CROWN GALL OF STONE FRUIT

II.* IDENTIFICATION AND NOMENCLATURE OF *AGROBACTERIUM* ISOLATES

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Summary

Twenty-seven local isolates of *Agrobacterium* and twenty-three isolates supplied by other laboratories were compared by biochemical tests, serology, protein patterns following gel electrophoresis, and by pathogenicity. All isolates fell into two distinct biotypes and this was supported by their serological reactions and by their protein patterns. Biotype 1 contained tumour-inducing, root-proliferating, and non-pathogenic forms; biotype 2 contained both these pathogenic forms but no non-pathogens. We believe the present division of the genus into species based on pathogenicity is untenable. If the genus is to be retained, we propose that it should consist of one species, *A. radiobacter*, with pathogenicity indicated by a varietal epithet and the biotype specified.

I. INTRODUCTION

Bergey's Manual (see Breed, Murray, and Smith 1957) lists seven species of *Agrobacterium*, four of which, *A. tumefaciens*, *A. radiobacter*, *A. rhizogenes*, and *A. rubi*, clearly belong to this genus whether or not they remain as separate species and three of which, *A. gypsophilae*, *A. pseudotsugae*, and *A. stellulatum*, should probably be excluded (DeLey 1968). The first four species are distinguished largely by their pathogenicity and host range. *A. tumefaciens* and *A. rubi* both cause crown gall, but the former has a wide host range while the latter is restricted to *Rubus* spp. *A. rhizogenes* causes root-proliferation in apple and many other plants, and *A. radiobacter* is non-pathogenic.

From galls on stone fruit trees, Kerr (1969*a*) isolated a tumour-inducing form of *Agrobacterium* which did not conform with any previously described species; such isolates he designated *Agrobacterium* sp.

This paper considers the relationship between *Agrobacterium* sp., other local isolates, and cultures of *A. tumefaciens*, *A. radiobacter*, *A. rhizogenes*, and *A. rubi* supplied by other laboratories.

II. MATERIALS AND METHODS

(*a*) *Cultures Tested*

Table 1 lists the cultures tested, their origins, the substrates from which they were isolated, the suppliers, and suppliers' numbers. Cultures were stored in sterile distilled water at 10°C (DeVay and Schnathorst 1963).

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(*b*) *Biochemical Tests*

Isolates Nos. 1, 15, and 34, representatives of *A. radiobacter*, *A. tumefaciens*, and *Agrobacterium* sp. respectively, were subjected to 90 biochemical tests and 28 of these were chosen, on the basis of likely value in differentiation, for testing all isolates. Details of the 28 tests are given below. Unless otherwise stated, light bacterial suspensions were used as inocula. The suspensions were prepared from cultures on yeast–mannitol agar incubated for 48 hr at 26°C.

(1) *Mode of Utilization of Glucose.*—The method of Hugh and Leifson (1953) as modified by Hayward and Hodgkiss (1961) was used.

(2) *Production of Acid from Carbohydrates and Related Materials.*—The method of Hayward (1964) was used for determining acid production from glucose, lactose, rhamnose, sorbose, sucrose, dulcitol, erythritol, glycerol, mannitol, and salicin. Cultures were kept for 21 days.

(3) *Utilization of Nitrogen Sources and Growth Factor Requirements.*—Bacteria from agar slopes were suspended in 10 ml sterile distilled water, centrifuged twice to remove nutrients, resuspended in sterile distilled water, diluted, and plated on agar containing 1% mannitol, inorganic salts, and a source of nitrogen with or without growth factors. Each isolate was plated on four separate media containing either (i) 0·4% $NaNO_3$, (ii) 0·4% $NaNO_3$ and 2 μg biotin per litre, (iii) 0·2% L-glutamic acid and 2 μg biotin per litre, or (iv) 0·2% L-glutamic acid and 0·01% yeast extract. Results were assessed after 3 days. Isolates which did not grow or formed very small colonies were marked negative.

(4) *Utilization of Citrate and Malonate.*—The methods of Simmons (1926)* and Leifson (1933)* respectively were used.

(5) *Growth in Ferric Ammonium Citrate Solution.*—The medium of Hendrickson, Baldwin, and Riker (1934)* was used. Production of a brown surface pellicle was the positive reaction.

