

**Final Report**  
**Serological detection of bacterial spearmint of calabrese**  
**HDC FV 104A**  
**J D Taylor**

## HDC FINAL REPORT

**Final report:** 31 March 1994

**Project Number:** FV 104A

**Project Title:** Serological detection of bacterial spear rot of calabrese

**Project Leader:** Dr J D Taylor

**Location:** HRI Wellesbourne - linked to main project at SAC,  
Edinburgh

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**Key Words:** Calabrese, spear rot, serological detection

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## Background

A study of factors affecting infection and symptom development in bacterial spear rot of calabrese was proposed to HDC by Drs. Harling and Chard of the Scottish Agricultural College, Edinburgh.

Their study sought to (1) identify the causal agents, probably *Erwinia* and *Pseudomonas* spp., by classical methods of pathogenicity tests and (2) to investigate the biology and epidemiology of the pathogen(s) in both field and growth chamber studies. This second component involving studies of pathogen populations would be difficult to carry out effectively without the availability of rapid and specific diagnostic tests. Although the original proposal made no provision for this, it was suggested that a diagnostic component be added to the programme. This diagnostic component, undertaken at HRI Wellesbourne, had as its main objective the preparation of serological reagents for use in the Edinburgh programme.

## Description of work

The project aimed to (1) prepare polyclonal antisera to a range of bacterial isolates supplied by SAC as being pathogens or potential pathogens of calabrese, (2) to test the specificity of the antisera against a test array of closely related organisms and (3) to conjugate the antisera with *Staphylococcus aureus* for use as a diagnostic reagent.

The 'conjugated *Staphylococcus aureus* slide agglutination test' can be used against a wide range of organisms from plants either from culture plates or directly from plant tissues (see Lyons N F & Taylor J D, 1990, Serological detection and identification of bacteria from plants by the conjugated *Staphylococcus aureus* slide agglutination test. Plant Pathology **39**, 584-590).

Antisera were screened for specificity against their homologous isolates and a test array of 41 bacterial isolates, mainly from brassicas, including pathogens and saprophytes. Test were made by two different methods, *Staphylococcus aureus* slide agglutination and ELISA,

## Results

Five different antisera were produced during the course of the study, these are listed together with a provisional identification of their homologous strains in the table below.

Antiserum No.	Homologous strain HRI No. (*)	Identification of strain
92/28	2949B (1015)	<i>Pseudomonas</i> group IVa
92/31	2950B (1065)	<i>Erwinia</i> ?
93/9	3359 (5067)	<i>Erwinia</i> ?
93/15	3491 (5038)	<i>Pseudomonas</i> group IVb
93/16	3492 (5049)	<i>Pseudomonas</i> group IVb

(\*) SAC number

Many of the antisera showed considerable specificity to their respective homologous strains. This was particularly so in ELISA tests (eg 2949B with antiserum 92/28, 2950B with antiserum 92/31, 3491 with antiserum 93/15 and 3492 with antiserum 93/16). The results were somewhat less clear cut with *Staphylococcus aureus* tests but some apparent cross-reactivity causing a 'stringy' (+s) agglutination could generally be distinguished from true agglutination. Only one antiserum 93/9 showed poor specificity in both ELISA and *Staphylococcus aureus* tests.

The full results of specificity testing with the five antisera and 41 isolates of the test array using *Staphylococcus aureus* slide agglutination and ELISA tests are shown in the Appendix.

## Conclusions

Antisera showing useful levels of specificity to their homologous strains were obtained to a number of isolates said to be pathogenic to calabrese. Unfortunately SAC were unable to arrive at a definite conclusion as to the causal agents, moreover, their target organisms appeared to change in different seasons and because of this the antisera we produced were said to have little or no practical value to the SAC programme. With hindsight it would appear that SAC's assessment of the pathogen situation at the outset of the project was insufficiently clear to justify the programme undertaken. It may be that the antisera we have produced will have some future value in a more systematic evaluation of the pathogenic ability of bacteria associated with spear rot.

The antisera produced, their homologous strains and the test array of bacteria used in specificity testing are available from low temperature storage at HRI Wellesbourne.

## Appendix

Results of specificity testing with the five antisera and 41 isolates of the test array using *Staphylococcus aureus* slide agglutination and ELISA tests



*Staphylococcus aureus* slide agglutination assay using 92/28/2 PAb (raised to isolate 2949B) against a selected range of brassica and other isolates.

