveness varied with the host. Dry weight per plant was significantly correlated with both nodule fresh weight and number of nodules per plant in *M. atropurpureum*, but not in *K. prostrata* (Table 4).

Serological affinities. (i) Relationships among wild legume isolates. Antisera to isolates As1 and Ax2 agglutinated only the homologous antigens, at titers of up to 1,600. Antiserum to isolate Ao1 agglutinated both the homologous antigen and isolate Po2 at titers of 1,600.

Cross-reactions between antigens and antisera were wider in immunodiffusion tests than in agglutination tests (Table 5 and Fig. 1 through 3). Each antiserum cross-reacted with all isolates from the same site, except for antiserum to Ao1, which did not cross-react with Kp5, Kp6, or Kp9. Antiserum to As1 from Gunnamatta did not cross-react with isolates from the other two sites, and no reciprocal cross-reactions occurred (Table 5). By contrast, cross-reactions were common between antisera and isolates from the other two sites (Table 5 and Fig. 2 through 3). Nonhomologous cross-reactions were due mainly to thermostable antigens which gave relatively faint bands near the antiserum wells (Table 5, type c). Isolates Ao1 and Po2 were very closely related serologically (Table 5 and Fig. 3). Heating antigen preparations enhanced the clarity of type *a* bands in cross-reactions with Ax2 and Ao1 antisera (Fig. 1 through 3).

Seven isolates (Kp7, Kp8, and Kp10 through Kp14) from *K. prostrata* at Cranbourne cross-reacted strongly with Ax2 antiserum (Fig. 4). The other three isolates (Kp5, Kp6, and Kp9) cross-reacted only with As1 antiserum, giving faint type *c* bands (Fig. 5).

(ii) Relationships with strains TA1, U45, and CB756. Strain TA1 cross-reacted with antiserum to As1 to give faint type *b* and *c* bands (Table 5 and Fig. 1). Strain CB756 cross-reacted similarly with antisera to both Ax2 and Ao1 (Table 5 and Fig. 2 through 3). Antiserum to strain U45 cross-reacted with As1 and Mi3, giving both type *b* and type *c* bands (Table 5 and Fig. 6).

DISCUSSION

Isolate Mi3 (from *M. indica*) was, as expected, a strain of *R. meliloti* (26).

Isolate Sl1 (from *S. lessertiifolia*) was culturally slow-growing (Table 1), specific in nodulation (Table 2), and yet serologically related only to fast-growing rhizobia (Table 5). Similar dispersed anomalous results have been noted in isolates from other *Swainsona* spp. (8, 10) and in other members of the tribe Galegeae B and Hf in Australia, New Zealand, and the United States (3, 4, 15, 27). Isolates from this tribe may represent an intermediate stage between slow-

TABLE 5. Types of precipitin bands formed in immunodiffusion tests

Antigen	Antiserum[a]											
	As1			Ax2			Ao1			U45		
	a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
Mi3	−	+	+	−	−	−	−	−	−	−	+	+
Sl1	−	+	+	−	−	−	−	−	−	−	−	−
Kp4	−	+	+	−	−	−	−	−	−	−	−	−
As1	+	+	+	−	−	−	−	−	−	−	−	+
Am1	−	−	−	−	+	+	−	+	−	−	−	−
Ax2	−	−	−	+	+	+	−	+	+	−	−	−
Ap1	−	−	−	−	+	+	−	+	+	−	−	−
Ao1	−	−	−	−	−	+	+	+	+	−	−	−
Ae1	−	−	−	−	−	+	−	−	+	−	−	−
Bc1	−	−	−	−	−	+	−	−	+	−	−	−
Ds1	−	−	−	−	−	+	−	−	+	−	−	−
Po2	−	−	−	−	−	+	+	+	+	−	−	−
U45 (*R. meliloti*)	−	−	−	−	−	−	−	−	−	+	+	+
TA1 (*R. trifolii*)	−	+	+	−	−	−	−	−	−	NT[b]	NT	NT
CB756 (*Rhizobium* sp.)	−	−	−	−	+	+	−	+	+	NT	NT	NT

[a] Results amalgamated from several replicate experiments. Band types: *a*, strong, thermostable, near antigen well; *b*, thermolabile; *c*, thermostable, near antiserum well.

[b] NT, Not tested.