(6) *Growth on Glycerophosphate Agar.*—The medium of Riker *et al.* (1930)* was used.

(7) *Growth of Sodium Selenite Agar.*—The medium of Hendrickson, Baldwin, and Riker (1934) was prepared by adding the sodium selenite either before or after autoclaving the other ingredients. Isolates were tested on both variations of the medium.

(8) *Hydrolysis of Starch.*—The method of Dye (1968) for detecting hydrolysis of soluble starch was used.

(9) *Hydrolysis of Gelatin.*—Nutrient gelatin (Oxoid) was stab-inoculated and incubated at 22°C for 21 days.

(10) *Lipolysis.*—The cottonseed-oil method reported by Dowson (1957) was used.

(11) *Catalase.*—The method of Hayward and Hodgkiss (1961) was used.

(12) *Oxidase.*—Twenty-four-hr cultures on nutrient agar supplemented with 1% glucose were used as inocula for the test described by Kovacs (1956).

(13) *Production of Nitrite from Nitrate.*—The method of Hayward and Hodgkiss (1961) was used.

(14) *Absorption of Congo Red and Aniline Blue.*—The methods of Riker *et al.* (1930) were used.

(15) *Oxidation–reduction.*—The method of Hamdi (1969) was used.

(16) *Action on milk.*—Bacto litmus milk was used.

(17) *3-Ketolactose production.*—The method of Bernaerts and DeLey (1963) was used.

(*c*) *Serology*

(1) *Preparation of Antisera.*—Isolates Nos. 1, 15, and 34 were grown in mannitol–nitrate solution consisting of mannitol, 10 g; $NaNO_3$, 4 g; K_2HPO_4, 0·5 g; $MgSO_4.7H_2O$, 0·2 g; NaCl, 0·2 g; biotin, 1 μg; distilled water 1 litre. After 24 hr, cells were separated from the culture medium by centrifugation, washed in normal saline, and recentrifuged twice before being taken up in buffered normal saline (pH 7·2) to give a suspension of approximately 10^8 cells per millilitre. The three isolates were then injected into three separate rabbits, each being given an injection once a week for 3 weeks. The first two injections, of 1 ml, were given subcutaneously

* Several of the isolates could not use inorganic nitrogen. In media marked * the inorganic nitrogen was replaced by or supplemented with 0·2% L-glutamic acid and 0·01% yeast extract.

TABLE 1

THE SOURCES OF 50 CULTURES OF *AGROBACTERIUM* SPP.

Isolate No.	Designation	Isolated from:	Origin	Supplier and Supplier's Number*
1 and 2		*Prunus cerasifera* gall	South Australia	
3		Peach gall	South Australia	
4		Pear gall	South Australia	
5–10		Soil	South Australia	
11	*A. radiobacter*		Canada (A. G. Lockhead: 426)	A.C. Parker (WU 10)
12			Canada (A. G. Lockhead: 590)	A. C. Parker (WU 11)
13				A. C. Parker (WU 80; ICPB: TR1)
14				A. C. Parker (WU 81; ICPB: TR4)
15		Soil	South Australia	
16–19		Peach gall	South Australia	
20 and 21		*Prunus cerasifera* gall	South Australia	
22			Wisconsin	R. Mushin (3)
23			London	R. Mushin (5)
24				A. C. Parker (WU 18)
25			Canada (W. H. Cook: B70)	A. C. Parker (WU 21)
26		Apple gall	Edinburgh (W. Blyth via D. W. Dye: A4)	A. C. Parker (WU 78)
27	*A. tumefaciens*	Poplar gall	Edinburgh A. M. Paton via D. W. Dye: A6)	A. C. Parker (WU 79)
28		Peach gall	New South Wales Forestry Commission (109A)	A. C. Parker (WU 89)
29		Peach gall	New Zealand (D. W. Dye: A1)	A. C. Parker (WU 75)
30		Peach gall	New Zealand (D. W. Dye: A3)	A. C. Parker (WU 77)
31			New Zealand	J. DeLey (NCPPB: 223)
32		Almond gall	Israel (Z. Volcani)	J. DeLey (ICPB: TT133)
33–37	*Agrobacterium* sp.	Peach gall	South Australia	
38–40		Plum gall	South Australia	
41 and 42		Almond gall	South Australia	
43	*A. rubi*			J. DeLey (ICPB: TR2)
44				J. DeLey (ICPB: TR3)
45	*A. rhizogenes*			M. P. Starr (ICPB: TR7)
46				J. DeLey (ICPB: TR101)
47				M. P. Starr (ICPB: TR105)
48				J. DeLey (ICPB: TR107)
49				M. P. Starr (ICPB: TR104)
50				M. P. Starr (ICPB: TR108)

