STUDIES ON PLANT PATHOGENIC BACTERIA

II. SEROLOGY

By Rose Mushin,* Jill Naylor,† and N. Lahovary*

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Summary

Fifteen species of plant pathogenic bacteria were used for the production of high-titre antisera. The serological relationships were determined for plant pathogenic and other bacteria belonging to the genera *Agrobacterium, Corynebacterium, Erwinia, Pseudomonas,* and *Xanthomonas* by slide and tube agglutinations and agglutinin-absorption tests. α and β antigens were not important in masking "O" antigens but other factors were found to interfere with some agglutinations.

It was concluded that diagnostic antisera can be useful in the identification of plant pathogenic bacteria, in epidemiological studies, and in screening isolates from plant pathogenicity tests.

I. Introduction

Part I of this series (Mushin, Naylor, and Lahovary 1959) was concerned with the cultural and biochemical characters of plant pathogenic bacteria endemic in Australia and of type cultures obtained from abroad. This paper presents investigations on the antigenic structure of these organisms with the aim of utilizing this character in systematic identification.‡

In some earlier studies of plant pathogenic bacteria often no distinction was made of various antigenic components of the cell. Antisera produced with organisms of poor antigenicity were of low titre, unsuitable for diagnostic work.

The limitation of antigenic analysis as the sole tool of identification is well known in certain genera, and it was also observed that some physiologically unrelated species may share antigenic factors. However, the value of serological characters as a distinguishing feature of plant pathogenic bacteria has been demonstrated (Burkholder and Starr 1948) and these tests have been employed by various workers.

II. Materials and Methods

Agglutination tests mainly with "O" antisera, and with "H" antisera to a much lesser extent, were used in the present investigation. The serological techniques were according to Kauffmann (1954) unless otherwise stated.

*School of Bacteriology, University of Melbourne.

† School of Bacteriology, University of Melbourne; present address: Research Laboratories, Kraft Foods Ltd., Port Melbourne.

‡Dr. Mushin would be grateful for cultures of plant pathogenic bacteria isolated in Australia for serological typing.

(*a*) *Preparation of "O" Antisera*

(i) *"O" Antigens for Injections*

To minimize the production of extracellular material, meat infusion or brain–heart (Difco) agar cultures without the addition of carbohydrates were used. Bacteria were harvested as soon as enough growth was available, after 24 hr incubation or longer when dealing with slower-growing organisms.

Adjuvants were introduced in view of the poor antigenicity of some bacteria.

(1) *Steamed (St) and Roschka's (R) Antigens.*—Roschka's alcohol–acetone-treated suspensions were standardized to opacity equivalent to 4000×10^6 organisms per ml.

(2) *Roschka's Antigen plus Paraffin Oil plus Tubercle Bacilli Adjuvants (R-Par).*—The technique was modified from Freund *et al.* (1948). The suspension was prepared by Roschka's method, the extract being made from 10 ml of saline suspension containing the growth from four petri dishes. Sterile, dried tubercle bacilli (obtained by the courtesy of Dr. A. Pound, Brisbane) were ground to a fine powder using a pestle and mortar. After a few preliminary tests with varying amounts of the reagents the mixtures adopted for further use consisted of 1 ml bacterial suspension, 1 ml "Eucerin" (emulsifying agent), 3 ml paraffin oil, and 2 mg tubercle bacilli. The material was broken up either in a supersonic disintegrator or in an electric homogenizer. The emulsions were stable at room temperature but were warmed prior to injections to reduce viscosity.

(3) *Steamed Antigen plus Sodium Alginate Adjuvant (St-Alg).*—Slavin's (1950) method was followed.

(ii) *Course of Injections*

The procedure varied according to the antigen used and the following methods were applied:

(1) *St-Conven.*—Steamed antigen was administered in a conventional manner.

(2) *R-Conven.*—Roschka's antigen was administered in a conventional manner at the earlier stage of this work in six intravenous injections, the first two in 0·2 ml doses and the remainder in 0·5 ml. The first four injections were given within a fortnight and the following doses at weekly intervals. The alternative was a course of six injections of 0·5 ml each at 4–5 days interval.

