Serological Relationship Between 50 S Ribosomal Subunits from Strains of *Agrobacterium* and *Rhizobium*

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ABSTRACT

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Antisera to 50 S ribosomal subunits of five strains of *Agrobacterium* were developed and tested for specificity against ribosomes from various bacterial species in immunodiffusion gels. Two methods of ribosome extraction were compared. When crude ribosomes from 34 *Agrobacterium* and four *Rhizobium* strains were tested against the five antisera, heterogenous precipitation patterns with multiple bands were produced. In contrast, when purified ribosomes from these strains were tested against the five antisera, a single precipitin band developed that was common to all 38 strains, showing that 50 S ribosomal subunits of *Agrobacterium* and *Rhizobium* are serologically identical. The five antisera did not react with species outside the Rhizobiaceae and were therefore specific to the *Agrobacterium-Rhizobium* group. Any of these antisera could be used in diagnostic tests to determine if an unknown isolate belonged to the *Agrobacterium-Rhizobium* group.

Comparative serology provides valuable information about relationships between prokaryotes (30) and has been helpful for rapid identification of various phytopathogenic bacteria (29). Unfortunately, in the case of *Agrobacterium* most of the serological studies are contradictory. In some reports serological specificity was observed at the species level (18,21,31), whereas in others it was at the subspecies level (2,7,22). Aside from an ambiguous taxonomy, this discrepancy probably arises from the use of antisera developed against a mosaic of different antigens (i.e., whole cells) and the presence of plasmids in *Agrobacterium* spp. that code for additional antigens (1,8,14,32,33).

Stable characters are essential for a practical classification that reflects true similarities among bacteria. Ribosomes appear to be an ideal choice to reveal serological relationships among the agrobacteria because these particles are 1) present in all cellular organisms, 2) simpler antigenically than whole cells, and 3) contain both highly conserved and moderately variable proteins (9). The potential utility of ribosomal serology was demonstrated initially by the development of specific ribosomal antisera (38), and subsequently this method was applied to some bacterial plant pathogens (27,28,34) but not *Agrobacterium*.

The objectives of the present research were to determine the serological specificity of ribosomes of *Agrobacterium* and, investigate the serological relationships between the agrobacteria and the closely related rhizobia. We have found that antisera to 50 S subunits of each of five different *Agrobacterium* strains gave reactions of identity with purified ribosomes from *Agrobacterium* and *Rhizobium* in Ouchterlony double diffusion tests. Furthermore, ribosomes from *Pseudomonas solanacearum*, *P. syringae*, *Xanthomonas campestris*, *Erwinia carotovora*, *Escherichia coli*, *Salmonella typhimurium*, *Bacillus subtilis*, and *Clavibacter michiganense* failed to react with these antisera.

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MATERIALS AND METHODS

Organisms and cultivation. To provide genetic diversity, 34 strains of *Agrobacterium* representing different species and biovars were isolated from different hosts grown in widely diverse geographical regions (Table 1). In addition, 13 strains of closely or distantly related bacterial species were included for comparative purposes (Table 2). The bacteria were maintained on potato-dextrose agar (Difco, Detroit, MI) supplemented with 5% (w/v) calcium carbonate and cultured in 2.8-L Fernbach flasks containing 1.5 L of 523 liquid medium (17) at 27 C on an orbital shaker. Because the yield of cells cultured in 523 was very low for *Agrobacterium* strains U11, K47, CG64, and 6/6, these strains had to be grown in YGP liquid medium (yeast-extract, 0.4%; glucose, 2.0%; peptone, 0.4%; and ammonium sulfate, 0.5%). *Rhizobium* strains were grown in yeast-mannitol liquid medium (36). Cells in exponential-growth phase were harvested by low-speed centrifugation (12,000 *g* for 15 min). The cell-pellets were washed in 0.85% sodium chloride, recentrifuged, and stored at −20 C.

