Neth. J. Pl. Path. 99 (1993) 51–60

Serological characterization of fluorescent Pseudomonas strains cross-reacting with antibodies against Erwinia chrysanthemi

J.M. VAN DER WOLF[1], J.R.C.M. VAN BECKHOVEN[1], E. DE BOEF[1] and N.J.M. ROOZEN[2]

[1] DLO Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, the Netherlands
[2] Research Station for Arable Farming and Field Production of Vegetables (PAGV), P.O. Box 430, 8200 AK Lelystad, the Netherlands

Accepted 5 January 1993

Abstract

Sixteen bacterial strains, cross-reacting with antibodies against *Erwinia chrysanthemi* (Ech), were isolated from potato peel extracts, ditch water, and the rhizosphere of wheat, onion, sugar beet and chicory using the immunofluorescence colony-staining procedure. Based on fatty acid profiles, isolates were classified as belonging to the *Pseudomonas fluorescens* group.

These strains, together with two previously isolated cross-reacting *P. fluorescens* strains, cross-reacted with polyclonal antibodies against Ech in immunofluorescence cell-staining, Ouchterlony double diffusion, and ELISA. Seventeen strains also reacted strongly with monoclonal antibodies against the lipopolysaccharides (LPS) of Ech in ELISA.

Cell envelopes (CE) and proteinase-K-treated CE (mainly LPS) of cross-reacting bacteria were further characterized with SDS-PAGE and Western blotting. Based on CE protein and LPS patterns, the cross-reacting bacteria were classified into two groups, each existing of two subgroups. Both CE and proteinase-K-resistant antigens strongly cross-reacted on immunoblots with antisera against a wild type strain of Ech. With an antiserum against a LPS O-chain lacking mutant of Ech only protein bands but no proteinase-K-resistant antigens were detected on immunoblots. These data suggest that in all cases the highly antigenic LPS 0-chain is responsible for the cross-reactions.

Additional keywords: ELISA, immunofluorescence cell-staining, Ouchterlony double diffusion, SDS-PAGE, Western blotting, lipopolysaccharides, fatty acid analysis.

Introduction

With the introduction of immunofluorescence colony-staining (IFC) as a novel method for detecting plant pathogenic bacteria, an excellent procedure was obtained also for the isolation of cross-reacting bacteria (Van Vuurde, 1987). Directed isolation of bacteria by puncturing fluorescing colonies in agar using (capillary) needles is, in theory, far more efficient than subsequent dilution plating techniques and screening of bacterial colonies for cross-reacting bacteria.

Once isolated, cross-reacting bacteria can be used to absorb antisera to improve specificity. Furthermore, knowledge of components that are responsible for cross-reactions is helpful for the production of more specific antibodies; antigens can be selected that are typical for the target bacterium.

Ecological research at the Research Station for Arable Farming and Field Production of Vegetables and at the DLO Research Institute for Plant Protection (IPO-DLO) on sources

responsible for contamination of seed potatoes with *Erwinia chrysanthemi* (Ech) in the field, has shown that IFC indeed is an adequate method for isolation of cross-reacting bacteria. Cross-reactants were isolated from ditch water (De Vries and Van Vuurde, 1992), potato peel extracts, and the rhizosphere of wheat, onion, sugar beet and chicory.

In this study 18 strains cross-reacting with antisera raised against Ech, 16 of which were isolated through IFC, were serologically characterized. Titers of cross-reacting strains were estimated in various serological tests as a measure of the risk of false positive reactions. The antigenic components responsible for the cross-reactions were analyzed with SDS-PAGE and Western blotting.

Materials and methods

Bacterial strains and growing conditions. Cross-reacting bacterial strains S44 and S45 (Table 1) were selected by randomly screening bacterial colonies that were grown on an

Table 1. Reactions of saprophytic Pseudomonas fluorescens spp. with antibodies against Ech in ELISA, Ouchterlony double diffusion (ODD) and immunofluorescence cell-staining (IF). Strains are classified on the basis of the cell envelope protein (CEP) pattern.