(3) *St-Shock or R-Shock.*—Steamed or Roschka's antigen was administered by "shock" treatment. In the preliminary experiments the type and dose of antigen and period of injections varied. Finally the procedure was standardized to six intravenous injections of Roschka's antigen within 8 days and bleeding after 3 days. The series of injections at short intervals was referred to as shock treatment.

(4) *R-Par.*—Roschka's antigen with paraffin oil and tubercle bacilli adjuvants, prepared as described above, was used. As a starting point two or three subcutaneous injections of 0·25 ml of this emulsion were administered simultaneously. This was followed by various procedures employed to supply comparative data. In some experiments an additional course of subcutaneous injections with or without the adjuvants was given within 6 weeks or longer. The next step was shock treatment or

the conventional course with Roschka's antigen. Additional details about the techniques used are supplied in Tables 1–3.

(5) *St-Alg.*—Injections of steamed antigen plus sodium alginate adjuvant were administered by Slavin's (1950) technique using the intraperitoneal route. After an interval of about 3 weeks, 0·5 ml Roschka's antigen was given three times in 4-day intervals or by shock treatment.

(*b*) *Preparation of "H" Antisera*

Flagella suspensions were prepared by passaging the bacteria several times through semisolid (0·4 per cent.) agar in Craigie tubes till active motility was induced. Formolized bean-extract broth cultures were injected into rabbits in the conventional manner.

(*c*) *Preparation of α and β Antisera*

Thermolabile surface α and β antigens were prepared with paracolon Fairbrother α strain (Stamp and Stone 1944) and paracolon 30β (Mushin 1949). Formolized nutrient broth cultures were injected into rabbits in the conventional manner.

All sera were preserved with glycerol.

(*d*) *Preparation of Antigens for Tube Agglutination Tests*

(i) *"O" Antigens.*—In preliminary agglutination tests the suitability of steamed, Roschka's, alcoholized, and autoclaved suspensions was compared. The first two suspensions proved to be the most sensitive antigens and for convenience steamed ones were used as a rule in routine work. They were prepared in a concentrated form, formolized, stored, and diluted suitably when required.

Modifications were introduced in the preparation of a few antigens. Some *Erwinia* suspensions were centrifuged lightly to dispose of the deposit showing in control tubes. Trypsin treatment was applied for *Xanthomonas* to digest the mucoid components which were responsible for impaired agglutinability. A trypsin preparation (B.D.H.) in 1 : 1000 final concentration was allowed to act on the antigen for 10 min at 37°C; the mixture was then boiled to inactivate the enzyme.

(ii) *"H", α, and β Antigens.*—These were prepared as previously described.

(*e*) *Agglutination Tests*

Slide agglutination tests were carried out with living suspensions and undiluted "O" antisera as a preliminary and rapid means of demonstrating a qualitative relationship between bacteria. Readings were taken within 1–2 min. α and β tube agglutinations were performed as described by Stamp and Stone (1944) and Mushin (1949). Absorbed antisera were prepared by standard techniques with living suspensions in 1 : 10 dilution and were preserved with "Merthiolate". In dealing with mucoid antigens such as *Xanthomonas*, steaming of the absorbing antigen for 2½ hr was helpful in removing the gummy material which otherwise formed a jelly-like mass with the antiserum.

TABLE 1

METHODS OF PRODUCTION AND TITRES OF C. MICHIGANENSE ANTISERA

The abbreviations used in Tables 1–3 represent the antigen composition and methods used for the course of injections as set out in Sections II(*a*)(i) and II(*a*)(ii) respectively

Expt. No.	Course of Injections of Antigens	Titre	Expt. No.	First Course of Injections of Antigens	Time between First and Second Course of Injections (days)	Second Course of Injections of Antigens	Duration of Expt. (weeks)	Titre
1	St-Conven	40	4	St-Alg	17	R-Shock	4	10,240
2	R-Conven	320	5	St-Alg	21	R-Conven	7	640
3	R-Shock	5120 (after 11 days)	6	R-Par*	10	3R-Conven‡	6	640
			7	R-Par	42†	3R-Conven‡	9	2,560
			8	R-Par	10	3R-Conven‡	6	2,560

*Without tubercle bacilli.

†Titre <20.

‡Numbers before the antigen show the number of injections, different to those indicated in the text.

In Tables 4–7 the origin of strains used as antigens are indicated by the following letters: A from Australia, C from Canada, E from England, I from Israel, and U from the United States.