Production of antisera. Five female New Zealand White rabbits were immunized with 50 S ribosomal subunits of *Agrobacterium* strains B6, C58, M63/79, U11, and CG64. These 50 S subunits were prepared by sucrose-gradient centrifugation of ammonium chloride-washed and dissociated 70 S ribosomes as described (28). Before beginning the immunization, preimmune sera were collected from marginal ear veins of each animal. Immunization consisted of intramuscular injections of emulsions prepared from equal volumes of 50 S subunits and incomplete Freund's adjuvant (Difco). Injections of 1.5, 2.5, 3.5, and 4.5 mg of 50 S subunits were administered sequentially at 10-day intervals; concentrations were determined as described (27). The five different antisera were harvested by ear-bleeding 10 and 14 days after the last injection. Antisera from the two bleedings were not combined, even though their serological activity was the same. For comparative purposes an antiserum to 70 S ribosomes of *E. coli*, pooled from six rabbits and prepared by Antibodies Inc. (Davis, CA), was provided by Dr. H. W. Schaup, Department of Biochemistry and Biophysics, Oregon State University.

Preparation of test-antigens. In contrast to the complex procedure required to prepare 50 S ribosomal subunits (used as immunogens for injects), a faster isolation method was needed for

TABLE 1. Source and biovar designation of *Agrobacterium* strains used as antigens

Strain	Species name[a]	Biovar affiliation[b]	Origin	Location	Source
T20/73	*radiobacter*	1	Rose	Oregon	1[c]
K84	*radiobacter*	2	Soil	Australia	7
K30	*tumefaciens*	1	Peach	Australia	7
B6	*tumefaciens*	1	Apple	Iowa	2
C58	*tumefaciens*	1	Cherry	New York	5
G2/79	*tumefaciens*	1	Cottonwood	Oklahoma	1
M63/79	*tumefaciens*	1	Cottonwood	Oklahoma	1
G18/79	*tumefaciens*	1	Poplar	Oklahoma	1
GA001	*tumefaciens*	1	Pecan	Georgia	1
GA002	*tumefaciens*	1	Pecan	Georgia	1
GA012	*tumefaciens*	1	Pecan	Georgia	1
GA015	*tumefaciens*	1	Pecan	Georgia	1
GA105	*tumefaciens*	1	Pecan	Georgia	1
H27/79	*tumefaciens*	1	Rose	Colombia	1
S1/73	*tumefaciens*	1	Lippia	Arizona	1
AB2/73	*tumefaciens*	2	Lippia	Arizona	1
B234	*tumefaciens*	2	INA[d]	California	4
GA003	*tumefaciens*	2	Pecan	Georgia	1
M3/73	*tumefaciens*	2	Birch	Oregon	1
U11	*tumefaciens*	2	Willow	Oregon	1
6/6	*tumefaciens*	3	Grapevine	Hungary	11
Ag63	*tumefaciens*	3	Grapevine	Greece	9
CG48	*tumefaciens*	3	Grapevine	New York	3
CG54	*tumefaciens*	3	Grapevine	New York	3
CG56	*tumefaciens*	3	Grapevine	New York	3
CG64	*tumefaciens*	3	Grapevine	New York	3
K47	*rhizogenes*	2	INA	Australia	7
UCBPP604	*rhizogenes*	2	INA	California	10
A4	*rhizogenes*	2	INA	California	6
RR5	*rubi*	1	Raspberry	Oregon	1
N2/79	*rubi*	2	Raspberry	Oregon	1
TR2	*rubi*	2	Raspberry	Washington	8
NT1	plasmid deficient mutant of C58				
A4R1	plasmid deficient mutant of A4				

[a]Species names based on Bergey's manual (19).
[b]Biovar affiliation based on physiological and biochemical tests (24).
[c]1 = Authors; 2 = R. Baker, Colorado State Univ.; 3 = T. Burr, New York St. Ag. Exp. Station; 4 = J. De Vay, Univ. California, Davis; 5 = R. Dickey, Cornell Univ.; 6 = R. Durbin, Univ. Wisconsin; 7 = A. Kerr, Waite Inst., Australia; 8 = E. Nester, Univ. Washington; 9 = C. Panagopoulos, Greece; 10 = M. Starr, Univ. California, Davis; 11 = S. Süle, Hungary.
[d]Information not available.

