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Reaction of saprophytic bacteria from potato peel extracts and plant pathogenic bacteria in ELISA with antisera to Erwinia chrysanthemi (serogroup O_1H_a)

J.M. VAN DER WOLF and G.C. GUSSENHOVEN

Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, the Netherlands

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Abstract

The specificity of two antisera raised to whole cells of *Erwinia chrysanthemi* (Ech), serogroup O_1H_a, was studied in double antibody sandwich (DAS-) ELISA with 100 strains of different plant pathogenic bacteria (PPB), including 39 Ech strains, and of one of these antisera with 900 saprophytic bacteria isolated from extracts of potato peelings of Dutch seed potatoes grown in several production areas.

All tested European Ech strains from potato reacted positively while no reactions were observed with any of the other plant pathogenic bacterial species. Two saprophytes (A254 and A256), both identified as pectinolytic *Pseudomonas fluorescens* species, cross-reacted strongly with polyclonal antibodies against Ech. Non-specific reactions were found in DAS-ELISA with 16 saprophytes. The detection limits for the individual saprophytes varied between c. 10^5 and 10^9 cells.ml^{-1}. The non-specific reactions were also found with monoclonal antibodies (mca 2A4) against a proteinase K resistent epitope of Ech and with antisera against other plant pathogens including an antiserum against potato virus Y^N. The non-specific reactions were observed in DAS-ELISA, but not in Ouchterlony double diffusion or immunofluorescence colony-staining, whereas A254 and A256 reacted in all tests, but only with antibodies against Ech. When in making dilution series potato peel extracts were used instead of phosphate buffered saline with 0.1% Tween 20, the 14 non-specifically reacting saprophytes only reacted at concentrations of 10^9 cells.ml^{-1} or higher. Only one of these 14 saprophytes was able to multiply on injured potato tuber tissue.

In contrast to most saprophytic strains, the saprophytes A254 and A256 reacted strongly in ELISA in dilutions series made with potato peel extracts. A256 was able to grow on potato tuber tissue but only under low oxygen conditions; A254 did not grow at all on potato tissue.

Defatted milk powder or bovine serum albumin added to the dilution buffer for the enzyme-conjugated antibodies, drastically reduced the non-specific reactions, but not the reactions with A254 and A256.

To reduce the cross-reaction with A254, an Ech antiserum was absorbed with A254. This resulted in a substantial drop in antibody reaction with the homologous antigen in Ouchterlony double diffusion.

Additional keywords: blocking agent, Ouchterlony double diffusion, immunofluorescence colony-staining, monoclonal antibodies.

Introduction

Erwinia chrysanthemi (Ech) is a pectinolytic plant pathogenic bacterium, belonging to the *Enterobacteriaceae*, that can infect a number of plant species including potato (*Solanum tuberosum*) (Graham, 1972). In areas with a warm and humid climate this pathogen may cause stem rot in potato plants.

Samson and Nassan-Agha (1978) classified strains of Ech, isolated from a variety of hosts, into different serogroups based on somatic (O) and flagellar (H) antigens. In Europe only serogroup O_1H_a of Ech has been found on potato (Samson et al., 1987, Janse and Ruissen, 1988).

In the Netherlands, seed potatoes are routinely tested by the General Netherlands Inspection Service for Agricultural Seeds and Seed Potatoes (NAK) for the presence of *Erwinia carotovora* subsp. *atroseptica* (Eca) in DAS-ELISA. For lots containing potato tubers with high absorbance values in ELISA, a correlation of 60-72% was found with the development of symptoms in the field (Endel and Van Vuurde, 1987).

Attempts to develop a reliable ELISA for Ech were less successful. There was only a weak positive correlation between ELISA-results and symptom expression in the field (E. Endel, personal communication). This can be explained in different ways. The occurrence of the disease in the field is highly dependent on environmental conditions such as temperature, humidity and soil oxygen concentration (Pérombelon and Kelman, 1980). Tubers contaminated with low concentrations of Ech ($< 10^3$ cells.ml^{-1}) may cause severe symptoms under favourable conditions, while under unfavourable conditions for disease development, severely contaminated material may give rise to plants without symptoms. Low concentrations of bacteria can not be detected by DAS-ELISA. For *Erwinia* species the detection limit of this assay is 10^5-10^6 cells.ml^{-1} (Caron and Copeman, 1984). The presence of Ech belonging to another serogroup than O_1 may also give rise to false-negative reactions.