Strain	Source[a]	ELISA threshold (cells ml^{-1})	ELISA titers[b]		ODD[c] titer	IF titer	ODD pattern	CEP (sub)-group
			8276B	mca 2A4				
S44	potato	10^5	ND	ND	64	800	p	A1
S45	potato	10^5	ND	ND	64	800	p	A1
S61	potato	10^5	8000	64000	128	200	f	A1
S65	onion	10^6	8000	64000	64	1600	p	A1
S66	sugar beet	10^6	16000	64000	64	1600	f	A1
S67	wheat	10^7	4000	1000	32	800	p	B1
S69	chicory	10^5	8000	32000	64	400	p	A1
S70	wheat	10^6	8000	16000	64	800	f	B1
S71	wheat	10^5	8000	32000	64	100	f	B2
S72	wheat	10^6	8000	64000	128	800	p	B1
S73	sugar beet	10^6	8000	16000	64	800	p	B1
S81	potato	10^6	16000	64000	128	400	p	B1
S83	potato	10^6	8000	>256000	64	100	p	A1
S84	potato	10^6	8000	16000	64	50	p	B1
S87	potato	10^5	16000	32000	128	200	p	B1
S88	potato	10^5	32000	32000	128	200	p	B1
S92	ditch water	10^5	8000	64000	64	1600	p	A2
S93	ditch water	10^5	8000	32000	128	1600	f	A2
Ech 502	potato	10^6	32000	>256000	128	1600		
Eca 161	potato	nc	nc	nc	<2	<50		

[a] Source of isolates: potato = potato peel extracts; onion, wheat, sugar beet = rhizosphere of these plants.
[b] Serial dilutions of the enzyme conjugated antibodies were tested in double antibody sandwich ELISA for 8276B and in triple antibody sandwich ELISA for mca 2A4.
[c] IF and ODD were carried out with polyclonal antiserum 9024C.
[d] Using Ech 502 as control: p = partial identical (spur forming); f = fusing lines.
ND = not determined; nc = negative control.

agar medium after dilution plating of potato peel extracts (Van der Wolf and Gussenhoven, 1992). S65 to S93 (Table 1) were isolated after immunofluorescence colony-staining, IFC (Van Vuurde and Roozen, 1990) using antisera against whole cells of Ech.

Bacteria were identified by the Plant Protection Service in Wageningen (J.D. Janse) by fatty acid profiling, using the Microbial Identification System (MIDI, Newark, DE, USA).

Erwinia chrysanthemi (Ech, IPO-DLO nr. 502 = Plant Protection Service (PD) nr. 226), was used as positive control in each test, while *E. carotovora* subsp. *atroseptica* (Eca, IPO-DLO nr. 161 = PD nr. 230) was used as negative control. Ech RH6065, a mutant of Ech 3937jRH (Schoonejans et al., 1987) that lacks the lipopolysaccharide (LPS) O-chain and a part of the LPS core region, was used for the preparation of antiserum 9053C. In all serological tests, strains were grown for 24 h at 27 °C on trypticase soy agar (TSA, BBL) unless otherwise stated.

The ability of bacteria to degrade pectate was tested on crystal violet pectate (CVP) medium (Cuppels and Kelman, 1974).

Antiserum preparation. Polyclonal antisera were produced following the immunization protocol of Vruggink and Maas Geesteranus (1975). Antiserum 9024C was prepared against a cell extract of Ech 502. Antiserum 8276B against Ech 502, antiserum 8567L against Eca 161 and antiserum 9053C against Ech RH6065 were prepared against whole cells. Monoclonal antibodies (mca 2A4) were prepared by S.H. De Boer (Vancouver, Canada) and were directed to the 0-chain of the LPS of Ech (unpublished results J.M. van der Wolf).

Immunoglobulins were purified from crude antisera as described by Steinbuch and Audran (1969). Purified immunoglobulins were conjugated to alkaline phosphatase for ELISA according to the procedure of Tobiás et al. (1982). FITC-conjugates were prepared as described by Allen and Kelman (1977).

Immunofluorescence cell-staining (IF). IF was performed according to Van Vuurde et al. (1983), using FITC-conjugated antibodies of 9024C or 9053C in a stock solution of 3 mg ml^{-1}. Microscope slides with 24 × 4 mm wells (Nutacon, 10-342-A) were used, which were filled with 10 µl per well. Titers were determined from preparations containing 50–500 cells per microscope field at a final magnification of 625. Preparations stained with serial dilutions of FITC-conjugated antiserum 8567L served as a negative control.