III. Results

(*a*) *Titre of Antisera Produced with Various Techniques*

Attempts were made to produce diagnostic antisera with simple methods but when inadequate titres were obtained more complicated techniques were applied. The merits of various procedures were assessed in relation to a genus or species. Titres of test bleedings were expressed for non-glycerolated sera.

(i) Agrobacterium.—Titres of two antisera prepared with Roschka's antigen from one strain of *A. tumefaciens* administered by the conventional and shock method were 5120.

(ii) Erwinia.—Steamed or Roschka's antigens administered in a conventional manner were used to prepare antisera with two strains of *E. atroseptica*, two strains of *E. carotovora*, and two antisera against one strain of *E. aroideae*. The sera obtained had titres of from 640 to 2560 and both types of antigen were of equal value.

(iii) Pseudomonas.—Antisera were prepared with the following strains: two of *Ps. medicaginis* var. *phaseolicola*, three of *Ps. syringae*, one each of *Ps. barkeri* and *Ps. solanacearum*, and two of *Ps. pisi*. Roschka's antigens were usually more satisfactory than steamed but the highest titre (20,000) was obtained with a steamed suspension of *Ps. medicaginis* var. *phaseolicola*.

(iv) Corynebacterium.—Table 1 shows the experimental data for *C. michiganense*. Roschka's and steamed suspensions applied in a conventional manner gave low-titre sera while shock treatment proved to be more efficient. Good results were obtained with adjuvants but the procedures took a longer time.

Experimental data for *C. flaccumfaciens* antisera are shown in Table 2 and again the superiority of shock treatment with Roschka's antigen was noticed. Earlier experiments with the use of adjuvants followed by shock treatment were more time-consuming. In one exceptional case good-titre serum was obtained with subcutaneous injections.

(v) Xanthomonas.—As shown in Table 3 satisfactory antisera were obtained for *X. carotae* and *X. juglandis* by various techniques. Variable results were recorded with *X. phaseoli*, and *X. campestris* gave only low-titre sera. However, higher titres were shown with Roschka's antigens, especially some old stock suspensions, than with steamed antigens. Working on the assumption that the impaired antigenic sensitivity may be due to the mucoid components of the bacteria, trypsin digestion was applied and a rise in titres was observed. This suggests that the mucoid fraction of the cell may be a critical factor.

(*b*) *α and β Antigens and Antibodies*

α antigens were not demonstrated in cultures of plant pathogenic bacteria and β antibodies were either absent or of a low titre in sera of normal rabbits and therefore of no significance.

TABLE 2

METHODS OF PRODUCTION AND TITRES OF C. FLACCUMFACIENS ANTISERA

Expt. No.	Course of Injections of Antigens	Titre	Expt. No.	First Course of Injections of Antigens	Time between First and Second Course of Injections (weeks)	Second Course of Injections of Antigens	Titre	Time between Second and Third Course of Injections (weeks)	Third Course of Injections of Antigens	Duration of Expt. (weeks)	Titre
1	St-Conven	160	4	R-Conven	5	R-Par	160 (after 9 weeks)	11	R-Shock	13	1280
2	9R-Shock*	640 (after 17 days)					20 (after 11 weeks)				
			5	St-Conven	1	St-Alg		4	R-Conven	8	1280
3	R-Shock	2560 (after 9 days) 640 (after 17 days) 160 (after 24 days)	6	R-Par	1	2R-Par, R-S/C†	1280 (after 7 weeks) 160 (after 10 weeks)	11	R-Shock	12	5120
			7, 8, 9	R-Par	1	R-Par (modified methods)	20–80		R-Shock		5120

*Numbers before antigen show the number of injections, different to those indicated in the text.

†S/C = subcutaneous injection, different to technique indicated in the text.

A few cultures gave weak positive slide agglutinations with β antisera but these were not confirmed in tube tests. Some *X. phaseoli* and *X. flaccumfaciens* cultures agglutinated in β antisera to titres of 256 and 128 respectively, but the reactions were delayed and of granular character and therefore non-specific. β agglutinations of titres up to 40 were recorded in some normal rabbit sera but the level of β antibodies did not increase during immunization.

It will be indicated later that some plant pathogens possessed surface antigens but these were of a different nature to α and β types.