TABLE 2. Bacterial species, other than *Agrobacterium*, that were used as antigens

Species	Strain	Origin	Source
Rhizobium meliloti	YA15	INA[a]	2[b]
Rhizobium leguminosarum	128A12	INA	9
Rhizobium leguminosarum bv. *trifolii*	162S7a	INA	9
Rhizobium leguminosarum bv. *phaseoli*	127K12b	INA	9
Pseudomonas solanacearum	51	Potato	8
Pseudomonas syringae pv. *syringae*	B3	Peach	4
Xanthomonas campestris pv. *campestris*	B24	Broccoli	1
Erwinia carotovora subsp. *carotovora*	EC105	INA	7
Escherichia coli	CDC01A	Swine	6
Salmonella typhimurium	E26	Mutant	3
Bacillus subtilis	J42	INA	1
Clavibacter michiganense pv. *michiganense*	1	INA	5
Unknown[c]	M9/79	INA	1

[a]Information not available.
[b]1 = Authors; 2 = L. Barber, Oregon St. Univ.; 3 = N. Bigley, Univ. Chicago; 4 = H. English, Univ. California, Davis; 5 = E. Echandi, North Carolina St. Univ.; 6 = W. Ewing, Center for Disease Control, Atlanta; 7 = R. Goodman, Univ. Missouri; 8 = A. Kelman, Univ. Wisconsin; 9 = R. Smith, Nitragin Co., Milwaukee, WI.
[c]This strain was mislabelled in our culture collection as *A. tumefaciens*.

testing ribosomal antigens from multiple bacterial strains against the 50 S subunit antisera. Two methods were compared: the differential centrifugation method developed by Schaad to obtain crude ribosomes (27) and a modification of Kurland's procedure to prepare salt-washed ribosomes (20). In the latter procedure, the cell lysate was centrifuged and ammonium sulfate was added to the supernatant to a concentration of 20% to precipitate nonribosomal proteins. The precipitate was removed by a low-speed centrifugation and the ammonium sulfate concentration of the supernatant was raised to 40%. After another low-speed centrifugation, the ribosome pellet was resuspended in TSM buffer (10 mM Tris base, 3 mM succinic acid, 10 mM $MgCl_2$, 6 mM 2-mercaptoethanol, pH 8). The salt was removed by overnight dialysis against TSM, and the ribosome solution was then adjusted to 0.6 M ammonium sulfate. The ribosomes were pelleted by high-speed centrifugation (3 hr at 180,000 *g*) and subsequently resuspended in TSM. After clarification of the suspension by another low-speed centrifugation, the concentration of ribosomes was derived from absorbance at 260 nm and adjusted to 3 mg/ml as previously described (27).

Gel immunodiffusion. Ouchterlony double-immunodiffusion (25) was used for the serological analysis. Gels were prepared with 8.5 g of NaCl, 7.5 g of SeaKem ME agarose (FMC Bioproducts, Rockland, ME), 2 g of $MgCl_2 \cdot 6H_2O$, 0.2 g of NaN_3, 10 ml of 1% trypan blue solution and 990 ml of distilled water. The suspension was autoclaved and 15-ml aliquots were poured in 100-mm-diameter plastic petri dishes. Wells (3.5 mm diameter) were cut in the gel; the central well was filled with 10–25 μl of antiserum and the outer well was filled with 5–25 μl of test-antigen. The gels were then incubated in a moist chamber at room temperature for 3 days before being read. To investigate the serological relatedness of the 50 S ribosomal subunits from the different bacterial strains tested, crude and purified ribosomes from each strain were reacted with each of the five antisera. Terminology and interpretation of results of immunodiffusion tests have been described (5). Because spur formation in immunodiffusion tests is dependent on the antigen-antibody ratio, the agar diffusion method of Piazzi (26) was used to determine the optimal antigen and antiserum concentrations.

RESULTS

Purified ribosome preparations from 34 *Agrobacterium* and four *Rhizobium* strains reacted identically with all five antisera to 50 S ribosomal subunits of *Agrobacterium* strains B6, C58, M63/79, U11, and CG64 (Table 3). No serological differences were detected between the 50 S ribosomal subunits of the *Agrobacterium* and *Rhizobium* strains. In all instances a single, sharply defined, reproducible precipitin band developed midway between the antigen and antiserum wells (Fig. 1). Furthermore, precipitin band junctions among the antigens were confluent, illustrating the serological identity of the ribosomes from this group of bacteria.