Number	Isolate	Identification	Origin	Staph
1	916C	<i>P. viridiflava</i>	Calabrese	-
2	1171	"	Brassica seed	-
3	65B	<i>P. syr. pv. maculicola</i>	Mustard	-
4	101	"	NCPPB 952	-
5	1809A	"	Cauliflower	-
6	2948B	<i>Pseudomonas sp.</i>	Calabrese	+
7	151B	"	Mustard	-
8	722A	"	Cauliflower	-
9	722B	"	"	-
10	753A	"	Cabbage	-
11	753C	"	"	+s
12	754B	"	"	+s
13	755A	"	"	+s
14	755D	"	"	+s
15	842	"	?	+/-
16	848	"	?	+s
17	981	"	Cabbage seed	+s
18	982	"	"	+s
19	983A	"	"	+s
20	983B	"	"	-
21	731A	"	Lettuce	+s
22	732A	"	"	-
23	1152B	"	"	-

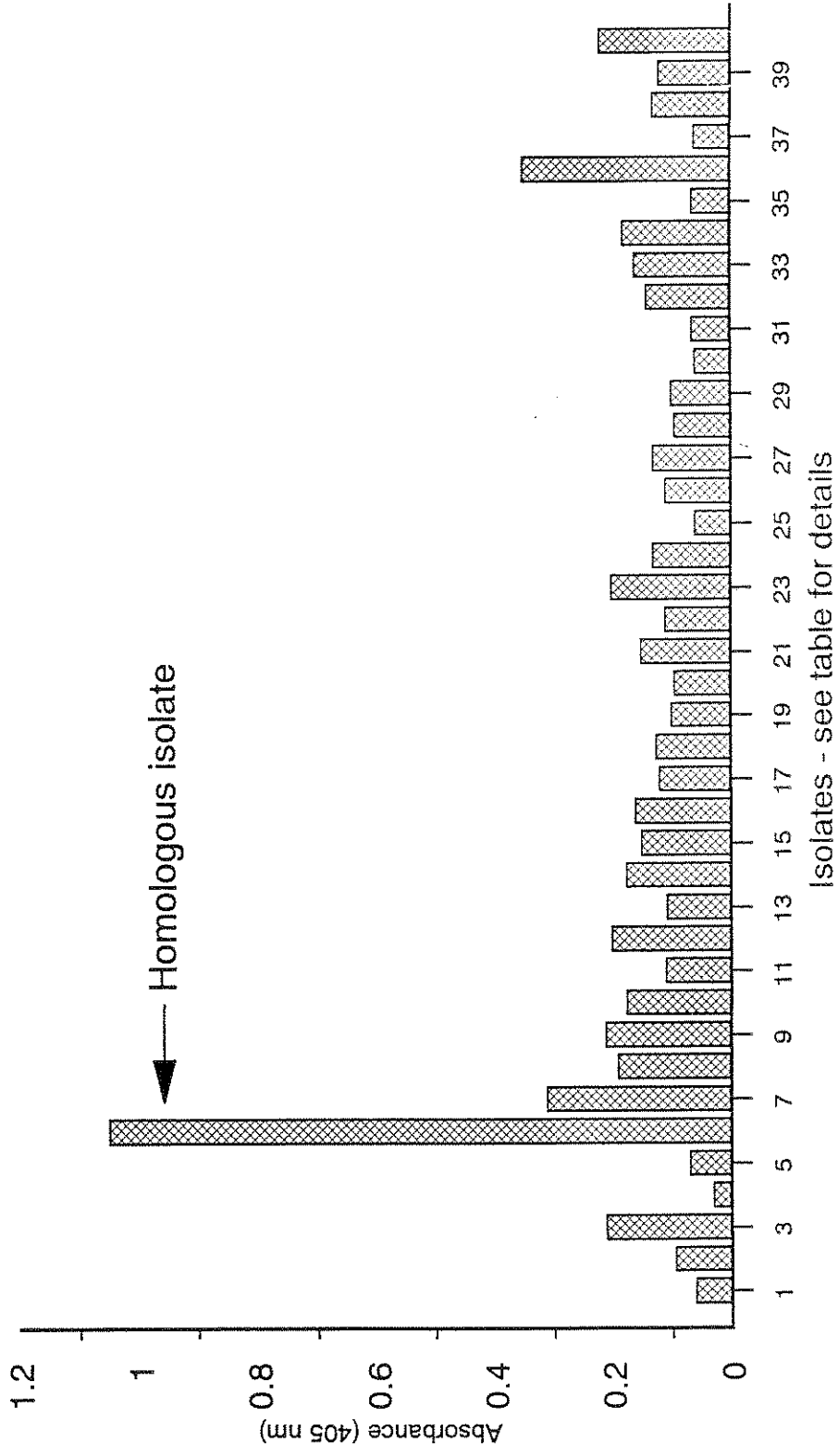
24	1222A	"	"	-
25	743A	X. campestris pv. campestris	Broccoli	+s
26	919A	E. carotovora	Swede	-
27	936D	"	Brussels sprout	+s
28	1001A	"	Lettuce	-
29	1143B	"	"	-
30	1326A	"	"	-
31	1328C	"	"	-
32	1333G	"	"	-
33	984A	Saprophyte	Cabbage seed	-
34	1089	"	"	+s
35	1119A	"	"	+s
36	765A	"	Broccoli	+s
37	765F	"	"	+s
38	1851A	"	Cauliflower	-
39	1852A	"	Brussels sprout	NT
40	1210F	"	Cauliflower	+s