TABLE 3

METHODS OF PRODUCTION AND TITRES OF XANTHOMONAS ANTISERA

Species	Expt. No.	Course of Injections of Antigens	Titre
X. carotae	1	St-Conven	320
	2	R-Conven	1280
X. juglandis	3	R-Shock	2560 (after 11 days)
	4	St-Alg and R-Shock	2560 (after 4 weeks)
X. campestris	6–10	Various (see Tables 1 and 2)	20–160, 1280*
X. phaseoli	11–16	Various (see Tables 1 and 2)	160–640, 2560*

*Trypsin-treated antigens.

(c) *Slide Agglutination Tests with "O" Antisera*

In slide agglutination tests of living suspensions with 14 homologous antisera no reaction was observed with several organisms apparently due to the presence of blocking surface antigens. This frequently occurred within the genus *Erwinia* but agglutination was shown when suspensions were boiled to destroy the heat-labile components or by choosing subcultures deficient in surface antigens. Also some *Xanthomonas* and *Ps. syringae* cultures possessed surface mucoid antigens which blocked agglutination with homologous somatic antisera. Distinct agglutinations were obtained with several organisms such as *A. tumefaciens*, *Ps. medicaginis* var. *phaseolicola*, *Ps. syringae*, *Ps. solanacearum* while other bacteria gave non-specific fine granular clumping.

Various species within one genus were found to cross-agglutinate, e.g. strains of *Ps. solanacearum* agglutinated in *Ps. syringae* serum; *Ps. barkeri* and *Ps. viridiflava* in *Ps. solanacearum* serum. Also some bacteria agglutinated in sera prepared with organisms from different genera apparently due to sharing of minor antigens, e.g. between *C. michiganense* and *Ps. pisi*, *X. campestris* and *Ps. syringae*, *X. carotae* and *Ps. syringae*. Frequently reactions were unilateral, not characteristic for species as strain differences were encountered, and of a low enough titre to be regarded as negative in subsequent tube agglutination tests.

Living strains of *A. tumefaciens*, *C. flaccumfaciens*, all *Erwinia*, *X. phaseoli*, *Ps. barkeri*, *Ps. solanacearum*, and *Ps. aeruginosa* did not react in any of the heterologous antisera. However, this may not indicate lack of antigenic relationships because, as previously mentioned, some living organisms, e.g. *Erwinia* were inagglutinable even in their homologous antisera.

It was concluded that results of slide agglutinations with living organisms and "O" antisera can be unreliable and should be confirmed by tube agglutination tests.

TABLE 4

TITRES OF "O" AGGLUTINATIONS OF CORYNEBACTERIUM

Capital letters used in Tables 4–7 are explained in the text (see p. 237)

Antigen	Antiserum from:	
	C. flaccumfaciens U	*C. michiganense* C
C. flaccumfaciens E	1280	—
A	1280	—
U	2560	—
C	640	—
C. michiganense A	—	2560
C	—	5120
E	—	640
C. fascians	—	—
C. simplex	—	—
Corynebacterium, 7 spp.*	—	—
C. diphtheriae mitis	20	—

*Human and animal pathogens and saprophytes.

(d) *Tube Agglutinations of "O" Antigens and "O" Antisera*

The antigenic relationships will be discussed within the appropriate genera and titres will be expressed for glycerolated stock sera. Titres less than 20 were recorded as negative. In all, 50 somatic antisera were produced from 15 bacterial species, namely, *A. tumefaciens* (2 antisera), *C. michiganense* (8), *C. flaccumfaciens* (9), *E. aroideae* (2), *E. atroseptica* (2), *E. carotovora* (1), *Ps. barkeri* (1), *Ps. medicaginis* var. *phaseolicola* (2), *Ps. pisi* (3), *Ps. solanacearum* (1), *Ps. syringae* (3), *X. campestris* (5), *X. carotae* (2), *X. juglandis* (2), *X. phaseoli* (6).

(i) *Serology of* Agrobacterium.—*A. tumefaciens* was represented by three strains, two received from England and one from Canada, and all gave full-titre agglutinations (2560) with two antisera. These strains were antigenically closely related and no cross-agglutinations were recorded with heterologous antisera.