ELISA. The detection threshold for each strain was determined in double antibody sandwich (DAS)-ELISA with antiserum 8276B. DAS-ELISA was performed as described previously (Van der Wolf and Gussenhoven, 1992), with the following modifications. Bacteria were grown in trypticase soy broth for 24 h at 27 °C. The cells were washed with demineralized water and lyophilized. Serial dilutions of freeze-dried bacteria (initial concentration 1 µg ml^{-1} = c. 10^9 cells ml^{-1}) were prepared in PBS (0.27 M NaCl, 0.04 M Na_2HPO_4, 2.9 mM KH_2PO_4, 5.4 mM KCl, 0.01% NaN_3, pH 7.2) supplemented with 0.1% Tween 20 (PBST).

The titers with mca 2A4 were determined in a triple antibody sandwich (TAS)-ELISA: microplate wells were coated with immunoglobulins from antiserum 8276B as described for DAS-ELISA. Coated plates were subsequently incubated with 10^8 bacterial cells ml^{-1} for 18 h at 4 °C, with mca 2A4 at 2.8 µg ml^{-1} for 2 h at 27 °C and with goat-anti-mouse immunoglobulins conjugated with alkaline phosphatase (Dakopatts, D314, diluted 1000 times) for 1 h at 27 °C. Each incubation was carried out in PBST. Visualization of the reaction was carried out as described for DAS-ELISA.

ELISA-values were considered to be positive when they exceeded the mean absorbance values plus 3 times standard deviations of the highest concentration of negative control bacteria.

Ouchterlony double diffusion (ODD). Ouchterlony double diffusion was performed according to Van der Wolf and Gussenhoven (1992) using antiserum 9024C. Bacteria were tested with the addition of 50 µl of liquid phenol per ml of suspension. To estimate the titer, the peripheral wells were filled with two-fold serial dilutions in PBS (1/2...1/512) and the center well was filled with a bacterial suspension ($OD_{620} > 1.0$), treated with phenol.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was carried out as described by Laemmli (1970) in 12.5% (w/v) separation gels and 3% (w/v) stacking gels in a mini Protean Cell apparatus (BioRad). Proteins were stained with Coomassie blue and lipopolysaccharides were stained with silver using a silver stain kit (BioRad). Low molecular weight standards (Sigma) were used as markers and biotinylated markers (Sigma) in gels for Western blots.

Western blotting. Transfer of bacterial components from acrylamide gels onto Immobilon 6-membranes (Millipore) was carried out according to Towbin et al. (1979) using a semi-dry blot method. By incubation of the blot for 30 min at room temperature in PBST + 5% skimmed milk powder, the reactive groups on the membrane were blocked. The membrane was washed twice for 5 min in PBST, and then incubated for 2 h with a 200-fold dilution of 9024C or a 100-fold dilution of 9053C in PBST + 0.1% BSA. After a washing for 3 times 5 min with PBST, the membranes were incubated with a goat-anti-rabbit alkaline phosphatase conjugate (Sigma, A9919) diluted 1000 times in PBST + 0.1% BSA, supplemented with 2000 times diluted streptavidin alkaline phosphatase (Boehringer). Serological reactions were visualised on blot using 0.06 mg ml^{-1} 5-bromo-4-chloro-3-indolylphosphate (4 mg ml^{-1} in methanol and acetone, 2:1) and 0.1 mg ml^{-1} nitro-blue tetrazolium in 0.1 M ethanolamine, 4 mM $MgCl_2$, pH 9.6.

Results

Identification of bacteria. All 18 strains were identified as bacteria belonging to the heterogenous *Pseudomonas fluorescens* group as defined by Janse et al. (1992). This group consists of oxidase positive, fluorescent rod-shaped bacteria which may or may not be pectolytic or cause soft rot of potato tissue. The *P. fluorescens* group includes the related *P. aurofaciens*, *P. chlororaphis*, *P. marginalis*, *P. putida* and *P. tolaasii* and their (biochemical) intermediates.

Reaction of bacteria in ODD, ELISA and IF. In ODD the *Pseudomonas* strains cross-reacted strongly with antiserum 9024C against Ech (Table 1). Six isolates, S61, S72, S81, S87, S88 and S93, had the same titer as the homologous strain Ech 502.