In the *Staphylococcus aureus* slide agglutination test +s indicates a 'stringy' form of reaction. Isolates marked + gave strong granular agglutination, +/- indicates slight agglutination, NT not tested.

(LYONS\ELISA\92-28-2.WP)

# PAb 92/28/2 raised to isolate 2949B (SAC 1015)

## Cross-reactivity with selected brassica and other isolates



Antigen standardised at 0.4 OD at 620 nm

*Staphylococcus aureus* slide agglutination assay using 92/31/3 PAb (raised to isolate 2950B) against a selected range of brassica and other isolates.

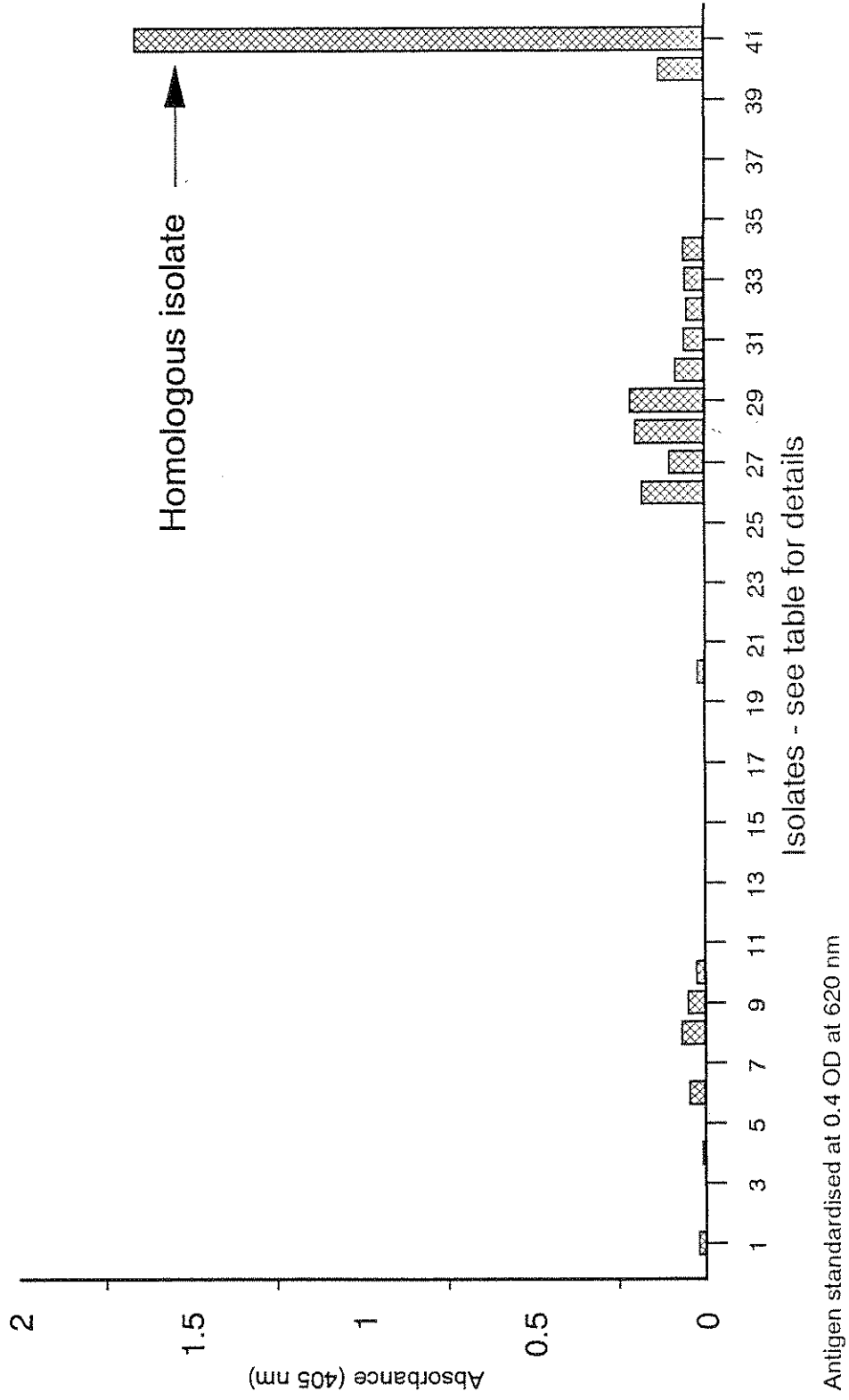
Number	Isolate	Identification	Origin	Staph
1	916C	<i>P. viridiflava</i>	Calabrese	NT
2	1171	"	Brassica seed	-
3	65B	<i>P. syr. pv. maculicola</i>	Mustard	-
4	101	"	NCPPB 952	-
5	1809A	"	Cauliflower	-
6	2948B	<i>Pseudomonas sp.</i>	Calabrese	-
7	151B	"	Mustard	+s
8	722A	"	Cauliflower	-
9	722B	"	"	-
10	753A	"	Cabbage	-
11	753C	"	"	-
12	754B	"	"	+s
13	755A	"	"	+s
14	755D	"	"	+s
15	842	"	?	+s
16	848	"	?	-
17	981	"	Cabbage seed	-
18	982	"	"	+s
19	983A	"	"	+s
20	983B	"	"	-
21	731A	"	Lettuce	-
22	732A	"	"	-
23	1152B	"	"	-