(ii) *Serology of* Corynebacterium.—As shown in Table 4, four strains of *C. flaccumfaciens* and three of *C. michiganense* agglutinated to full or high titre in their homologous antisera and failed to react with heterologous antisera.

No affinity was shown between the above species and *C. fascians*, *C. simplex*, *C. diphtheriae gravis* (2 strains), *C. xerosis*, *C. hoffmanii*, *C. equi*, *C. ovis*, and a *Corynebacterium* sp. from soil. A strain of *C. diphtheriae mitis* gave an insignificant agglutination. Thus the serological distinction of *C. michiganense* and *C. flaccumfaciens* was demonstrated.

Agglutinin-absorption tests were carried out with *C. michiganense* antiserum prepared with a type culture from Canada. Absorption was complete with the homologous and the local strain but the strain from England did not exhaust the serum. Thus, *C. michiganense* strains were found to be serologically closely related in sharing a major group antigen while strains from Australia and Canada possessed an additional factor.

TABLE 5

TITRES OF "O" AGGLUTINATIONS OF ERWINIA

Antigen		Antiserum from:				
		E. aroideae	*E. atroseptica*		*E. carotovora*	
		E	U	A	E	C
E. aroideae	E	1280	160	20	320	160
E. atroseptica	U	20	320	20	160	40
	I	—	—	—	—	—
	A	20	40	640	320	160
E. carotovora	E	20	80	20	640	320
	C	20	80	20	640	640
	I	—	—	—	—	—
Paracolons and coliforms, 28 spp		—	—	—	—	—
Paracolon sp.		10	10	10	40	40
Paracolons and coliforms, 34 spp.						—

(iii) *Serology of* Erwinia (*soft-rot group*).—All strains except two from Israel proved to be interrelated as indicated by agglutination tests with five antisera (Table 5). This group was difficult to examine due to antigenic variation in suspensions prepared from subcultures of the same strain. Also, the readings of the tests were often unsatisfactory because end-point agglutinations were not distinct.

Agglutinin-absorption tests were attempted with a few cultures but this met with little success as controls indicated that absorptions of sera with homologous organisms were not complete. Many variations in time and temperature in the absorption stage were tried and also in the kind and amount of absorbing antigen. Tests were set up with concentrated amounts of the absorbing antigen using steamed suspensions which were prepared from the growth on 64 petri plates. Those sus-

TABLE 6

TITRES OF "O" AGGLUTINATIONS OF PSEUDOMONAS

Numbers in parenthesis refer to number of strains giving a particular reaction

Antigen*	No. of Strains	Antisera from:								
		Ps. medicaginis var. *phaseolicola*		*Ps. syringae*			*Ps. pisi*		*Ps. solanacearum*	*Ps. barkeri*
		A_1	A_2	A_1	E	A_2	C	E	E	E
Ps. medicaginis var. *phaseolicola*	37	80– 160 (6) 320– 640 (11) 1280–10000 (20)	40– 80 (11) 160– 320 (18) 640–2560 (8)	—	—	—	— (22) 20–80 (15)	— (20) 20–80 (16) 640 (1)	— (5)	—
Ps. syringae	17	—	—	— (3) 80 (1) 320–1280 (13)	— (3) 320 (1) 640–2560 (13)	— (3) 320 (1) 640–2560 (13)	—	—	20– 40 (2) — (3)	—
Ps. solanacearum	9	—	—	—	—	—	—	—	40– 80 (2) 160– 320 (3) 640–2560 (4)	—
Ps. pisi	2	160– 320	—	—	—	—	2560	2560	80	—
Ps. barkeri	1	—	—	—	—	—	—	—	—	640

**Ps. cerasi* (2 strains), *Ps. viridiflava* (1), *Ps. aeruginosa* (1), and *Ps. fluorescens* gave negative reactions.

pensions were mixed with 1 ml of serum in four absorption stages of 2 hr each. The difficulties encountered may have been due to the age of the cultures and fresh isolates may give satisfactory results.

A number of paracolons and coliforms tested (Table 5) showed no serological relationship with *Erwinia* except for one paracolon strain. It was noticed that *Erwinia* agglutinated in *X. carotae* serum but not vice-versa, and also a slight reaction was recorded in *Ps. syringae* serum.