False-positive reactions occur due to the presence of high concentrations of dead bacterial cells in the test samples (Cother and Vruggink, 1980). Van Vuurde (pers. comm.) has shown that Eca cells rapidly die when present on the outside of potato tubers, because they only survive when they are protected in special 'niches' in or on the periderm. False-positive reactions can also be caused by cross-reacting organisms present in the tubers.

In this study the specificity of antisera against Ech in DAS-ELISA was investigated. The ability for cross-reacting organisms to grow on potato tuber tissue was studied in order to assess the risk on false-positive reactions. Methods were applied to reduce or to preclude observed cross-reactions.

Materials and methods

Media. All serological tests were performed with bacterial strains grown for 24 h at 27 °C on trypticase soy agar (TSA, BBL) unless otherwise stated. TSA and Merck Standard Nährbouillon Agar (NBA) at 1/6 of the concentration prescribed by the manufacturer (Trolldenier, 1972) were used for the isolation of saprophytes from the potato peel extracts. The ability of the saprophytes to degrade pectate was tested with Crystal Violet Pectate (CVP) medium (Cuppels and Kelman, 1974). Saprophytes were stored in a medium containing glycerin (15% v/v) and nutrient broth (0.8% w/v) at −20 °C.

Strains of plant pathogenic bacteria. For Ech the IPO strain nr. 502 (Ech 502) (= PD nr. 226) was used for antibody production, and it served as positive control in all tests. Eca IPO strain nr. 161 (Eca 161) (= PD nr. 230) was used as a negative control.

The following bacteria were tested in DAS-ELISA: *Erwinia chrysanthemi*: 9 strains isolated from potato in Europe; 10 strains isolated from potato outside Europe; 10 strains isolated from other hosts in Europe; 10 strains isolated from other hosts outside Europe, *Erwinia carotovora* subsp. *atroseptica*: 20 strains, *E.c.* subsp. *carotovora* (Ecc): 19 strains, *Agrobacterium tumefaciens*: 1 strain, *Pseudomonas* spp.: 11 strains, *Bacillus* spp.: 2 strains, *Corynebacterium* spp.: 3 strains.

Isolation of saprophytes from potato peel extracts. Saprophytes were collected from 20 potato lots, using tubers of 10 different potato cultivars grown on sand, clay or peat in different seed potatoes production areas in the Netherlands.

After harvesting, potato tubers were stored for a maximum of one week at 4-6 °C before use for isolation of saprophytic bacteria. Adhering soil was removed from the tubers without wetting them and a piece of the peel (2 × 9 cm), including the hylum end, was extracted with a Pollähne press, which was sterilized with ethanol between samples. The extract from each tuber was collected separately in a sterile tube and stored on ice. Six tubers were sampled from each of 20 tuber lots used. The extracts were diluted 1/10 and 1/100 in RP (Ringer + 0.4% Pepton) and plated on TSA and NBA. Plates were incubated for 24 h at 27 °C and thereafter for 24 h at 6 °C. Five to ten colonies that grew on TSA or NBA from each tuber peel extract, differing in size, colour or morphology were transferred to TSA to obtain pure cultures.

Growth of saprophytes on potato tuber tissue. Potato tubers (cv. Bintje) were surface sterilized in 0.1% hypochlorite during 5 min followed by three 5 min washes in sterile water. Sterility was confirmed by pressing the tubers on TSA-plates and observing for microbial growth. For tests under aerobic conditions tubers were cut in c. 1 cm thick slices and put on water soaked filter paper in Petri dishes. The slices were inoculated with 50 μl of a bacterial suspension (c. 10^5 or 10^7 cells.ml^{-1}) in 0.85% NaCl. Inoculation with *Xanthomonas campestris* pv. *pelargonii* (Xcp) served as a negative control. The plates were sealed with Parafilm and incubated in plastic bags at 27 °C in the dark for 1 week. The potato slices were visually examined for rotting and discoloration each day.