24	1222A	"	"	+s
25	743A	X. campestris pv. campestris	Broccoli	-
26	919A	E. carotovora	Swede	-
27	936D	"	Brussels sprout	-
28	1001A	"	Lettuce	-
29	1143B	"	"	-
30	1326A	"	"	-
31	1328C	"	"	-
32	1333G	"	"	+s
33	984A	Saprophyte	Cabbage seed	-
34	1089	"	"	-
35	1119A	"	"	-
36	765A	"	Broccoli	+s
37	765F	"	"	-
38	1851A	"	Cauliflower	-
39	1852A	"	Brussels sprout	-
40	1210F	"	Cauliflower	-
41	2950B	Homologous isolate	Calabrese	+

In the *Staphylococcus aureus* slide agglutination test +s indicates a 'stringy' form of reaction. Isolates marked + gave strong granular agglutination, +/- indicates slight agglutination, NT not tested.

(LYONS\ELISA\92-31-3.WP)

**PAb 92/31/3 raised to isolate 2950B (SAC 1065)**  
**Cross-reactivity with selected brassica**  
**and other isolates**



Indirect ELISA and *Staphylococcus aureus* slide agglutination using 93/9/2 PAb (raised to isolate 3359) against a selected range of brassica and other isolates.

Number	Isolate	Identification	Origin	ELISA	Staph
1	916C	<i>P. viridiflava</i>	Calabrese	-	+
2	1171	"	Brassica seed	+	+
3	65B	<i>P. syr. pv. maculicola</i>	Mustard	+	-
4	101	"	NCPFB 952	+	+
5	1809A	"	Cauliflower	-	-
6	2948B/1	<i>Pseudomonas sp.</i>	Calabrese	+	-
7	2949B/2	"	"	-	+/-
8	151B	"	Mustard	-	+/-
9	722A	"	Cauliflower	-	+
10	722B	"	"	-	-
11	753A	"	Cabbage	-	-
12	753C	"	"	-	-
13	754B	"	"	-	+s
14	755A	"	"	-	+s
15	755D	"	"	-	+s
16	842	"	?	-	+s
17	848	"	?	-	-
18	981	"	Cabbage seed	+	+s
19	982	"	"	+	+s
20	983A	"	"	+	+s
21	983B	"	"	+	+s
22	731A	"	Lettuce	-	+
23	732A	"	"	-	+s