In contrast, when suspensions of crude ribosomes were tested against the same antisera, the number and sharpness of the precipitin bands differed greatly among the strains and spurs developed (Fig. 2). This increase in number of bands with different migration patterns suggested that multiple serological groups existed among the agrobacteria and rhizobia (Table 3). However, the idea of multiple serogroups became questionable when different preparations of crude ribosomes, extracted at different times from the same strains, produced different reactions (Fig. 2D).

The differences in the serological reaction between crude and purified ribosomes were also reflected in their $A_{260/235nm}$ ratios. Crude ribosomes had a variable ratio ranging from 1.0 to 1.7 whereas purified ribosomes had a reproducible ratio of about 1.8.

Antisera to 50 S subunits of *Agrobacterium* were specific to purified and crude ribosomes from the *Agrobacterium-Rhizobium* group; these five antisera did not react with species outside this group (Table 3). Ribosomes from agrobacteria and rhizobia did not react with antiserum to ribosomes of *E. coli*. However, the *E. coli* antiserum did react with ribosomes extracted from *Erwinia carotovora*; both *E. coli* and *Erwinia carotovora* belong to the Enterobacteriaceae.

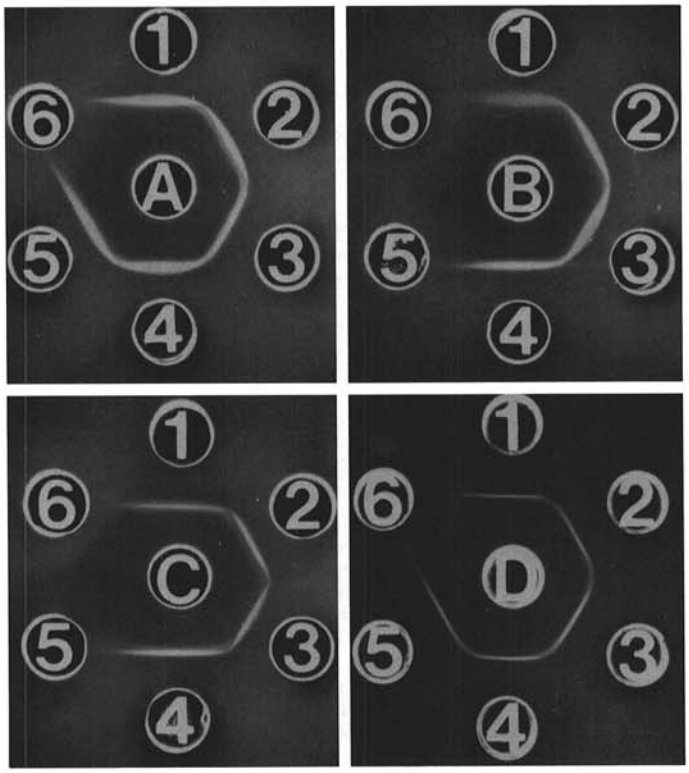

Fig. 1. Immunodiffusion patterns of antisera to 50S ribosomal subunits of *Agrobacterium* against purified ribosomes. Center wells of **A, B, C, and D** contain antiserum, respectively, to M63/79, U11, CG64, and C58. Outer wells of **A** contain M63/79 (1), T20/73 (2), A4 (3), CG56 (4), *Rhizobium meliloti* YA15 (5), and *Clairbacter michiganense* (6). Outer wells of **B** contain U11 (1), K30 (2), CG56 (3), *Rhizobium leguminosarum* 127K12b (4), *Pseudomonas syringae* (5), and M9/79 (identity unknown) (6). Outer wells of **C** contain CG64 (1), Ag63 (2), GA002 (3), AB2/73 (4), *Bacillus subtilis* (5), and *Erwinia carotovora* (6). Outer wells of **D** contain C58 (1), B6 (2), K84 (3), N2/79 (4), A4 (5), and *Pseudomonas solanacearum* (6). This precipitin band is represented by the third subcolumn of each antiserum in Table 3.