For tests under low oxygen conditions, bacterial slime was taken from a culture grown on TSA-slants at 27 °C for 24 h with a sterile tooth-pick and inserted into the tuber to a depth of c. 1.5 cm. The tooth-pick was broken off at the surface of the tuber. The tuber was then wrapped in a wet paper towel and covered with 3 layers of plastic film and incubated at 27 °C in the dark. After 3 days of incubation, the tubers were cut through at the site of inoculation and were visually examined for rotting and discoloration. Discoloured and unaffected tissues directly under the peel were tested for the presence of bacterial growth by inoculation onto TSA-plates.

Antiserum production. Antisera 8575E and 8276B against Ech 502, 8567G and 8567L against Eca 161 and 8362 against *Xanthomonas campestris* pv. *begoniae* IPO strainnr. 206 (Xcb 206) were produced according to Vruggink and Maas Geesteranus (1975). A control antiserum against potato virus Y^N (isolate PVY^N CH 605 of P.

Gugerli, Nyon) was kindly provided by D.Z. Maat (IPO-DLO, Wageningen).

Antibodies were purified from crude antisera as described by Steinbuch and Audran (1969). Purified antibodies were conjugated with alkaline phosphatase (Boehringer, nr. 567 752) according to the method described by Tobiás et al. (1982).

For conjugation with fluorescein isothiocyanate (FITC) (Sigma, F7250) the immunoglobulins were purified and conjugated with FITC according to the method of Allen and Kelman (1977).

Monoclonal antibody 2A4 to Ech 502 was prepared by S.H. De Boer (Vancouver, Canada) and was shown to react with a proteinase K resistent epitope (unpublished results).

Absorption of antiserum. Several bleedings of antiserum 8575E were absorbed with A254 by mixing bacterial growth (bacteria grown on TSA for 24 h at 27 °C) in the antisera and incubating for 30 min at 37 °C. The agglutinates were removed by centrifugation (10 min, 10 000 *g*). This procedure was repeated till no more agglutination could be observed. The absorption was checked by Ouchterlony double diffusion.

DAS-ELISA. DAS-ELISA was performed essentially as described by Clark and Adams (1977). Microplates (Inotech, 11041E) were coated with purified immunoglobulins (c. 0.5 μg.ml^{-1}) for 24 h at 4-6 °C in sodium carbonate buffer (0.05 M CO_3^{2-}, pH 9.6). A volume of 150 μl per well was used in all incubation steps. The microplates were washed with a plate washer, using tap water between each incubation step.

In the first test with 900 saprophytes from potato peelings, the isolates were grown on TSA-plates and bacterial growth of individual colonies was suspended in PBST (0.27 M NaCl, 0.04 M Na_2HPO_4, 2.9 mM KH_2PO_4, 5.4 mM KCl, 0.01% NaN_3, 0.1 % Tween 20, pH 7.2) to obtain OD_{620}-values of 0.3-1.0. In all other tests the bacteria were grown on slopes with TSA and suspended in PBST to obtain OD_{620}-values of 0.8 (c. 10^9 cells.ml^{-1}). These suspensions were used for making serial dilutions in PBST. In one experiment the serial dilution was made in an extract from potato peelings. Bacterial suspensions were incubated in antibody-coated microplates for 24 h at 4-6 °C.

The enzyme-conjugated antibodies (c. 0.3 μg.ml^{-1}) were incubated 2 h at 27 °C. In the blocking experiments 1% bovine serum albumin (Sigma, A4378) or 0.1%, 1.0% or 5.0% defatted milk powder was added to the PBST. Para-nitrophenyl phosphate (p-NPP, Sigma 105-104) was used as the enzyme substrate at a concentration of 0.75 mg.ml^{-1}. Substrate conversion was measured with an EAR-400 spectrophotometer (SLT) after different incubation times, and expressed as the difference between the absorbance at 405 nm and the absorbance at 645 nm (reference value).

Ouchterlony double diffusion. Ouchterlony double diffusion tests (ODD) were performed according to De Boer et al. (1979) with a few modifications. Wells, 4 mm in diameter, were 4 mm apart from each other. All saprophytes were tested with and without added phenol (50 μl of liquefied phenol per ml of suspension).