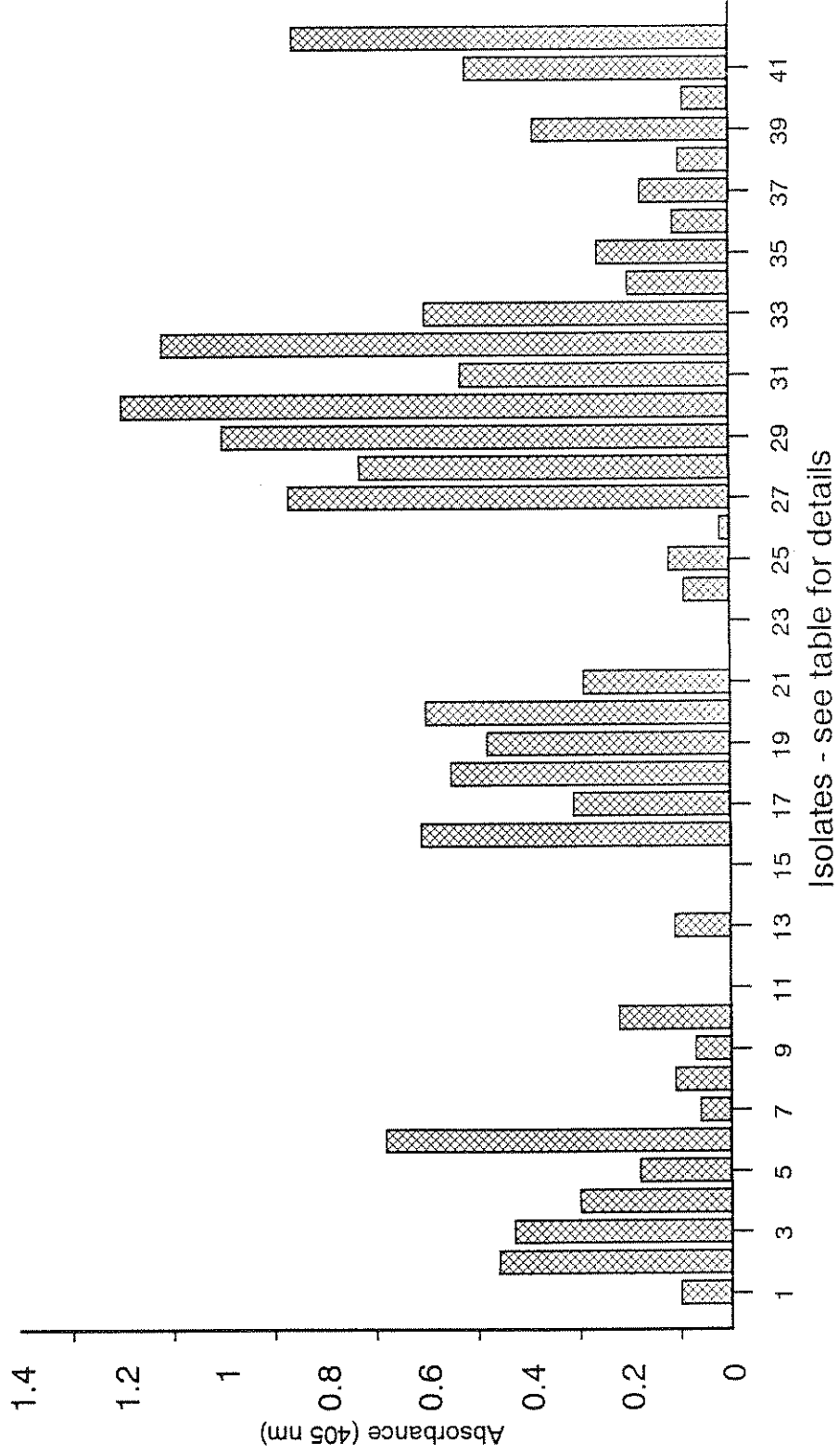
24	1152B	"	"	-	-
25	1222A	"	"	-	+s
26	743A	X. campestris pv. campestris	Broccoli	-	+s
27	919A	E. carotovora	Swede	+	-
28	936D	"	Brussels sprout	+	-
29	1001A	"	Lettuce	+	-
30	1143B	"	"	+	+
31	1326A	"	"	+	-
32	1328C	"	"	+	-
33	1333G	"	"	+	+s
34	984A	Saprophyte	Cabbage seed	-	-
35	1089	"	"	+	+s
36	1119A	"	"	-	+s
37	765A	"	Broccoli	-	-
38	765F	"	"	-	-
39	1851A	"	Cauliflower	+	+s
40	1852A	"	Brussels sprout	-	+
41	1210F	"	Cauliflower	+	-
42	3359	Homologous isolate	Calabrese	+	+

In the *Staphylococcus aureus* slide agglutination test +s indicates a 'stringy' form of reaction. Isolates marked + gave strong granular agglutination, +/- indicates slight agglutination.