In ELISA, both polyclonal and monoclonal antibodies against Ech cross-reacted with the *P. fluorescens* strains. Mca 2A4 reacted weakly with S67 and strongly with S83 compared with 8276B. Apart from S67, the threshold levels of the cross-reacting strains were similar to the level of the homologous strain (c. 10^5–10^6 cells ml^{-1}) using antiserum 8276B. The threshold level with S67 was 10^7 cells ml^{-1}.

In IF the titers for the *P. fluorescens* strains varied between 50 and 1600 with antiserum 9024C, compared with a titer of 1600 for Ech. Relatively low titers (< 400) were found

for strains S61, S71, S83, S84, S87 and S88. Using antiserum 9053C against the O-chain lacking mutant of Ech, no cross-reactions (titers < 10) were found with six representative strains (S44, S65, S67, S70, S71 and S92), while the titer with the homologous strain 3937jRH was 320.

All strains degraded pectin on CVP.

Analysis of cross-reacting components in ODD, SDS-PAGE and Western blotting. In ODD the precipitation lines of five *Pseudomonas* strains (S61, S66, S70, S71 and S93) fused with the line of Ech 502. The precipitation lines of the 13 other strains developed a spur, indicating a partial similarity with Ech.

Preparations of cell envelopes of the 18 strains were analyzed by SDS-PAGE. Based on banding patterns of the major proteins, the strains were divided in two main groups (A and B), which were both divided in two subgroups: A1 containing seven strains, A2 containing two strains, B1 containing eight strains and B2 a single strain (Table 1). The protein profiles of six *Pseudomonas* strains, representing the four subgroups and strains of Eca 161 and Ech 502 are shown in Fig. 1. Only small differences in molecular weight of the major proteins distinguished subgroup A1 from A2. Group B1 had a 22 kD protein, which is not present in group B2. Furthermore, group B2 had a 48 kD protein instead of the 51 kD protein present in group B1. All *Pseudomonas* strains possesed the typical 19 kD protein band that was also found by De Weger et al. (1986) in *Pseudomonas* strains from different sources.

The cell envelopes of these strains were also analyzed by Western blotting. A predominant smear in a part of the lanes was detected on blots incubated with all strains except Eca 161 using antiserum 9024C (Fig. 2). No background smear was observed on blot using antiserum 9053C against the O-chain lacking mutant of Ech (Fig. 3). The intensely stained low molecular bands in the cell envelope extract of strain Ech 502 and Eca 161, for Eca 161 only partially visible in Fig. 2, probably reflects the LPS core

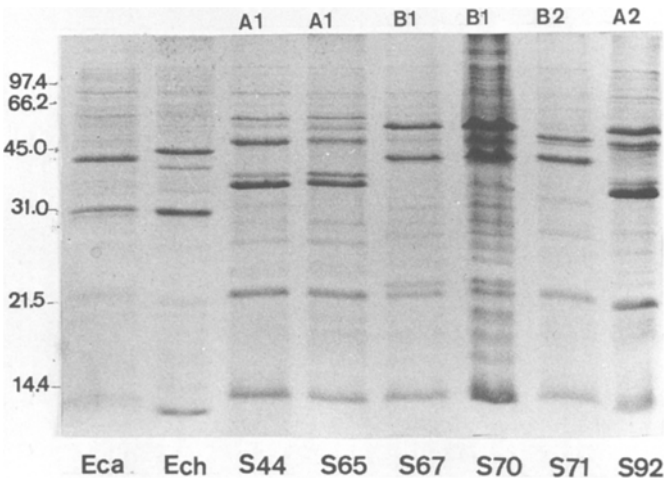

Fig. 1. SDS-polyacrylamide gel electrophoresis of cell envelopes of Eca 161, Ech 502 and *Pseudomonas fluorescens* strains cross-reacting with Ech antisera. Labels above the lanes refer to the cell envelope protein group and labels below lanes are isolate designations. On the left, the positions of the molecular weight markers with the molecular weights (in kD) are given.