(iv) *Serology of* Pseudomonas.—The results of agglutinations of nine *Pseudomonas* species against nine antisera are shown in Table 6. All 37 strains of *Ps. medicaginis*, 13 strains of *Ps. syringae*, one of *Ps. solanacearum*, and one of *Ps. viridiflava* were local isolates while the remainder was imported from abroad.

Ps. medicaginis var. *phaseolicola* (Pmp) and *Ps. pisi*: As some affinity was shown between these two species they will be discussed under one heading. Agglutination tests against two antisera labelled Pmp A_1 and Pmp A_2 with 37 *Ps. medicaginis* strains, including isolates from the same plant, showed variation in titres. *Ps. pisi* strains were identical in reciprocal agglutinin-absorption tests and therefore only one strain and antiserum will be referred to in further examinations. *Ps. pisi* showed reciprocal agglutination with Pmp A_1 but not with Pmp A_2.

In agglutinin-absorption tests it was evident that Pmp A_2 strain had an additional factor, not possessed by Pmp A_1, as there was only partial absorption of Pmp A_2 serum with Pmp A_1 antigen while Pmp A_2 strain completely absorbed Pmp A_1 serum. Tests with the remaining Pmp strains against the absorbed sera showed sharing of a major antigen and presence or lack of minor antigens.

Absorption of Pmp A_1 and Pmp A_2 with *Ps. pisi* did not exhaust these antisera. Absorption of *Ps. pisi* antiserum with Pmp A_1 and Pmp A_2 antigens lowered its titre for *Ps. pisi* although no agglutination was noticed of *Ps. pisi* in Pmp A_2 unabsorbed serum. This will be discussed later.

On the basis of agglutinin-absorption tests the following antigenic patterns can be suggested:

Ps. medicaginis var. *phaseolicola* A_1 strain had factors 1, 3
Ps. medicaginis var. *phaseolicola* A_2 strain had factors 1, 2, 3
Ps. pisi strains had factors 3, 4

Several *Ps. medicaginis* var. *phaseolicola* and both *Ps. pisi* strains cross-agglutinated in *X. carotae* antiserum to a low titre.

Ps. syringae: The majority of strains showed a close serological affinity. *Ps. syringae* A_2 antiserum agglutinated its homologous antigen to a much lower titre than some other strains, thus indicating their antigenic variability. Agglutinin-absorption tests were carried out with three strains of *Ps. syringae*, labelled A_1, E, and A_2, and the corresponding antisera. Strains E and A_2 seemed to be identical and contained an additional factor compared with A_1. Agglutination tests of several *Ps. syringae* strains with the absorbed sera gave similar patterns, thus indicating the presence or absence of minor antigenic factors.

Ps. syringae strains agglutinated in *X. carotae* antiserum; the reactions were unilateral and only a minor antigenic relationship was recorded.

Ps. solanacearum: The nine strains of this species were not homogeneous, as shown in Table 6. *Ps. pisi* and some *Ps. syringae* strains agglutinated in *Ps. solanacearum* antiserum to a low titre.

Other pseudomonads: The remaining *Pseudomonas* strains did not agglutinate in the diagnostic antisera.

TABLE 7

TITRE OF "O" AGGLUTINATIONS OF XANTHOMONAS

Antigen		Antiserum from:			
		X. campestris E	*X. carotae* E	*X. phaseoli* C_1	*X. juglandis* E
X. campestris	E	40	—	80	—
	E*	640			
	C	40	—	80	—
	I	—			
X. carotae	E	20	640	160	640
X. phaseoli	E	—	—	20	80
	C_1	40	20	640	160
	C_1*			1280	
	C_2	—	—	40	—
	C_3			20	
	U			—	
X. vesicatoria	A	—	—	40	—
X. incanae	A	—	—	20	—
X. juglandis	E	160	320	160	1280
	C	160	160	80	640
X. albilineans	E	40	—	80	—

*Trypsin-treated antigens.

(v) *Serology of* Xanthomonas.—*X. campestris* and *X. phaseoli* gave low-titre sera but antigens exposed to trypsin digestion produced better reactions (Table 7). *X. juglandis* strains were closely related to each other and shared antigens with other members of the genus. *X. carotae* agglutinated to a full titre in *X. juglandis* antiserum, to a significant titre with *X. phaseoli*, slightly with *X. campestris*, and not with antisera from other genera. On the other hand, many organisms reacted to a low titre in *X. carotae* serum.