(LYONS\ELISA\93-9-2.WP)



**PAb 93/9/2 raised to isolate 3359**  
**Cross-reactivity with selected brassica**  
**and other isolates**



Antigen standardised at 0.4 OD at 620 nm

Indirect ELISA and *Staphylococcus aureus* slide agglutination using 93/15/2 PAb (raised to isolate 3491) against a selected range of brassica and other isolates.

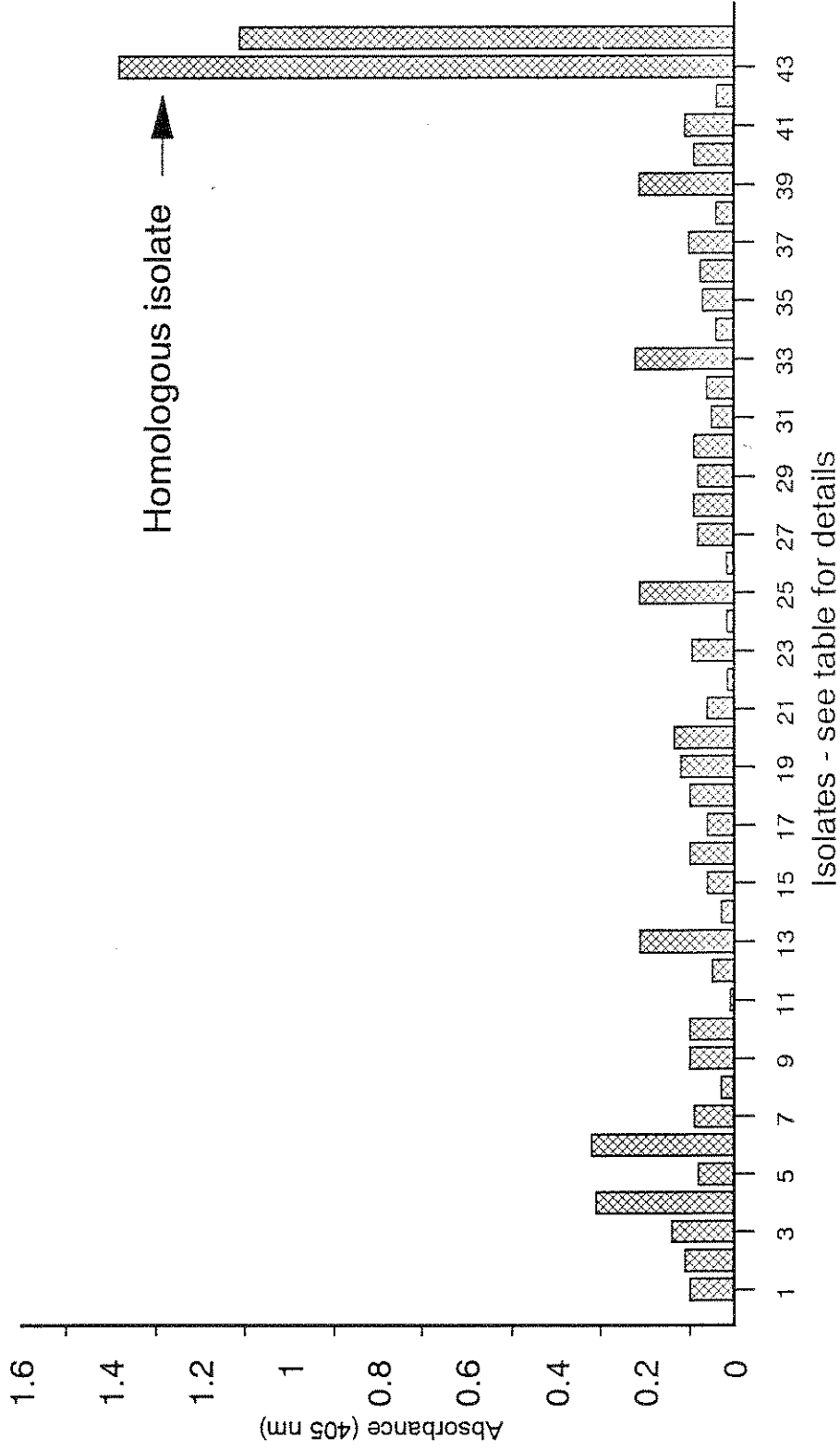
Number	Isolate	Identification	Origin	ELISA	Staph
1	916C	<i>P. viridiflava</i>	Calabrese	-	-
2	1171	"	Brassica seed	-	-
3	65B	<i>P. syr. pv. maculicola</i>	Mustard	-	-
4	101	"	NCPFB 952	-	-
5	1809A	"	Cauliflower	-	-
6	2948B/1	<i>Pseudomonas sp.</i>	Calabrese	-	+/-
7	2949B/2	"	"	-	-
8	151B	"	Mustard	-	-
9	722A	"	Cauliflower	-	-
10	722B	"	"	-	-
11	753A	"	Cabbage	-	-
12	753C	"	"	-	-
13	754B	"	"	-	-
14	755A	"	"	-	+/-
15	755D	"	"	-	+s
16	842	"	?	-	-
17	848	"	?	-	-
18	981	"	Cabbage seed	-	-
19	982	"	"	-	-
20	983A	"	"	-	-
21	983B	"	"	-	-
22	731A	"	Lettuce	-	-
23	732A	"	"	-	+