* ICPB, International Collection of Pathogenic Bacteria; NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, England.

and consisted of an emulsion of equal proportions of bacterial suspension and Freund's adjuvant, and the last, of 2 ml of bacterial suspension, was injected intravenously. Ten days after the final injection each rabbit was bled and antiserum obtained by allowing the blood to clot and centrifuging the serum at 4000 *g* for 10 min to remove blood cells.

(2) *Preparation of Antigens.*—Isolates were grown in mannitol–casamino acid solution consisting of distilled water, 1 litre; mannitol, 10 g; casamino acids (Difco), 6·7 g; KH_2PO_4, 0·1 g; $CaCl_2$, 0·2 g; NaCl, 0·2 g; $MgSO_4.7H_2O$, 0·2 g; FeEDTA, 2 ml of a solution containing $FeSO_4.7H_2O$, 0·278 g; Na_2EDTA, 0·372 g; 100 ml distilled water. After 48 hr, cells were washed by centrifugation and then either suspended in saline buffer (10^8 cells per millilitre) for tube agglutination testing or disrupted for 5 min in an MSE ultrasonic disintegrator for gel-diffusion testing.

(3) *Tube Agglutination Test.*—Successive twofold dilutions of antisera were prepared in buffered saline; 0·5-ml aliquots of the various dilutions of antisera were dispensed into Pyrex serology tubes and 0·5 ml bacterial suspension added. Tubes were incubated for 3 hr at 37°C and then left overnight at 4°C before agglutination end-points were determined.

(4) *Gel-diffusion Test.*—The method of Ouchterlony (1961) was used. Undiluted antisera were added to central wells and dense suspensions of ultrasonically disintegrated bacteria to the peripheral wells.

(5) *Fluorescent Antibody Test.*—Antiserum against isolate No. 34 was conjugated with fluorescent dye (Hill and Gray 1967) and stored at −40°C. Bacteria to be tested were smeared on a clean glass slide, heat-fixed, and a drop of labelled antiserum added. The slide was incubated for 45 min at 28°C in a moist chamber, rinsed in several changes of buffered saline (pH 7·5), and blotted dry. Preparations were mounted in carbonate-buffered glycerol [glycerol–water 9 : 1 (v/v); pH 9·5] and examined under oil immersion using transmitted light. Ultraviolet light was provided by a 200-W mercury vapour lamp, matched with a BG38 (4 mm) and two BG12 (5 and 1 mm) primary filters. A Leitz K510 suppression filter was used.

(*d*) *Electrophoresis*

Protein patterns following disk electrophoresis were determined for 41 of the isolates. Most isolates were grown in a liquid medium, as used for preparation of antigens except that casamino acids were replaced by 2·0 g L-glutamic acid and 2·0 μg biotin per litre. Two isolates, Nos. 43 and 44, also required nicotinic acid and calcium pantothenate, both at 100 μg/ml (Starr 1946). Two isolates, Nos. 49 and 50, did not grow satisfactorily without the inclusion of 0·01% yeast extract. Aliquots of 200 ml were dispensed into 1-litre flasks, inoculated, and incubated on a rotary shaker for 2-days. (Preliminary investigation indicated that there was no significant difference in the protein patterns of bacteria harvested after 24 and 60 hr.) Cells were harvested by centrifugation, washed in sterile distilled water, spun down, and stored at −40°C.

For protein extraction, the bacterial paste was thawed and diluted in protein extraction medium (Staples and Stahmann 1963), cooled in an ice-bath, and disrupted by 5 min treatment in an MSE ultrasonic disintegrator. Larger debris was spun down (25,000 *g* for 30 min) and the protein content of the supernatant determined by the biuret method (Gornall, Bardawill, and David 1949).