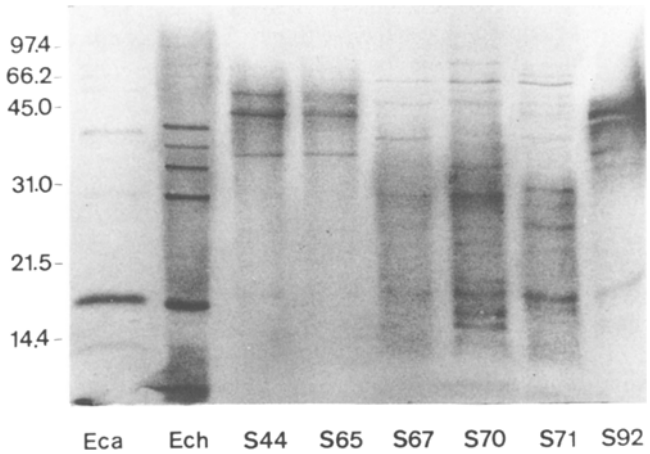

Fig. 2. Immunoblot of a similar gel as shown in Fig. 1, probed with antiserum 9024C against a total cell extract of Ech 502.

(Schoonejans et al., 1987). This band was also detected in proteinase-K-treated cell envelopes of Ech 502, but not of Eca 161 (Fig. 5).

All *Pseudomonas* strains had cross-reacting protein bands. A 13.5 kD protein band was detected in the cell envelope extracts of all tested strains with antiserum 9053C, while using antiserum 9024C only 13.5 kD protein bands were detected in S67 and S70. Antiserum 9053C strongly reacted with two bands of c. 31 kD of strains belonging to subgroup B. The 52.5 kD protein band of the *Pseudomonas* strains reacted non-specifically with the goat-anti-rabbit alkaline phosphatase conjugate (data not shown). Antiserum 9053C strongly reacted with a 35.5 kD protein band of Ech.

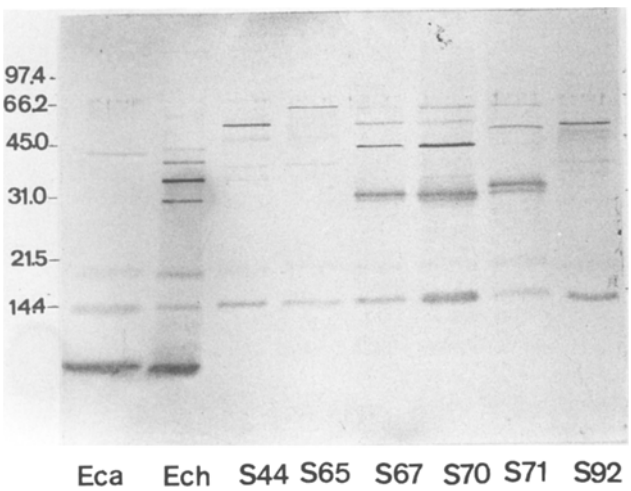

Fig. 3. Immunoblot of a similar gel as shown in Fig. 1, probed with antiserum 9053C against a LPS O-chain lacking mutant mutant of Ech.

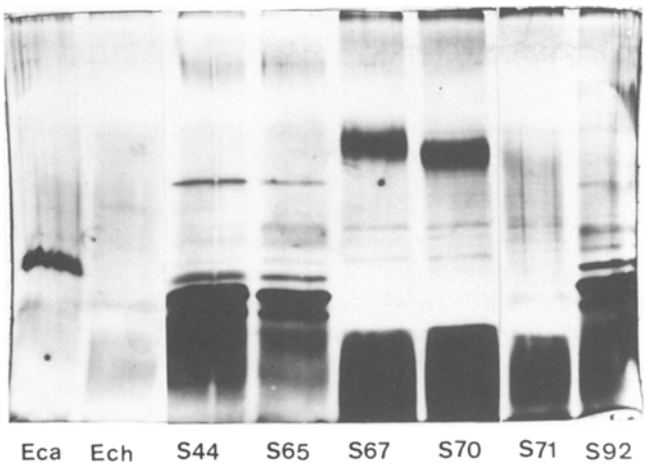

Fig. 4. Silver stained SDS-polyacrylamide gel of proteinase-K-treated cell envelopes of Eca 161, Ech 502 and *Pseudomonas fluorescens* strains cross-reacting with Ech antisera.