DISCUSSION

The 50 S ribosomal subunits from *Agrobacterium* strains of diverse origin and taxonomic affiliation were serologically identical, indicating that these subcellular particles have conserved antigenic components. These data, obtained using purified ribosomes as test-antigens, are in agreement with the reported structural similarities among ribosomes of closely related species (38).

Similarly, 50 S ribosomal subunits of *Agrobacterium* and *Rhizobium* strains were serologically identical in tests with antisera to 50 S ribosomal subunits of *Agrobacterium*, which corroborates the close relatedness between *Agrobacterium* and *Rhizobium* previously demonstrated by different methods of analysis (6,10–13,15,23,31,37,39). The data from the above reports and the present study show that *Agrobacterium* and *Rhizobium* are closer to each other than is reflected by the current nomenclature presented in the most recent edition of Bergey's manual (16), a nomenclature based primarily on pathogenic-symbiotic differentiations.

In contrast to the single homogeneous precipitin band obtained with purified ribosomes, the crude ribosomal preparations resulted in inconsistent and heterogeneous precipitation patterns. The lower $A_{260/235nm}$ ratio in crude ribosomal extracts suggests the presence of nonribosomal proteins, and the wide range in the ratios among crude ribosomal extracts indicates varying amounts of

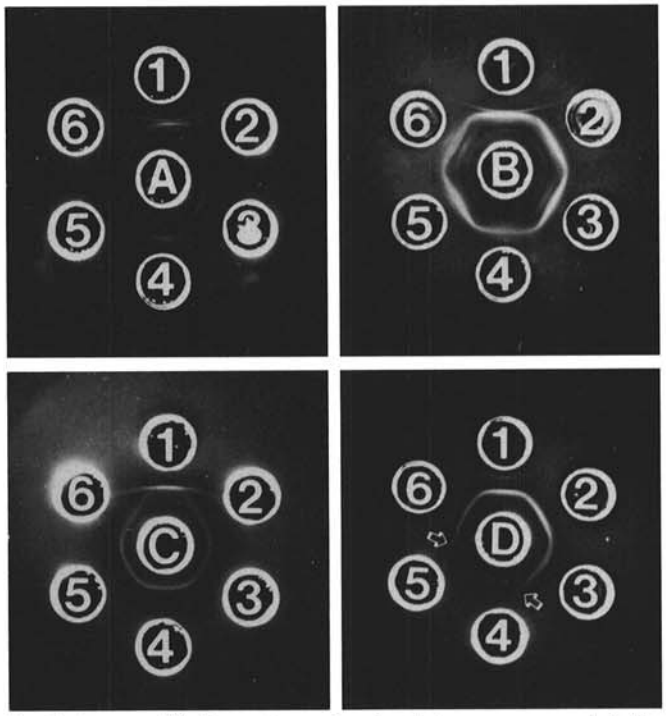

Fig. 2. Immunodiffusion patterns showing heterogeneous precipitation bands when antisera to 50 S ribosomal subunits of *Agrobacterium* were tested against crude ribosomes. Center wells **A and B** contain antiserum to B6, and center wells **C and D** contain antiserum to M63/79. Outer wells of **A** contain B6 (1,4), AB2/73 (2), G2/79 (3), K84 (5), and U11 (6). Outer wells of **B** contain B6 (1), CG48 (2), M63/79 (3), A4 (4), and C58 (5,6). Outer wells of **C** contain M63/79 (1), AB2/73 (2), U11 (3), K84 (4), CG48 (5), and G2/79 (6). Outer wells of **D** contain M63/79 (1) and crude ribosomes extracted from A4 at one time (2,3,6) and another time (4,5). Spur formation (arrows) indicates loss of antigens during the second extraction (4,5), illustrating a lack of reproducibility between different preparations of crude ribosomes. The precipitin band that is continuous between the various *Agrobacterium* and *Rhizobium* strains shown in this figure is identical to the single band shown in Fig. 1 above (data shown in reference 4). Differences in band intensity are due to varying ribosome concentrations in the crude ribosomal preparations.