Disk electrophoresis was carried out by the method of Davis (1964) in an apparatus essentially the same as that described by Ornstein (1964). Each gel was 6 mm in diameter and consisted of 1 cm coarse-pore gel over 5 cm fine-pore gel; approximately 60 μg protein nitrogen were added to each gel. A drop of bromophenol blue was added to the buffer (Tris–glycine, pH 8·3) in the upper (cathode) reservoir and a current of 4 mA per tube applied until the dye marker band had reached 5 mm from the end of the gel. This usually took 1 hr 20 min.

The protein bands were fixed in 12·5% trichloroacetic acid and stained with Coomassie brilliant blue (Chrambach *et al.* 1967). Protein patterns were photographed using transmitted light.

Each isolate was grown, harvested, and tested on two separate occasions.

(*e*) *Pathogenicity*

Most isolates were tested for pathogenicity to tomato (Kerr 1969*a*); exceptions were isolates of *A. rhizogenes* imported under quarantine regulations which forbade glasshouse testing.

III. Results

(*a*) *Biochemical Tests*

All isolates utilized glucose oxidatively, were catalase-positive, produced acid from glucose, rhamnose, sucrose, mannitol, and salicin, could not hydrolyse gelatin or starch, and were not lipolytic. The following tests were extremely variable and could not be used to classify isolates: production of acid from lactose, sorbose, dulcitol, and glycerol, production of nitrite from nitrate, absorption of aniline blue, and the test for oxidation–reduction. Absorption of congo red was not entirely satisfactory because five different categories were distinguished and categorization was subjective and not always easy. Results of the remaining tests divided all isolates into two distinct biotypes, as follows:

	Biotype 1	Biotype 2		Biotype 1	Biotype 2
Erythritol	−	+	Glycerophosphate	+	−
Growth factor requirements	+ or −	+	Selenite	+	−
Citrate	−	+	Oxidase	+	−
Malonate	−	+	3-Ketolactose	+	−
Ferric ammonium citrate	+	−	Litmus milk	Alkaline	Acid

Biotype 1 included isolates 1–28 and 49–50; biotype 2 included isolates 29–48.

Although growth factor requirements did not give a complete differentiation of biotypes, they were very useful. No isolate of biotype 2 could utilize nitrate unless biotin were supplied; a few required L-glutamic acid and biotin; and two, isolates 43 and 44, both authentic cultures of *A. rubi*, required L-glutamic acid and yeast extract. Only two isolates of biotype 1 required growth factors. They were isolates 49 and 50, both supplied cultures of *A. rhizogenes* which required L-glutamic acid and yeast extract.

All isolates grew on our modified Simmons citrate medium but with biotype 1 isolates the medium remained green or turned yellow, whereas biotype 2 isolates turned the substrate blue. Only the latter reaction was considered positive.

The glycerophosphate test was quite specific in distinguishing biotypes 1 and 2 but the results differed from those obtained by Riker *et al.* (1930). All isolates grew well on our modified medium whereas Riker *et al.* (1930) reported that *A. rhizogenes* did not grow. In the original medium, the only source of nitrogen was nitrate, which *A. rhizogenes* cannot utilize (Starr 1946). As a result, the reaction to glycerophosphate was confounded with utilization of nitrate. In our test, a white precipitate around a colony was considered a positive reaction and no precipitate was considered negative. Only three cultures produced a brown diffusible pigment as described by Riker *et al.* (1930). It was of no taxonomic value and certainly did not distinguish *A. tumefaciens* from *A. radiobacter*.

The sodium selenite medium completely inhibited all biotype 2 isolates and supported good growth of biotype 1 isolates, whether the selenite was added before or after autoclaving the other ingredients. This was surprising because our previous unpublished work had shown that many biotype 2 isolates were not inhibited by the same concentration of sodium selenite in a different medium. There must be an interaction between sodium selenite and other ingredients in the medium.