Also proteinase-K-treated cell envelopes of Ech 502, Eca 161, S44, S65, S67, S70, S71 and S92 were analyzed with SDS-PAGE (Fig. 4). The patterns of proteinase-K-resistant components of strains within group A were very similar to one another, each containing three distinct bands above a broad band at a 7 kD level. Also the patterns of strains belonging to subgroup B1 had a high level of homology. Strain S71 belonging to subgroup B2 lacked the intensely stained broad band just above the middle of the lane seen in strains of subgroup B1. The pattern of strain Eca 161 and Ech 502 differed from all other strains. The amount of proteinase-K-treated cell envelopes used in the silver stained gel shown in Fig. 4 is the same as used for the Western blot in Fig. 5. Compared with the *P. fluorescens*

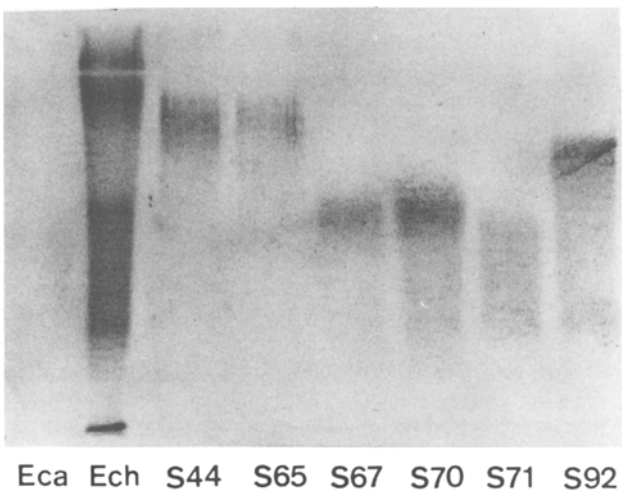

Fig. 5. Immunoblot of a similar gel as shown in Fig. 4, probed with antiserum 9024C against a total cell extract of Ech 502.

strains, Ech 502 proteinase-K-resistant components, from which only a small amount was applied according to the silver stained gel, reacted very strongly on blot with antiserum 9024C.

Based on the (diffuse) patterns of proteinase-K-treated cell envelopes on Western blots, using antiserum 9024C, the 18 cross-reacting strains could be divided in four groups which corresponded with the four (sub)groups based on cell envelope protein patterns. Patterns of the six representative strains S44, S65, S67, S70, S71 and S92 are shown in Fig. 5. No distinct ladder patterns could be obtained in spite of the attempts to improve the resolution on the immunoblots. In proteinase-K-treated cell envelopes the core of strain Eca 161 did not cross-react with the Ech antibodies.

Mca 2A4 against the LPS O-chain of Ech 502 reacted weakly in Western blotting, even at a mca concentration of 10 $\mu g\ ml^{-1}$ (data not shown). Strains S67 and Eca 161 did not react with mca 2A4 on blot at all.

Discussion

Reactions in ODD, ELISA and IF. Seventeen out of eighteen fluorescent *P. fluorescens* strains cross-reacted strongly with polyclonal antisera against Ech 502 in ODD and ELISA (Table 1). Relatively strong reactions were also obtained with mca 2A4 against the LPS of Ech 502 in ELISA. The IF titers varied more between individual strains than in ODD and ELISA.

S67 reacted relatively weakly with polyclonal antisera in ODD and ELISA, but strongly on immunoblot (Fig. 5) and in IF, indicating that S67 produced strongly cell-wall-linked cross-reacting antigens. Low reaction levels in ELISA and ODD may be due to poor solubilization of the cross-reacting component.

Whereas in the Netherlands large-scale indexing of seed potatoes is carried out with ELISA, it is important to know whether the concentration of cross-reacting *P. fluorescens* strains can exceed the detection threshold of ELISA (c. 10^5 cells ml^{-1}), leading to false-positive results. Geels and Schippers (1983) estimated the maximum concentration of two individually tested *P. fluorescens* strains on tuber periderm at 10^4 cfu cm^{-2}, which is equivalent to c. 5.10^4 cfu ml^{-1} potato peel extract. The total concentration of *P. fluorescens* on tuber periderm was estimated at a maximum of c. 5.10^5 cells ml^{-1} potato peel extract. However, on wounded tuber surfaces, cell concentrations may exceed the detection threshold of ELISA. The fact that all cross-reacting *Pseudomonas* strains degraded pectin, may indicate that some of these bacteria can cause rotting of wounded plant tissue, as was demonstrated for S45 already (Van der Wolf and Gussenhoven, 1992).