TABLE 3. Immunodiffusion reactions of antiserum to 50S ribosomal subunits from five *Agrobacterium* strains against crude and purified ribosomes

Antigens		Antiserum to 50S subunits of *Agrobacterium*														
		B6[a]			C58			M63/79			U11			CG64		
Crude ribosomes																
Agrobacterium-Rhizobium:																
B6[b]	(1)[c]	I	I	I	I	I	I	–	I	I	III	III	I	I	I	I
GA105	(1)	III	I	I	III	I	I	–	I	I	III	III	I	III	I	I
S1/73	(2)	III	I	I	III	I	I	–	III	I	III	I	I	–	I	I
M63/79	(1)	–	I	I	I	I	I	I	I	I	III	III	I	I	I	I
C58	(11)	–	I	I	I	I	I	–	I	I	III	III	I	I	I	I
A4	(8)	–	I	I	I	I	I	–	I	I	III	I	I	III	I	I
CG48	(1)	–	I	I		nt[d]		–	–	I	–	–	I	–	I	I
K30	(2)	III	–	I	III	I	I	–	–	I	II	I	I	–	III	I
TR2	(1)	II	III	I	III	I	I	–	I	I	II	I	I	–	I	I
CG54	(1)	–	III	I	III	I	I	–	I	I	III	I	I	–	I	I
U11	(1)	–	–	I	–	I	I	–	–	I	I	I	I	–	III	I
K84	(5)	–	–	I	–	I	I	–	–	I	III	I	I	–	III	I
AB2/73	(1)	–	–	I	III	I	I	–	–	I	III	I	I	–	III	I
G2/79	(1)	–	–	I	III	I	I	–	–	I	II	III	I	–	III	I
128A12	(1)	–	–	I	–	I	I	–	–	I	–	–	I	III	I	I
Others[e]		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Purified ribosomes																
Agrobacterium-Rhizobium		–	–	I	–	–	I	–	–	I	–	–	I	–	–	I
Others		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Key to symbols: I = Reaction of complete fusion, II = noninteraction (precipitin lines cross), III = partial fusion (spur), – = no band of precipitation.

[a] At the optimum antigen-antibody ratio, up to four precipitin bands could be observed with crude ribosomes (Fig. 2), whereas a single band developed with purified ribosomes of the *Agrobacterium-Rhizobium* group (Fig. 1). Three subcolumns are used to illustrate the reaction and position of the three bands nearest the antigen well: left (band nearest the antigen well), middle (intermediate band), and right (band farthest from the antigen well).

[b] Representative strain for a particular serological group (3). Those *Agrobacterium* and *Rhizobium* strains that had the same precipitin patterns were grouped together. Based on the pattern combinations shown in this table, 15 serogroups were identified.

[c] Number in parenthesis is the total number of *Agrobacterium* and *Rhizobium* strains belonging to that particular serological group (footnote b). Strain YA15 is included in the C58 group, whereas strains 127K12b and 162S7a were grouped with A4.

[d] Not tested.

[e] *Pseudomonas solanacearum, P. syringae, Xanthomonas campestris, Erwinia carotovora, Escherichia coli, Salmonella typhimurium, Bacillus subtilis, Clavibacter michiganense,* and M9/79 (identity unknown).

these impurities. The presence of contaminants in crude ribosomes is probably responsible for the inconsistency in the precipitin patterns of crude ribosomes. This serological diversity was eliminated by removal of nonribosomal proteins during the purification process in the presence of ammonium sulfate. The presence of nonspecifically bound contaminant on ribosomes extracted by Schaad's method (4) probably explains the unexpected reaction of ribosomal antisera with whole cells of *Xanthomonas* in immunofluorescence staining (28) and the production in gel immunodiffusion of a common specific band between ribosomes and fixed whole cells (34). This specific band was reported to be a membrane glycoprotein (35). It will be of interest to know if this glycoprotein is equivalent to our contaminant; however, isolation and purification of the contaminant in our preparation is still in the preliminary stage.

The importance of using purified ribosomal particles to prepare an antiserum is emphasized in our study. However, obtaining pure ribosomes for immunization is the major drawback of ribosomal serology. Once antisera to purified ribosomes are available, crude ribosomes, which are extracted faster, may be preferred as test-antigens. The value of ribosomal serology lies in the uniqueness of bacterial ribosomes which elicit specific antisera. The present data demonstrate that 50 S ribosomal subunits are serologically identical and conserved in the *Agrobacterium-Rhizobium* group. Furthermore, antigenicity of the 50 S subunits was not altered by the presence or absence of plasmids as observed when whole cells were used as immunogens (8,14,33). In contrast, 50 S ribosomal subunit antisera provides a reliable tool for identification of strains of the *Agrobacterium-Rhizobium* group isolated from nature.