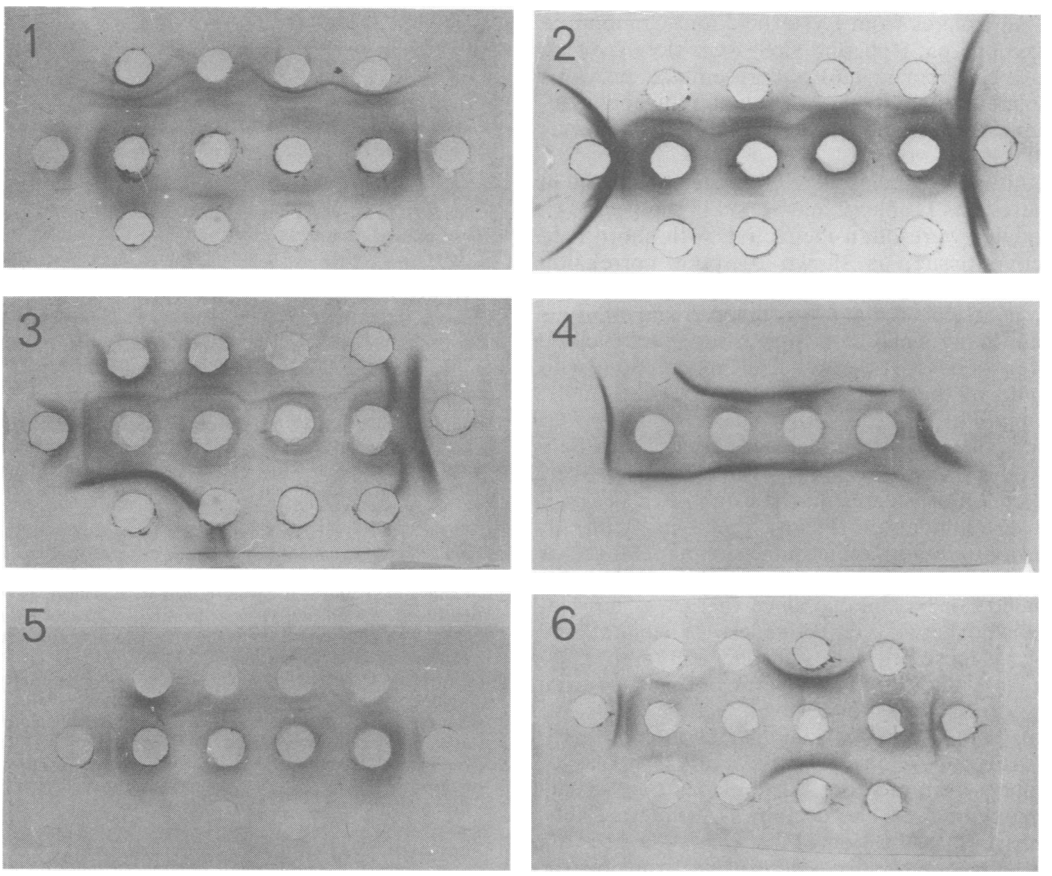

FIG. 1–6. Precipitation bands formed in immunodiffusion assays with cultures in outer wells and antisera in inner wells as detailed below. Except for 4, wells on top row and left side contained broken unheated cells; wells on bottom row and right side contained broken cells steamed for 30 min at 100°C. For 4, all outer wells contained broken cells steamed as above. 1, Top and bottom rows, left to right, TA1, Kp4, Mi3, Sl1; left and right wells, As1; antiserum, As1. 2, Top and bottom rows, left to right, CB756, Bc1, Am1, Ap1; left and right wells, Ax2; antiserum, Ax2. 3, Top and bottom rows, left to right, Po2, CB756, Ae1, Ds1; left and right wells, Ao1; antiserum, Ao1. 4, Top row, left to right, Kp4, Kp7, Kp8, Kp10; bottom row, left to right, Kp14, Kp13, Kp12, Kp11; left and right wells, Ax2; antiserum, Ax2. 5, Top and bottom rows, left to right, Kp4, Kp5, Kp6, Kp9; left and right wells, As1; antiserum, As1. 6, Top and bottom rows, left to right, As1, Kp4, Mi3, Sl1; left and right wells, U45; antiserum, U45.

and fast-growing rhizobia, corresponding to the evolutionary position of the tribe Galegeae between tribes which are hosts for fast- or slow-growing rhizobia (15, 20).

Isolates As1 (from *A. longifolia* var. *sophorae*) and Kp4 through 6 and Kp9 (from *K. prostrata*) were culturally and serologically fast growers (Tables 1 and 5) but were nonspecific in nodulation (Tables 2 and 4) and nodulated *M. atropurpureum* (Table 4). Culturally fast-growing isolates from *Acacia* species have been isolated previously (4, 6, 23), but most have shown some degree of specificity. Culturally fast- and slow-growing isolates are known in the tribe Phaseoleae B and Hf (25), to which the genus *Kennedia* Vent belongs, but these data show that both fast and slow growers may be isolated from nodules on the same plant. The promiscuous but ineffective nodulation of a wide range of legumes means that these isolates resemble most those fast-growing rhizobia from *Leucaena leucocephala* (22) and some other legumes (4). All previous isolates from *Acacia* and *Kennedia* species in Australia were slow growing (7, 12), and this was thought to be due to host selectivity and a preponderance of slow-growing rhizobia in the soils (15, 25). The isolation of these fast-growing isolates from two sites with markedly different soil types indicates that host selectivity may be more important than soil type but that soil type may influence strongly the type of rhizobia most common in the soil (14, 15).

All isolates from Lysterfield and Cranbourne (except Kp5, Kp6, and Kp9) were slow-growing cowpea rhizobia (26) and resembled previous isolates from other Australian indigenous legumes (7, 12) and some African *Acacia* species (6).

Although species were often well nodulated in these tests (Tables 2 and 3) and in the field (14), nodules were often ineffective with short-lived active tissue, as shown by poor correlation between nitrogen content and nodule abundance or acetylene reduction (Tables 3 and 4), thus confirming field data suggesting poor nodule effectiveness (14). Artificial inoculation with some of the isolates might be possible in *A. longifolia* var. *sophorae* and *A. melanoxylon*, since they showed significant variations in effectiveness among isolates (Table 2). However, selection of isolates on the established bases of host taxonomy, symbiotic behavior with *M. atropurpureum*, foliar nitrogen percentage, or serological affinities with effective strains (26) would not be suitable since there was no relationship between effectiveness of isolates and any of these properties (Tables 2, 3, and 5).

The wide range in properties of isolates from only 11 species and seven genera of native Australian legumes demonstrates the need to classify the genus *Rhizobium* on the basis of isolates from a wider range of naturally occurring symbioses, the usefulness of internal antigens in 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.

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