Pre-immune serum and a heterologous bacterium served as controls. The plates were incubated at 27 °C for 48 h and examined with a stereomicroscope (Wild M7S) with dark-field illumination at c. 6 times magnification.

Immunofluorescence colony-staining. Immunofluorescence colony-staining (IFC) was performed in 24-well tissue-culture plates as described by Van Vuurde and Roozen (1990). Agar films with 50-500 bacterial colonies were stained with FITC-conjugated antibodies (8276B) against Ech 502. Staining of the films with FITC-conjugated Eca antibodies (8567L) served as a control.

Results

Reactions of saprophytes in DAS-ELISA. In DAS-ELISA no cross-reactions of Ech antisera 8276B and 8575E with any of the strains of plant pathogenic bacteria were observed. All 39 Ech strains, all isolated in Europe, reacted with the antisera. However, 8 out of 20 Ech strains isolated from plants grown outside Europe did not react. Five of them were isolated from potato plants grown in Australia.

Eighteen of the 900 saprophytes, isolated from potato peel extracts reacted with Ech antiserum 8575E in DAS-ELISA. These strains together with 27 strains that did not react in the first screening were tested again in DAS-ELISA with antiserum 8276B and mca 2A4 against Ech 502. The results (Table 1, part A) confirmed the data of the first screening. The 18 strains reacted with antisera 8575E and 8276B, whereas the other 27 did not (data of the latter 27 strains not shown). Antiserum 8276B reacted with the saprophytes almost to the same extent as 8575E. The reactions of various saprophytes with certain antisera in DAS-ELISA differed largely. A254 and A256 reacted at concentrations of c. 10^5 cells.ml^{-1} with antiserum 8276B, while A402, A429, A468, and A632 only reacted at concentrations of c. 10^9 cells.ml^{-1}. Mca 2A4 reacted even stronger with 10 of the 18 saprophytes than with the homologue Ech 502.

These 18 saprophytes were tested with antisera against Ech, Eca, Xcb and PVY in DAS-ELISA, to investigate whether these reactions were due to non-specific bindings with immunoglobulins (Table 1, part B). Seven saprophytes reacted with the four antisera tested, whereas seven isolates reacted only with three of them; no reactions with antiserum 8567G were obtained with these strains. Compared with the other antisera, the Eca antiserum reacted generally weak with the saprophytes. In contrast to the non-specifically reacting saprophytes, A254 and A256 reacted only with antibodies against Ech. The values of the individual saprophytes were in many cases different from the values listed in Table 1, part A. Two of the saprophytes, viz. A751 and A818, that reacted in the first screening did not react with the Ech antibodies in this experiment.

The 18 saprophytes did not produce typical *Erwinia* pits on CVP. However two of these isolates viz. A254 and A256, formed shallow depressions in CVP. Cross-reacting strains A254 and A256 were identified by the Dutch Plant Protection Service as *Pseudomonas fluorescens*; the non-specifically reacting saprophytes A404 and A726 as gram-variable bacteria, probably *Bacillus species*, while A632 was identified as *Bacillus pumilus* (J.D. Janse, pers. comm.)

Reactions of saprophytes in ODD and IFC. The reactions of 16 out of 18 saprophytic strains with antisera to different groups of plant pathogens indicated that these reactions in DAS-ELISA were not due to antigen-antibody interactions, but were caused by a non-specific type of binding. To confirm this hypothesis, the saprophytes were tested in other serological tests.

In ODD a precipitin line with Ech antiserum 8575E was only formed with A254 and

Table 1. Reaction of bacterial isolates from potato peel extracts in DAS-ELISA subsequently tested with antibodies against Ech 502 (A) and antisera against various plant pathogens (B).