LITERATURE CITED

1. Banerjee, D., Basu, M., Tiwari, R. K., and Chatterjee, G. C. 1983. Evidence for the presence of an antigenically distinct protein in outer membrane of virulent *Agrobacterium tumefaciens* cells. Folia Microbiol. 28:87-90.
2. Berquist, K. R., and Elrod, R. P. 1948. The somatic antigens of the genus *Agrobacterium*. Proc. SD Acad. Sci. 27:104-111.
3. Bouzar, H. 1983. A survey of *Agrobacterium* strains associated with Georgia pecan trees and an immunological study of the bacterium. M.S. thesis. Oregon State University.
4. Bouzar, H., Moore, L. W., and Schaup, H. W. 1986. Importance of ribosome purity in ribosomal serology. Phytopathology 76:(In press).
5. Chaparas, S. D., Lind, A., Ouchterlony, Ö., and Ridell, M. 1983. Terminology guidelines for serotaxonomic studies using immunodiffusion and immunoelectrophoresis. Int. J. Syst. Bacteriol. 33:414-416.
6. De Smedt, J., and De Ley, J. 1977. Intra- and intergeneric similarities of *Agrobacterium* ribosomal ribonucleic acids and cistrons. Int. J. Syst. Bacteriol. 27:222-240.
7. Digat, B. 1978. Antigen specificity in *Agrobacterium radiobacter* var. *tumefaciens*. Pages 321-326 in: Proc. 4th Int. Conf. Plant Path. Bact., Angers, France.
8. El-Kady, S., and Süle, S. 1982. Serological comparison between strains of *Agrobacterium radiobacter*. Acta Phytopathol. Acad. Sci. Hung. 17:1-6.
9. Geisser, M., Tischendorf, G. W., and Stoffler, G. 1973. Comparative immunological and electrophoretic studies on ribosomal proteins of Bacillaceae. Mol. Gen. Genet. 127:129-145.
10. Gibbins, A. M., and Gregory, K. F. 1972. Relatedness among *Rhizobium* and *Agrobacterium* species determined by three methods of nucleic acid hybridization. J. Bacteriol. 111:129-141.
11. Graham, P. H. 1964. The application of computer techniques to the taxonomy of root-nodule bacteria of legumes. J. Gen. Microbiol. 35:511-517.
12. Graham, P. H. 1971. Serological studies with *Agrobacterium radiobacter, A. tumefaciens,* and *Rhizobium* strains. Arch. Mikrobiol. 78:70-75.
13. Heberlein, G. T., De Ley, J., and Tijtgat, R. 1967. Deoxyribonucleic acid homology and taxonomy of *Agrobacterium*, *Rhizobium*, and *Chromobacterium*. J. Bacteriol. 94:116-124.
14. Hochster, R. M., and Cole, S. E. 1967. Serological comparisons between strains of *Agrobacterium tumefaciens*. Can. J. Microbiol. 13:569-572.
15. Holmes, B., and Roberts, P. 1981. The classification, identification and nomenclature of agrobacteria. J. Appl. Bacteriol. 50:443-467.