In IF it will not be possible to exclude all cross-reactions by dilution of antiserum; four cross-reacting strains had the same titers as Ech, while the titers of five other strains were only one dilution step lower. The weak reactions of six strains in IF may be explained by a masking of the cross-reacting component, e.g. by capsular polysaccharides. On the basis of Western blots results, it is unlikely that the weak reacting strains possess smaller quantities of cross-reacting components or less reactive components.

Analysis of cross-reacting components in ODD, SDS-PAGE and Western blotting. In ODD the precipitation lines of the cross-reacting bacteria S61, S66, S70, S71 and S93 fused with the line of Ech 502. In these cases ODD was unable to discriminate between homologous and heterologous reactions. Furthermore, ODD was unable to discriminate between different (sub)groups of cross-reactants; the six isolates represent all four distinguishable subgroups.

The strains were classified in SDS-PAGE on the basis of major proteins in the cell

envelopes (Fig. 1). The proposed classification of four (sub)groups was justified by the results of the Western blottings and the analysis of proteinase-K-treated cell envelopes with SDS-PAGE (Figs. 2, 3, 4 and 5). The various groups found in these experiments correlated well with the grouping based on cell envelope protein pattern. The correlation between LPS type and cell envelope protein pattern suggests a functional relation between LPS and cell envelope proteins. Furthermore, data obtained from a comparison of fatty acid profiles of eight cross-reacting strains concurred to some extend with the classification in cell envelope protein pattern (sub)groups. A strong homology in fatty acid profile was found for the strains S87 and S88 (subgroup B1). Some homology was found for the profiles of S72, S81 and S84 (subgroup B1) and on the other hand for the profiles of S44, S45 and S69, belonging to subgroup A1.

All *Pseudomonas* strains from wheat belonged to group B, but no relation was found between (sub)group and the source from which the other strains were isolated (Table 1).

Analysis of proteinase-K-treated cell envelopes of the *Pseudomonas* strains in Western blotting using antiserum 9024C, resulted in smears, indicating that the cross-reactive components were not proteins (Fig. 5). The reactions of the *Pseudomonas* strains with mca 2A4 directed against the LPS of Ech makes it likely that the smear is formed by the LPS O-chain. By using antiserum 9053C raised against an O-chain lacking mutant of Ech, further evidence was obtained that the smear was caused by a reaction of Ech-antibodies with the LPS O-chain. Only distinct cell envelope protein bands were detected with this antiserum (Fig. 3). Therefore, the LPS O-chain of the *Pseudomonas* strains is considered to be responsible for the cross-reactions with Ech-antibodies for at least 17 out of 18 strains in the different serological tests.

It is unlikely that the cell envelope proteins of the *Pseudomonas* strains that cross-react on blot play an important role in the cross-reactions found in IF and ELISA. Antiserum 9053C, cross-reacting with proteins of *Pseudomonas* on blot, did not react with these strains in IF. Furthermore, cell envelope proteins of Eca showed cross-reactions on blot too, but did not cross-react in IF and ELISA. Possibly LPS O-chain moieties prevent access of antibodies to membrane proteins anchored in the cell wall, as was also suggested by Jessop and Lambert (1985) for antibodies against outer membrane proteins of *Serratia marcescens*.

A similar remark can be made for the antibodies against the LPS core present in antiserum 9024C (Fig. 3). No cross-reactions of Ech-antibodies with Eca are found in serological assays in which intact bacterial cells are detected, while on blot strong cross-reactions are noticed with the LPS core of Eca in cell envelopes. Therefore it is unlikely that antibodies against the LPS core are important in cross-reactions in IF and ELISA. Antibodies directed against the LPS core are often only genus specific (Luk et al., 1991). The relatively strong reactions of antiserum 9053C with a 13.5 kD membrane protein of six representative *Pseudomonas* strains and of 31 and 32 kD proteins of three of them (S67, S70 and S71) as compared with the reaction levels of antiserum 9024C, may be explained by the increased attainableness of these proteins to the immune system, due to the absence of LPS O-chain moieties in Ech RH6065.

Acknowledgments

We wish to extend our thanks to Dr S.H. De Boer (Vancouver, Canada), Dr H. Huttinga and Dr J.W.L. van Vuurde for critical reading of the manuscript. Thanks are indebted to Dr. J.D. Janse (Dutch Plant Protection Service) for the identification of the bacteria and to Dr. D. Expert (INRA, Paris) for the generous gift of the mutant bacteria.