Strainnr.	A			B			
	8276B	8575E	2A4	8567G (Eca)	8362 (Xcb)	PVY^N	8276B (Ech)
A237	8[1]	8	8	-[2]	9	8	9
A254	5	5	5	–	–	–	5
A256	5	5	5	–	–	–	5
A402	9	7	7	8	7	7	7
A404	8	7	7	7	7	6	6
A429	9	7	7	8	7	7	8
A447	8	7	7	9	7	8	8
A468	9	8	8	9	9	9	9
A480	6	6	6	–	8	8	9
A554	8	8	8	–	9	9	9
A607	7	7	7	–	8	8	8
A632	9	8	8	9	8	9	8
A726	7	7	7	6	6	7	7
A751	7	7	6	–	8	8	–
A818	7	7	7	–	–	–	–
A826	7	7	6	–	7	8	8
A839	8	7	7	–	7	7	8
A857	7	7	6	–	7	8	8
Ech 502	6	6	8	NT[4]	NT	NT	5
Eca 161	C	C	C	5	C[5]	C	C
Xcb 206	NT	NT	NT	NT	6	NT	NT
PVY^N	NT	NT	NT	NT	NT	+[3]	NT

[1] The reaction is expressed as the ^{10}log of the minimum number of bacteria.ml^{-1} at which a significant reaction was obtained (data based on duplicates). The Ech values are based on 10 replicates. A reaction is considered to be significant positive when the A_{405} is at least twice the A_{405} of the same dilution of Eca (negative control).

[2] – = no reaction.

[3] + = positive reaction.

[4] NT = not tested.

[5] C = negative control.

A256. A spur was observed in both reactions with the homologue, indicating a distinct serological relationship between the two saprophytes and Ech (Fig. 1). The fusion of the precipitation lines between A254 and A256 indicated a full serological homology between these saprophytes.

In IFC none of the tested saprophytes reacted except A254 and A256. The results with Ech 502, Eca 161, A254 and A256 are given in Table 2.

ELISA-reactions of saprophytes diluted in potato extracts. Serial dilutions of non-specifically reacting saprophytes in potato peel extracts were tested in DAS-ELISA, to investigate whether these saprophytes can cause false-positive reactions in seed

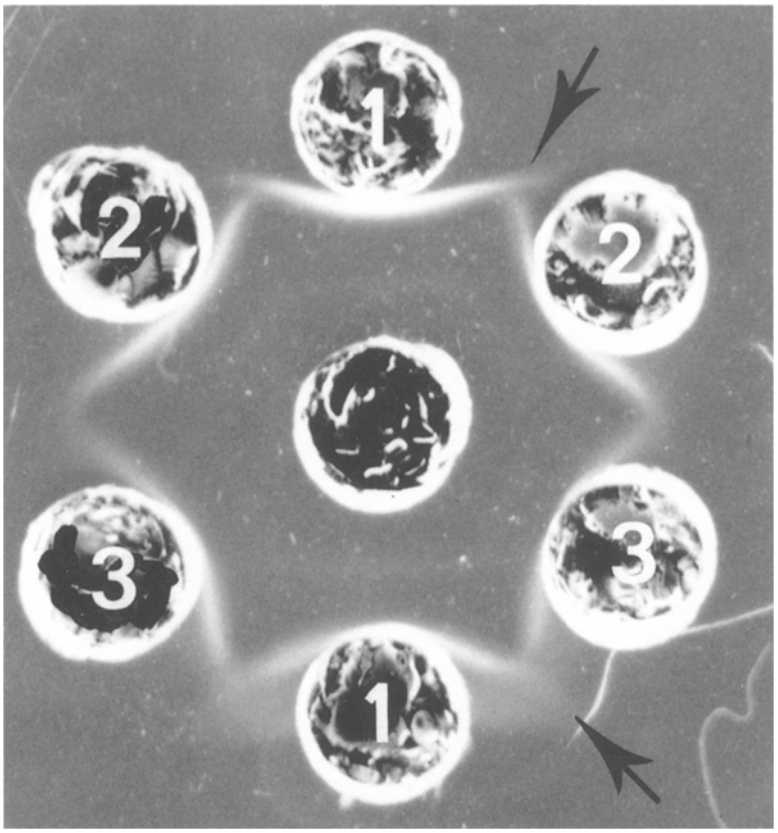

Fig. 1. Reaction of saprophytic bacteria A254 and A256 with antiserum 8575E (center hole) against *Erwinia chrysanthemi* (Ech) in an Ouchterlony double diffusion test.
1) Ech 502, 2) A254, 3) A256. Arrows indicate the spurs between Ech and both saprophytes.

Table 2. Reaction of saprophytic bacteria A254 and A256, in immunofluorescence colony-staining with Ech and Eca FITC-conjugated antibodies.