TABLE 2: CHARACTERISTICS OF THE 50 ISOLATES OF *AGROBACTERIUM*

+++, strong reaction; ++, moderate reaction; +, weak reaction; −, no reaction. NP, not pathogenic; TI, tumour-inducing; RP, root-proliferating (not tested by us); n.t., not tested

Isolate	Biotype	Reaction to Antisera of:		Reaction to Fluorescent Antibody of Isolate 34	Protein Patterns Group*	Pathogenicity
		Isolate 15	Isolate 34			
1	1	+++	−	−	A	NP
2	1	+++	−	n.t.	n.t.	NP
3	1	++	−	n.t.	n.t.	NP
4	1	++	−	n.t.	n.t.	NP
5	1	++	−	−	A	NP
6	1	+	−	−	A	NP
7	1	++	−	−	A	NP
8	1	++	−	n.t.	n.t.	NP
9	1	++	−	−	A	NP
10	1	++	−	−	A	NP
11	1	++	−	−	A	NP
12	1	++	−	−	A	NP
13	1	++	−	−	A	NP
14	1	++	−	−	A	NP
15	1	+++	−	−	A	TI
16	1	++	−	n.t.	n.t.	TI
17	1	+++	−	−	A	TI
18	1	+++	−	n.t.	n.t.	TI
19	1	++	−	−	A	TI
20	1	++	−	−	A	TI
21	1	++	−	n.t.	n.t.	TI
22	1	++	−	−	A	TI
23	1	++	−	−	A	TI
24	1	++	−	−	A	TI
25	1	+	−	−	A	TI
26	1	++	−	−	A	TI
27	1	++	−	−	A	TI
28	1	++	−	−	A	TI
29	2	−	+	−	B	TI
30	2	−	+	−	B	TI
31	2	−	+	+	B	TI
32	2	−	++	+	B	TI
33	2	−	++	+	B	TI
34	2	−	+++	+	B	TI
35	2	−	++	+	B	TI
36	2	−	++	n t.	n.t.	TI
37	2	−	++	+	B	TI
38	2	−	+++	+	B	TI
39	2	−	+++	+	B	TI
40	2	−	+++	+	B	TI
41	2	−	++	+	B	TI
42	2	−	++	n.t.	n.t.	TI
43	2	−	−	−	C	TI
44	2	−	−	−	C	TI
45	2	−	+	+	B	RP
46	2	−	++	+	B	RP
47	2	−	+	−	B	RP
48	2	−	+	−	B	RP
49	1	+	−	−	D	RP
50	1	−	−	−	D	RP

* For explanation of A, B, C, and D, see Section III(*c*).

All biotype 1 isolates were oxidase-positive, producing a dark-blue colour reaction with 1% tetramethylparaphenylenediamine dihydrochloride within 10 sec; biotype 2 isolates produced a colour reaction only after 30 sec.

(*b*) *Serology*

Results of the tube-agglutination tests with isolates 1, 15, and 34 are given below:

Antiserum	Antigen	Titre
A. tumefaciens (isolate 15)	*A. tumefaciens*	4096
	A. radiobacter	4096
	Agrobacterium sp.	<4
A. radiobacter (isolate 1)	*A. radiobacter*	8192
	A. tumefaciens	2048
	Agrobacterium sp.	<4
Agrobacterium sp. (isolate 34)	*Agrobacterium* sp.	512
	A. tumefaciens	<4
	A. radiobacter	<4

It is clear that isolate 34 (*Agrobacterium* sp.) is distinct serologically from isolates 15 (*A. tumefaciens*) and 1 (*A. radiobacter*), and that the latter two are closely related.

Antisera of isolates 15 (biotype 1) and 34 (biotype 2) were tested against antigens of all isolates by gel diffusion. Results are given in Table 2 and support the evidence from physiological tests. Antigens of biotype 1 isolates never gave a specific reaction with isolate 34 antiserum although one non-specific band was frequently