24	1152B	"	"	-	-
25	1222A	"	"	-	+/-
26	743A	X. campestris pv. campestris	Broccoli	-	-
27	919A	E. carotovora	Swede	-	-
28	936D	"	Brussels sprout	-	-
29	1001A	"	Lettuce	-	-
30	1143B	"	"	-	-
31	1326A	"	"	-	-
32	1328C	"	"	-	-
33	1333G	"	"	-	+
34	984A	Saprophyte	Cabbage seed	-	-
35	1089	"	"	-	+
36	1119A	"	"	-	-
37	765A	"	Broccoli	-	+s
38	765F	"	"	-	-
39	1851A	"	Cauliflower	-	-
40	1852A	"	Brussels sprout	-	-
41	1210F	"	Cauliflower	-	-
42	3359		Calabrese	-	-
44	3491	P. putida	Calabrese	+	+/-
44	3492	Homologous isolate	Calabrese	+	+

In the *Staphylococcus aureus* slide agglutination test +s indicates a 'stringy' form of reaction. Isolates marked + gave strong granular agglutination and those marked +/- indicates slight agglutination.

(LYONS\ELISA\93-15.2.WP)

**PAb 93/15/2 raised to isolate 3491 (SAC 5038)**  
**Cross-reactivity with selected brassica**  
**and other isolates**



Antigen standardised at 0.4 OD at 620 nm

Indirect ELISA and *Staphylococcus aureus* slide agglutination using 93/16/2 PAb (raised to isolate 3492) against a selected range of brassica and other isolates.