Strain	IFC reaction	
	8276B (Ech)	8567L (Eca)
Ech 502	4[1]	1
Eca 161	1	4
A254	3	1
A256	3	1

[1] 1 = brown/green stained colonies (weak fluorescence); 2 = yellow/green fluorescence; 3 = green fluorescence; 4 = brilliant green fluorescence.

Table 3. Reaction of saprophytic bacteria diluted in potato peel extracts or PBST in DAS-ELISA with antiserum 8276B, and the blocking effect of defatted milk powder (5% w/v) on the non-specific reaction.

Isolate	Without milk powder		With milk powder	
	bacteria in a potato extract	bacteria in PBST	bacteria in a potato extract	bacteria in PBST
A237	–[2]	9[1]	–	–
A254	8	7	8	7
A256	6	5	6	5
A402	–	6	–	–
A404	–	8	–	–
A429	9	7	–	–
A447	9	7	–	–
A468	–	8	–	–
A480	9	7	–	–
A607	–	8	–	–
A632	9	9	–	–
A726	–	6	–	–
A751	9	7	–	–
A818	9	7	9	–
A826	9	7	NT[3]	–
A839	9	7	–	–
A857	9	7	–	–
Ech 502	5	5	5	5

[1] The reaction is expressed as the ^{10}log of the minimum number of bacteria.ml^{-1} at which a significant reaction was obtained (data based on duplicates). A reaction is considered to be significant positive when the A_{405} is at least twice the A_{405} of the same dilution of Eca (negative control).

[2] – = no reaction.

[3] NT = not tested.

indexing programs. Saprophytes diluted in PBST served as a control.

Non-specific reactions of the saprophytes with the Ech antiserum 8276B were strongly reduced when they were diluted with an extract of potato peelings instead of with PBST (Table 3). In potato extracts no reaction was detected with A402 and A726, while in PBST even concentrations of 10^6 cells.ml^{-1} could be detected.

Growth of saprophytes on injured potato tubers. The ability of the non-specifically reacting and cross-reacting saprophytes to grow on potato tuber tissue was tested to determine whether or not these saprophytes could reach high densities, thus increasing the risk of false-positive reactions in DAS-ELISA. Under aerobic conditions only A632 was able to cause rot on potato slices. Bacterial slime was clearly visible and the tuber tissue was decayed at the point of inoculation. The potato slices inoculated with A256 became brown at the edges of the inoculation site, but no decay of the parenchyma occurred. No symptoms developed on the slices inoculated with the other

saprophytes. The slices inoculated with Ech 502 and Eca 161 were discoloured and showed extensive rotting, while Xcp 272 did not affect the potato slices.

Under low oxygen conditions A632 caused extensive decay of the potato tuber, while A256 only caused a slight decay of the tubers. The other saprophytes did not affect the tubers. The tubers used for this test were not completely sterile after the treatment with hypochlorite. Potato tubers incubated under low oxygen conditions also developed large (1-10 cm^2), randomly spread, dark brown areas on the surface. In these areas the number of bacteria was comparable to that in the not-discoloured areas. Tubers inoculated with Ech 502 and Eca 161 were completely macerated around the site of inoculation, but no discoloration occurred. Xcp 272 had no effect on the tuber tissue.

Reduction of reactions caused by saprophytes. To reduce non-specific reactions with the saprophytes in DAS-ELISA, the effect of the blocking agents defatted milk powder and bovine serum albumin (BSA) was investigated by adding them to the solution with the enzyme-conjugated antibodies.

The addition of milk powder or BSA drastically reduced the reaction of non-specifically reacting saprophytes in DAS-ELISA, whereas the reaction of Ech 502 and A254 was not affected (Table 4). No effect of the milk powder concentrations (5%, 1% or 0.1%) was found on the detection threshold of the homologous reaction, but the reaction tended to be higher at the lower concentrations of milk powder (data not shown).

The milk powder further reduced the reaction of the saprophytes diluted in potato peel extracts (Table 3). Only A818 still reacted weakly after addition of the milk powder.

Cross-absorption of Ech antiserum 8575E with A254 caused a fourfold reduction in titre in ODD.