(*e*) *Tube Agglutinations of "H" Antigens and "H" Antisera*

Ps. syringae (14 strains) agglutinated to high titres, from 640 to 5120, thus apparently sharing major flagellar antigens. *Ps. medicaginis* var. *phaseolicola* agglutinated to lower titres ranging from 40 to 640 but this may have been due to the difficulty of enhancing motility in some strains.

IV. Discussion

In this investigation several methods were applied to obtain high-titre antisera in rabbits. Antigens in the form of polysaccharide extracts gave good results with *Agrobacterium*, *Erwinia*, and *Pseudomonas*.

Various studies have shown *Corynebacterium* to be poor antigens (Sniezko and Bonde 1943). Woodroffe (1950) used a quick succession of injections with *C. equi* and recorded low-titre sera while Rosenthal and Cox (1953) were more successful with a similar technique. In the present experiments good-titre sera were produced with adjuvants (Freund *et al.* 1948; Slavin 1950). The shock treatment with Roschka's antigen subsequently adopted was equally successful and had the advantage of using a simpler antigen and a shorter time of administration.

The *Xanthomonas* strains are notorious for their mucoid substances whose polysaccharide nature was investigated by various workers (Leach *et al.* 1957). These exudates were found to give non-specific serological reactions and cross-agglutinations (Elrod and Braun 1947*a*, 1947*b*, 1947*c*). In this work the mucoid fractions were removed by trypsin digestion and thus the somatic antigens became more specific.

Slide agglutination tests with living organisms served as a preliminary basis for classification of species within the identified genus. The interpretation of both positive and negative results had to be made with caution and confirmed by tube agglutinations using steamed suspensions to eliminate blocking components.

A. tumefaciens strains showed a close somatic relationship. An extracellular substance in these bacteria was found by Pinkes and Neill (1957) to possess a serological affinity with *Pneumococcus* type 27 and *Leuconostoc mesenteroides* B 512.

The inclusion of plant pathogens in the genus *Corynebacterium* was criticized by Conn and Dimmick (1947) on taxonomic grounds. In the present studies *C. michiganense* and *C. flaccumfaciens* species appeared to be well defined, homogeneous, and not related to other *Corynebacterium* species tested. Similar patterns were described by Rosenthal and Cox (1953, 1954).

The *Erwinia* soft-rot group which comprises strains biochemically similar to coliforms and paracolons showed a marked antigenic variability and cross-agglutinations. This was previously noticed by Elrod (1941). The serological interrelationships of strains confirm Dowson's (1957) suggestion of uniting the three species of the soft-rot group into one. In the present study a minor antigenic relationship was encountered with a paracolon strain while Elrod (1946) recorded an affinity with *Shigella*. With the new knowledge of the antigenic pattern of Gram-negative bacteria it should be possible to bring a serological order into this group on the lines indicated by Kauffmann (1954).

Cross-reactions were recorded in the genus *Pseudomonas* and this was noticed by other workers (Friedman 1953). Of great interest were *Ps. medicaginis* var. *phaseolicola* and *Ps. syringae* strains as most were locally isolated. *Ps. syringae* strains were obtained mostly from beans while one culture with only a slight antigenic affinity to the other strains was recovered from lemon and thus may have displayed a host specificity. *Ps. medicaginis* var. *phaseolicola* strains showed a great heterogenicity and some were related to *Ps. pisi*. Pmp A_2 and *Ps. pisi* did not show relationship in a direct agglutination test but an affinity was demonstrated by agglutinin absorption. It is possible that the distribution of components on the bacterial cell was such that the primary antigen–antibody contact was impaired. Variations in physicochemical behaviour of the organisms may be responsible for the many unilateral reactions encountered in this study.

In the genus *Xanthomonas* the treatment of the polysaccharide envelope of the bacterial cells with trypsin provided specific somatic antigens. This method suggested a promising line of investigation for further studies.

It is concluded that serology cannot replace other bacteriological techniques but it can be very helpful as an additional tool for characterization of species or strains of plant pathogenic bacteria. Serology may be useful in epidemiological studies in tracing the spread of infection and as a rapid means of identification of isolates in plant pathogenicity tests.

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VI. References

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