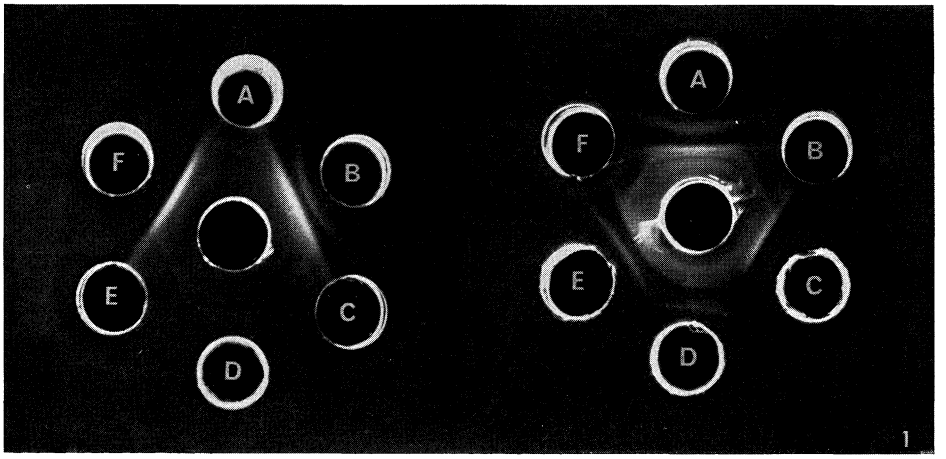


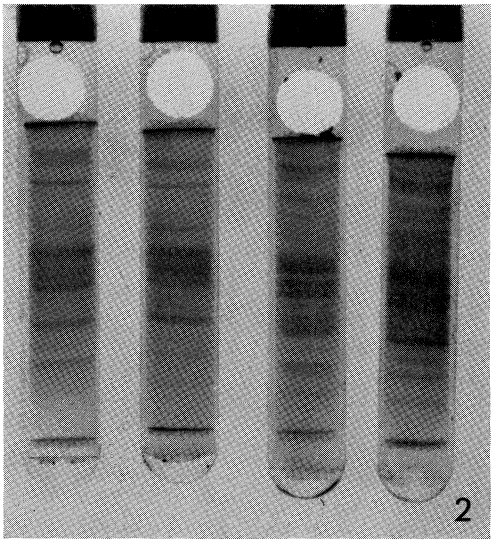
Fig. 1.—Serological reactions in gel diffusion tests. Peripheral wells contain antigens of isolate 34 (A), isolate 15 (B), isolate 46 (C), isolate 32 (D), isolate 39 (E), and isolate 19 (F). Centre wells contain antisera to isolate 15 (left) and isolate 34 (right).

present; similarly, negative results were obtained with biotype 2 antigens and isolate 15 antiserum. Most biotype 1 isolates produced specific precipitation bands when tested against isolate 15 antiserum, although the strength of the reaction and number of bands varied from isolate to isolate; the only exception was isolate 50 which gave no detectable reaction. Similar results were obtained between antigens of biotype 2 isolates and isolate 34 antiserum. The only isolates which did not react were nos. 43 and 44. Typical results are shown in Figure 1.

Antiserum of isolate 34 conjugated with fluorescent dye was tested against a range of isolates; results are given in Table 2 and largely confirm those of gel diffusion. Four isolates which gave a weak serological reaction did not fluoresce.

(*c*) *Electrophoresis*

About 30 protein bands could be distinguished in all the gels. Results again confirmed the evidence from biochemical tests. Most biotype 2 isolates formed a very well-defined group (B); all the protein patterns, with two exceptions, were very similar to those of isolates 30, 32, 38, and 46 illustrated in Figure 2. The exceptions were isolates 43 and 44, both designated *A. rubi*, which produced protein patterns (group C) distinct from those of all other isolates.



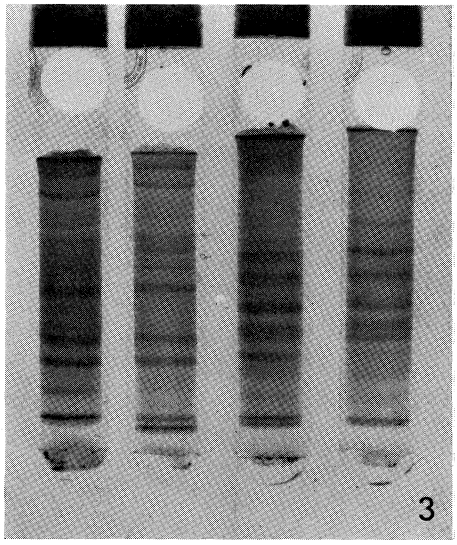


Fig. 2.—Protein patterns of biotype 2 isolates following disk electrophoresis. From left to right, isolates 46, 32, 39, 30.

Fig. 3.—Protein patterns of biotype 1 isolates following disk electrophoresis. From left to right, isolates 15, 1, 20, 14.

Most biotype 1 isolates formed a more variable group (A) in which it was impossible to distinguish *A. tumefaciens* from *A. radiobacter*. This is illustrated in Figure 3 where the protein patterns of two isolates of *A. tumefaciens* (Nos. 15 and 20) and two of *A. radiobacter* (Nos. 1 and 14) are shown. The similarity between the species is much greater than within them. *A. rhizogenes* isolates in biotype 1 (Nos. 49 and 50) formed distinct patterns (group D).