References

Allen, E. & Kelman, A., 1977. Immunofluorescent stain procedures for detection and identification of *Erwinia carotovora* var. *atroseptica*. Phytopathology 67: 1305–1312.

Cuppels, D. & Kelman, A., 1974. Evaluation of selective media for isolation of soft rot bacteria from soil and plant tissue. Phytopathology 64: 468–475.

De Vries, Ph.M. & Van Vuurde, J.W.L., 1992. Detectie van pathogene *Erwinia* spp. van aardappel in oppervlaktewater in de periode 1988–1991. IPO-DLO Report nr. 92-01.

De Weger, L.A., Van Boxtel, R., Van den Burg, B., Gruters, R.A., Geels, F.P., Schippers, B. & Lugtenberg, B., 1986. Siderophores and outer membrane proteins of antagonistic, plant growth-stimulating, root-colonizing *Pseudomonas* spp. Journal of Bacteriology 165: 585–594.

Geels, F.P. & Schippers, B., 1983. Selection of antagonistic fluorescent *Pseudomonas* spp. and their root colonization and persistence following treatment of seed potatoes. Phytopathologische Zeitschrift 108: 193–206.

Janse, J.D., Derks, J.H.J., Spit, B.E. & Van der Tuin, W.R., 1992. Classification of fluorescent soft rot *Pseudomonas* bacteria, including *P. marginalis* strains, using whole cell fatty acid analysis. Systematic and Applied Microbiology 15: 538-553.

Jessop, H.L. & Lambert, P.A., 1985. Immunochemical characterization of the outer membrane complex of *Serratia marcescens* and identification of the antigens accessible to antibodies on the cell surface. Journal of General Microbiology 131: 2343–2348.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685.

Luk, J.M.C., Lind, S.M., Tsang, R.S.W. & Lindberg, A.A., 1991. Epitope mapping of four monoclonal antibodies recognizing the hexose core domain of *Salmonella* lipopolysaccharide. Journal of Biological Biochemistry 226: 23215–23225.

Schoonejans, E., Expert, D. & Toussaint, A., 1987. Characterization and virulence properties of *Erwinia chrysanthemi* lipopolysaccharide defective 0EC2-resistant mutants. Journal of Bacteriology 169: 4011–4017.

Steinbuch, M. & Audran, R., 1969. The isolation of IgG from mammalian sera with the aid of caprylic acid. Archives of Biochemistry and Biophysics 134: 171–183.

Tobiás, I., Maat, D.Z. & Huttinga, H., 1982. Two Hungarian isolates of cucumber mosaic virus from sweet pepper (*Capsicum annuum*) and melon (*Cucumis melo*): identification and antiserum preparation. Netherlands Journal of Plant Pathology 88: 171–183.

Towbin, H., Staehelin, T. & Gordon, J., 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proceedings of the National Academy of Science USA 76: 4350–4354.

Van der Wolf, J.M. & Gussenhoven, G.C., 1992. Reaction of saprophytic bacteria from potato peel extracts and plant pathogenic bacteria in ELISA with antisera to *Erwinia chrysanthemi* (serogroup O_1H_a). Netherlands Journal of Plant Pathology 98: 33–44.

Van Vuurde, J.W.L., 1987. New approach in detecting phytopathogenic bacteria by combined immunoisolation and immunoidentification assays. EPPO Bulletin 17: 139–148.

Van Vuurde, J.W.L. & Roozen, N.J.M., 1990. Comparison of immunofluorescence colony-staining in media, selective isolation on pectate medium, ELISA and immunofluorescence cell staining for detection of *Erwinia carotovora* subsp. *atroseptica* and *E. chryanthemi* in cattle manure slurry. Netherlands Journal of Plant Pathology 96: 75–89.

Van Vuurde, J.W.L., Van den Bovenkamp, G.W. & Birnbaum, Y., 1983. Immunofluorescence microscopy and enzyme-linked immunosorbent assay as potential routine tests for the detection of *Pseudomonas syringae phaseolicola* and *Xanthomonas campestris* pv. *phaseoli* in bean seed. Seed Science & Technology 11: 547–559.

Vruggink, H. & Maas Geesteranus, H.P., 1975. Serological recognition of *Erwinia carotovora* subsp. *atroseptica*, the causal organism of potato blackleg. Potato Research 18: 546–555.