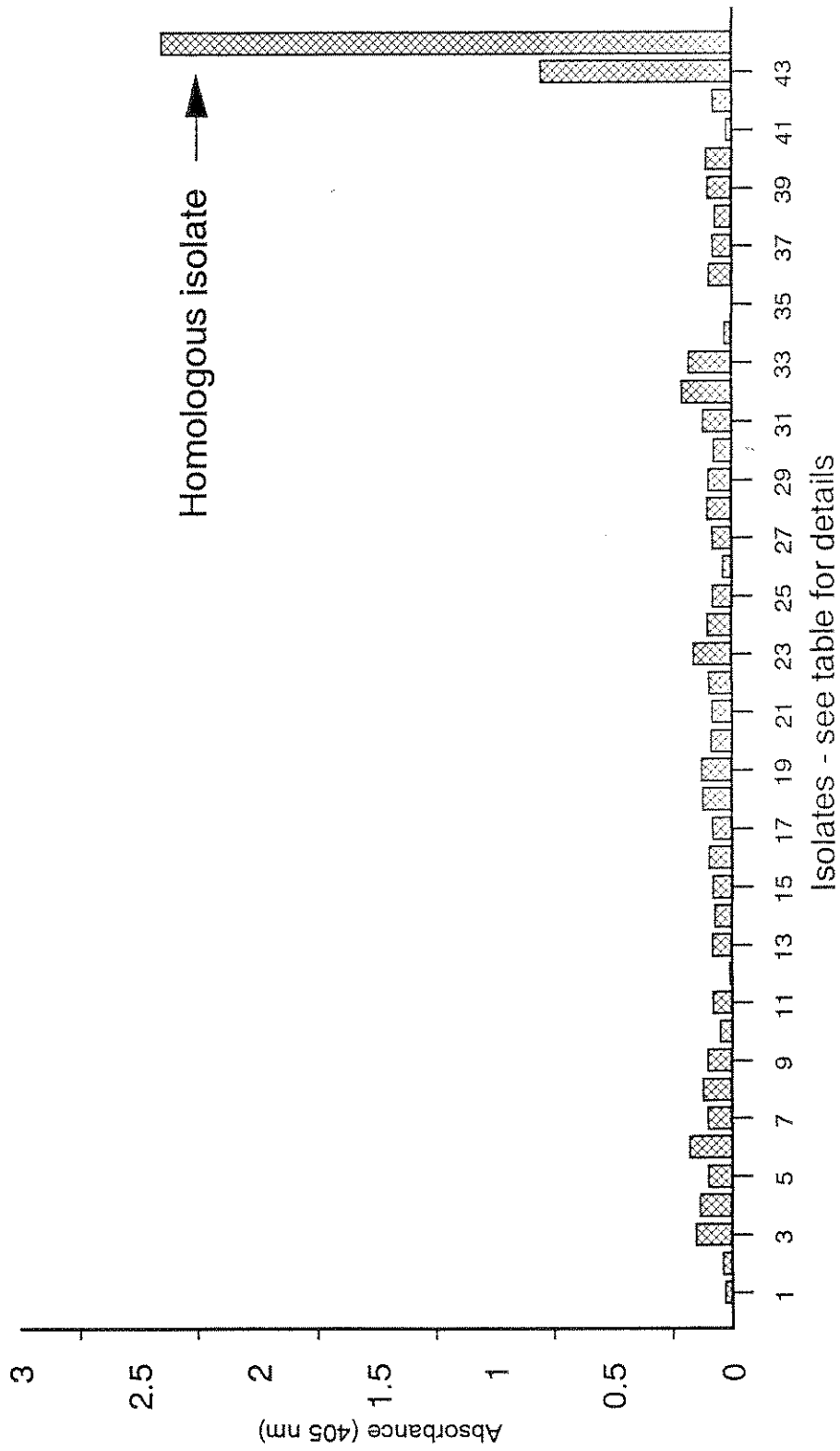
Number	Isolate	Identification	Origin	ELISA	Staph
1	916C	<i>P. viridiflava</i>	Calabrese	-	-
2	1171	"	Brassica seed	-	-
3	65B	<i>P. syr. pv. maculicola</i>	Mustard	-	-
4	101	"	NCPPB 952	-	+/-
5	1809A	"	Cauliflower	-	-
6	2948B/1	<i>Pseudomonas sp.</i>	Calabrese	-	+/-
7	2949B/2	"	"	-	+
8	151B	"	Mustard	-	-
9	722A	"	Cauliflower	-	+/-
10	722B	"	"	-	+/-
11	753A	"	Cabbage	-	-
12	753C	"	"	-	-
13	754B	"	"	-	-
14	755A	"	"	-	+s
15	755D	"	"	-	+s
16	842	"	?	-	-
17	848	"	?	-	-
18	981	"	Cabbage seed	-	-
19	982	"	"	-	-
20	983A	"	"	-	-
21	983B	"	"	-	-
22	731A	"	Lettuce	-	+/-s
23	732A	"	"	-	+s

24	1152B	"	"	-	-
25	1222A	"	"	-	+s
26	743A	X. campestris pv. campestris	Broccoli	-	+s
27	919A	E. carotovora	Swede	-	-
28	936D	"	Brussels sprout	-	-
29	1001A	"	Lettuce	-	-
30	1143B	"	"	-	-
31	1326A	"	"	-	-
32	1328C	"	"	-	-
33	1333G	"	"	-	+/-
34	984A	Saprophyte	Cabbage seed	-	-
35	1089	"	"	-	+/-
36	1119A	"	"	-	-
37	765A	"	Broccoli	-	+s
38	765F	"	"	-	-
39	1851A	"	Cauliflower	-	-
40	1852A	"	Brussels sprout	-	-
41	1210F	"	Cauliflower	-	-
42	3359		Calabrese	-	-
44	3491	P. putida	Calabrese	+	+
44	3492	Homologous isolate	Calabrese	+	+

In the *Staphylococcus aureus* slide agglutination test +s indicates a 'stringy' form of reaction. Isolates marked + gave strong granular agglutination, +/- indicates slight agglutination.

(LYONS\ELISA\93-16-2.WP)

**PAb 93/16/2 raised to isolate 3492 (SAC 5049)**  
**Cross-reactivity with selected brassica**  
**and other isolates**



Antigen standardised at 0.4 OD at 620 nm