(*d*) *Pathogenicity*

Results are given in Table 2. All *A. radiobacter* isolates were non-pathogenic; all isolates of *A. tumefaciens*, *A. rubi*, and *Agrobacterium* sp. induced tumour formation in tomatoes following inoculation.

IV. Discussion

Results of biochemical tests indicated that all isolates studied formed two distinct biotypes. This was largely confirmed by serological reactions and by protein patterns following disk electrophoresis.

Biotype 1 contains tumour-inducing, root-proliferating, and non-pathogenic forms. Isolates Nos. 1–14 are non-pathogenic and appear to be typical of *A. radiobacter*. Isolates 15–28 induce tumour formation in tomatoes but otherwise cannot be distinguished from isolates 1–14 and we do not believe that a separate species, *A. tumefaciens*, is justified. It would seem more logical and perfectly adequate to indicate pathogenicity by a varietal epithet. The position of the remaining isolates in biotype 1, Nos. 49 and 50, both designated *A. rhizogenes*, is not so clear. They differ from the other isolates in biotype 1 in requiring growth factors, in their protein patterns, and in their weak or negative serological reaction. However, except in their root-proliferating characteristics, they are quite different from *A. rhizogenes* as described by Riker *et al.* (1930) and biochemically are much closer to other biotype 1 isolates than to isolates 45–48, also designated *A. rhizogenes*. The most satisfactory solution would again seem to be a varietal epithet to specify pathogenicity.

Biotype 2 contains both tumour-inducing and root-proliferating forms. On a biochemical basis, biotype 2 coincides with the definition (Riker *et al.* 1930) of *A. rhizogenes*, but this is untenable because on the one hand it includes tumour-inducing forms and on the other some root-proliferating isolates would be excluded. Most tumour-inducing isolates in biotype 2 are indistinguishable from root-proliferating isolates 45–48 except by pathogenicity. Although pathogenicity of *A. rhizogenes* isolates was not tested by us, isolates 45, 46, and 48 have recently been tested by DeLey *et al.* (1966); isolates 45 and 46 did not induce tumour formation in tomatoes and isolate 48 gave an equivocal reaction. The position of the two *A. rubi* isolates is doubtful. They can be distinguished from other isolates in biotype 2 by their negative serological reaction, by their distinct protein patterns, and by their growth-factor requirements. Many more isolates would have to be tested to clarify the position, but it is clear that the pathogenicity of *A. rubi* is not restricted to *Rubus* spp. In biochemical tests *A. rubi* isolates were identical with other biotype 2 isolates and on present evidence the retention of a separate species would seem unjustified.

The division of the genus *Agrobacterium* into species according to pathogenicity is unsatisfactory for several reasons. We have shown, on the one hand, that isolates which would be designated *A. radiobacter* under the present system of classification are indistinguishable biochemically, serologically, and electrophoretically from others which would be designated *A. tumefaciens*. On the other hand, some isolates which induce tumour formation are readily distinguished by the above tests from other tumour-inducing isolates. Under the present system, they would all be designated *A. tumefaciens*. The recent report (Kerr 1969*b*) that *A. radiobacter* can be converted to *A. tumefaciens* through transfer of virulence is further evidence that pathogenicity is an unsatisfactory basis for speciation. We believe that *A. radiobacter*, *A. tumefaciens*, *A. rhizogenes*, and *A. rubi* should be included in one species and, according to the International Code of Nomenclature of Bacteria and Viruses (1958), this should be *A. radiobacter* unless *Agrobacterium* is incorporated into *Rhizobium* as proposed by Graham (1964) and DeLey (1968).

All the cultures we have studied could be accurately defined by giving a varietal epithet to indicate pathogenicity and by specifying biotype. The 50 isolates would be grouped as follows:

A. radiobacter var. *radiobacter* biotype 1—isolates 1–14
A. radiobacter var. *tumefaciens* biotype 1—isolates 15–28
A. radiobacter var. *tumefaciens* biotype 2—isolates 29–44
A. radiobacter var. *rhizogenes* biotype 1—isolates 49–50
A. radiobacter var. *rhizogenes* biotype 2—isolates 45–48

V. Acknowledgments

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