Table 4. Effect of BSA and milk powder, added as blocking agents to enzyme-conjugated antibodies against Ech, on the reaction with Ech and non-specifically reacting saprophytes, in DAS-ELISA.

	Strains				
	Ech 502	A254	A402	A429	A480
PBST	5[1]	5	7	7	8
+ 5% milk powder	5	5	–[2]	–	–
+ 1% milk powder	5	NT[3]	–	–	–
+ 0.1% milk powder	5	NT	–	–	–
+ 1% BSA	5	NT	–	–	–

[1] The reaction is expressed as the ^{10}log of the minimum number of bacteria.ml^{-1} at which a significant reaction was obtained (data based on duplicates). A reaction is considered to be significant when the A_{405} is at least twice the A_{405} of the same dilution of Eca (negative control).

[2] – = no reaction.

[3] NT = not tested.

Discussion

Cross-reactions of saprophytic bacterial isolates with antisera against plant pathogenic bacteria are reported already for a number of different plant pathogenic bacteria (Schaad, 1979; Miller, 1984; De Boer et al., 1979). This is the first report in which cross-reactions with antisera against Ech are described. From a collection of 900 saprophytic bacterial isolates from potato two *Pseudomonas fluorescens* strains (A254 and A256) strongly cross-reacted in all serological tests that were applied. The ODD results indicated that A254 and A256 were serologically only partially related with Ech. Although A254 and A256 were isolated from the same potato sample, and in ODD the precipitation lines of A254 fused with A256 using antibodies against Ech (Fig. 1), protein profiles of cell extracts of A254 and A256 in sodium dodecyl sulphate polyacrylamide gelelectrophoresis showed that the strains were different (data not shown).

Apart from these serological cross-reactions, in DAS-ELISA also non-specific reactions were observed with 16 saprophytes. Saprophytes A751 and A818 did not consistently react with Ech antisera in all experiments. Strong evidence was obtained that these reactions were not caused by a typical antigen-antibody interaction:

1. Fifteen isolates also reacted with antisera against PVY^N and *Xanthomonas campestris* pv. *begoniae* (Table 1, part B).
2. The isolates belonged to different groups of bacteria (Gram-positive and Gram-negative). Therefore it is not likely that these different isolates possess the same type of dominant antigen.
3. The isolates did not react with Ech antisera in other serological procedures such as ODD or IFC (Table 2).
4. In contrast with the serological reactions, these non-specific reactions could be inhibited easily with blocking agents (Tables 3 and 4), indicating a relatively weak binding. The variation in results between individual experiments in Table 1 (columns 1 and 7) may be explained by this fact.

It is not likely that a reaction of protein A or a similar type of component in the bacterial cell wall with the F_c-region of the antibodies caused the non-specific binding, because no reactions were observed in other serological tests in which the same antibodies were used.

The probability of a false-positive result in DAS-ELISA caused by the non-specifically reacting saprophytes in seed indexing programs seem to be limited for the following reasons:

1. Potato extract acts as a blocking agent (Table 3). Saprophytes that caused strong reactions in PBST only reacted in a potato extract at a level of c. 10^9 cells.ml^{-1}.
2. Only one of the 16 saprophytes (A632) was able to multiply on potato tuber tissue under aerobic or low oxygen conditions. Therefore there is only a small chance that high cell densities will develop in potato tubers. Only when soil material adhering to the tubers contains high numbers of non-specifically reacting saprophytes, the detection threshold may be crossed.

The strains A254 and A256 potentially offer more risks for false-positive results in serological tests. Both strains cause cross-reactions at low concentrations (c. 10^5 cells.ml^{-1}). Furthermore A256, a pectinolytic organism, is able to grow on potato tissue and can possibly multiply to a high cell density.

The addition of milk powder or BSA to the conjugate buffer further reduced non-specific reactions of the saprophytes remarkably, whereas the reaction with Ech was hardly affected (Table 3 and 4). Also the general background values dropped drastically as a result of the blocking. This decrease in background values improved the detection level of the test, because of an improved signal to noise ratio. Similar effects of milk powder on the reduction of background were found by Johnson et al. (1984).

Absorption of antisera to Ech with A254 was sufficient to eliminate the cross-reaction with A254 and also in A256, but resulted in a loss of activity for Ech.

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