16. Jordan, D. C. 1984. Rhizobiaceae Conn 1938. Pages 234-256 in: Bergey's Manual of Systematic Bacteriology, Vol. 1, N. R. Krieg, ed. Williams & Wilkins Co., Baltimore.
17. Kado, C. I., and Heskett, M. G. 1970. Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. Phytopathology 60:969-976.
18. Keane, P. J., Kerr, A., and New, P. B. 1970. Crown gall of stone fruit. II. Identification and nomenclature of *Agrobacterium* isolates. Aust. J. Biol. Sci. 23:585-595.
19. Kersters, K., and De Ley, J. 1984. *Agrobacterium* Conn 1942. Pages 244-254 in: Bergey's Manual of Systematic Bacteriology, Vol. 1, N. R. Krieg, ed. Williams & Wilkins Co., Baltimore.
20. Kurland, C. G. 1966. The requirement for specific sRNA binding by ribosomes. J. Mol. Biol. 18:90-108.
21. Lopez, M. 1978. Characteristics of French isolates of *Agrobacterium*. Pages 233-237 in: Proc. 4th Int. Conf. Plant Path. Bact., Angers, France.
22. Miller, H. J., and Vruggink, H. 1981. An assessment of biochemical and serological tests for *Agrobacterium radiobacter* subsp. *tumefaciens*. Phytopath. Z. 102:292-300.
23. Moffett, M. L., and Colwell, R. R. 1968. Adosonian analysis of the Rhizobiaceae. J. Gen. Microbiol. 51:245-266.
24. Moore, L. W., Anderson, A., and Kado, C. I. 1980. *Agrobacterium*. Pages 17-25 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria. N. W. Schaad, ed. Amer. Phytopathol. Soc., St. Paul, MN.
25. Ouchterlony, Ö. 1948. Antigen-antibody reactions in gels. Ark. Kemi, Mineral. Geol. Bd 26B:1-9.
26. Piazzi, S. E. 1969. A simple method for preliminary immunodiffusion test of antigen-antibody systems having unknown ratios or reaction. Anal. Biochem. 27:281-284.
27. Schaad, N. W. 1974. Comparative immunology of ribosomes and disc gel electrophoresis of ribosomal proteins from erwiniae, pectobacteria, and other members of the family Enterobacteriaceae. Int. J. Syst. Bacteriol. 24:42-53.
28. Schaad, N. W. 1976. Immunological comparison and characterization of ribosomes of *Xanthomonas vesicatoria*. Phytopathology 66:770-776.
29. Schaad, N. W. 1979. Serological identification of plant pathogenic bacteria. Annu. Rev. Phytopathol. 17:123-147.
30. Schleifer, K. H., and Stackebrandt, E. 1983. Molecular systematics of prokaryotes. Annu. Rev. Microbiol. 37:143-187.
31. Schroth, M. N., Weinhold, A. R., McCain, A. H., Hildebrand, D. C., and Ross, N. 1971. Biology and control of *Agrobacterium tumefaciens*. Hilgardia 40:537-552.
32. Sonoki, S., and Kado, C. I. 1978. Proteins conferred by the virulence-specifying plasmid of *Agrobacterium tumefaciens* C-58. Proc. Natl. Acad. Sci. USA 75:3796-3800.
33. Süle, S., and El-Kady, El-S. 1983. Analysis of plasmid-coded antigens of *Agrobacterium radiobacter* strain K84. Phytopath. Z. 107:92-95.
34. Thaveechai, N., and Schaad, N. W. 1984. Comparison of different immunogen preparations for serological identification of *Xanthomonas campestris* pv. *campestris*. Phytopathology 74:1065-1070.
35. Thaveechai, N., and Schaad, N. W. 1986. Immunochemical characterization of a subspecies-specific antigenic determinant of a membrane protein extract of *Xanthomonas campestris* pv. *campestris*. Phytopathology 76:148-153.
36. Vincent, J. M. 1970. A Manual for the Practical Study of Root-Nodule Bacteria. I.B.P. Handbook No. 15, Blackwell Scientific Publication, Oxford.
37. White, L. O. 1972. The taxonomy of the crown-gall organism *Agrobacterium tumefaciens* and its relationship to rhizobia and other agrobacteria. J. Gen. Microbiol. 72:565-574.
38. Wittmann, H. G., Stoffler, G., Kaltschmidt, E., Rudloff, V., Janda, H. G., Dzionara, M., Donner, D., Nierhaus, K., Cech, M., Hindennach, I., and Wittman, B. 1970. Protein, chemical and serolgocial studies on ribosomes of bacteria, yeast and plants. Fed. Eur. Biochem. Soc. Sympos. 21:33-46.
39. Woese, C. R., Stackebrandt, E., Weisburg, W. G., Paster, B. J., Madigan, M. T., Fowler, V. J., Hahn, C. M., Blanz, P., Gupta, R., Nealson, K. H., and Fox, G. E. 1984. The phylogeny of purple bacteria: The alpha subdivision. System. Appl. Microbiol. 5:315-326.