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Cross-reactions of Corynebacterium sepedonicum antisera with soil bacteria associated with potato tubers

H.J. MILLER

Plant Protection Service, P.O. Box 9102, 6700 HC Wageningen, the Netherlands

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Abstract

The occurrence of low numbers of fluorescing coryneform bacteria located by immunofluorescence microscopy (IF) in heel-end extracts of healthy potatoes was demonstrated to be a normal phenomenon. The number of bacteria found was highly dependent on the antiserum dilution. Bacteria isolated from other healthy potato material also cross-reacted, mostly at low antiserum dilutions compared with homologous isolates or bacteria of the same species, which reacted at a dilution of 1 : 1280. Only relatively small differences were found between the antisera tested. Failure to recognize the occurrence of cross-reacting unknown soil bacteria, indicated by the data presented in this paper, can only increase the dangers of incorrect interpretation of IF results.

Additional keywords: immunofluorescence microscopy, coryneform bacteria, antiserum dilution.

Introduction

Immunofluorescence microscopy (IF) in diagnostic phytobacteriology has recently been receiving much attention. However, IF is frequently incorrectly applied and cross-reactions which occur in such investigations are often incorrectly interpreted. This situation reflects our lack of knowledge in a developing field. An incorrect diagnosis can lead to serious national and international problems, especially when dealing with a quarantine disease such as ringrot of potatoes caused by *Corynebacterium sepedonicum* (Spieckerman & Kotthoff) Skaptason & Burkholder.

As the result of an improvement in the method for detection of *C. sepedonicum* by IF microscopy (Miller, 1984) and information regarding factors which influence the IF technique (Miller, 1983), it is possible to look at the question of interpretation more closely. It is the main purpose of this paper, therefore, to examine several results from recent cross-reactions studies.

Material and methods

Potato heel-end extracts (200 tubers of Dutch origin per sample), as well as an extract made from Canadian potatoes, were supplied by Professor U. Mazzucchi, Bologna, Italy.

Ten isolates of bacteria of bacteria found in or on potatoes and believed to cross-

Table 1. Immunofluorescence microscopy results using cultures of C. sepedonicum and potato heel-end extracts of healthy potatoes against two antisera prepared against *C. sepedonicum* PD 37 (Dutch antiserum) and NCPPB 2140 (Italian antiserum).

Material examined	Dutch antiserum			Italian antiserum		
	titre	approx. number[1] of cells/ml	number of cells/ml at 1 : 40 dilution	titre	approx. number of cells/ml	number of/ml at 1 : 40 dilution
C. sepedonicum						
PD 37	1 : 1280	10^6	n.d.[2]	1 : 1280	10^6	n.d.
NCPPB 2140	1 : 1280	10^6	n.d.	1 : 1280	10^6	n.d.
NCPPB 2136	1 : 1280	10^6	n.d.	1 : 1280	10^6	n.d.
Heel-end extracts						
N12 1-2192	1 : 160	40	160	1 : 320	150	8.10^2[3]
N19 1-1001	1 : 80	40	80	1 : 320	80	6.10^2
N23 1-2020	1 : 320	80	2.10^2	1 : 320	80	4.10^2
N64 8-3334	0	0	0	1 : 160	150	4.10^2
V19 5-2291	1 : 160	150	3.10^2	1 : 160	150	6.10^2
V23 1-0218	1 : 40	80	80	1 : 160	80	4.10^2
Bo15 1-0494	1 : 40	80	80	1 : 80	80	2.10^2
Postive extract (Canada)[4]	1 : 1280	5.10^6	n.d.	1 : 1280	5.10^6	n.d.

[1] Fluorescing coryneform cells.
[2] n.d. = not done.
[3] Our results as published by Mainolfi et al. (1982).
[4] Diluted 1 : 10.

react with *Corynebacterium sepedonicum* antiserum were obtained from Dr. H.P. Bræke, Ås, Norway (Table 3). All other bacteria were our own isolates or were obtained from the National Collection of Plant Pathogenic Bacteria, England (NCPPB numbers).

Pathogenicity to eggplant was determined as described by Miller (1984).

Isolates were grown on Difco Nutrient Agar for 2 to 5 days prior to suspension in 0.01M phosphate buffered saline pH 7.2 for serological testing. The immunofluorescence technique applied was the same as used by Miller (1983, 1984). Antiserum was either purchased from Le Service de la Protection des Végétaux, Angers, France, or obtained from Professor Mazzucchi, Bologna, Italy, or the same as used by Miller (1984).

Results

Table 1 contains the complete Dutch results of IF tests on heel-end extracts which had been prepared in Italy from Dutch potatoes. These results were presented at a meeting of the European Economic Community and were published in part by Mainolfi et al., (1982). Pathogenicity tests of all extracts originating from Dutch potatoes were negative in the eggplant test (c. 25 plants per extract). The extract from Canadian

Table 2. Cross-reactions with isolates of known plant pathogenic bacteria and unidentified soil coryneform bacteria isolated from potato tubers. Antisera used as in Table 1.

Isolates	Titre	
	Dutch antiserum	Italian antiserum
Corynebacterium sepedonicum (PD 37)	1 : 1280	1 : 1280
C. sepedonicum (NCPPB 2140)	1 : 1280	1 : 1280
C. michiganense (81/96)	1 : 80	1 : 80
C. insidiosum (NCPPB 1634)	1 : 40	1 : 40
C. tritici (NCPPB 1953)	–[1]	1 : 10
C. betae (PD 149)	–	–
C. poinsettiae (NCPPB 844)	–	–
C. nebraskense (PD 174)	1 : 10	1 : 40
C. fascians (80/804)	–	–
C. flaccumfaciens (NCPPB 2343)	–	1 : 40
Erwinia carotovora subsp. *carotovora* (80/727)	–	–
E. carotovora subsp. *atroseptica* (81/91)	–	–
E. chrysanthemi	–	–
Soil coryneform (PD 72)	1 : 20	1 : 40
Soil coryneform (77/1212)	–	1 : 20
Soil coryneform (80/625)	1 : 10	1 : 10
Soil coryneform (79/39)	1 : 10	1 : 160
Soil coryneform (80/626)	1 : 160	1 : 80

[1] – = negative reaction at an antiserum dilution of 1 : 10

Table 3. Characteristics and cross-reactions of ten Norwegian bacterial isolates from potato with Dutch antiserum (see Table 1) and a French antiserum prepared against *C. sepedonicum* NCPPB 2140.

Isolate	Gram stain reaction	Cell morphology	Titre	
			Dutch antiserum	French antiserum
2	−[1]	n.a.[2]	1 : 80	1 : 10
7	+	not coryneform	< 1 : 10	< 1 : 10
8	+	not coryneform	1 : 40	1 : 40
9	+	coryneform	< 1 : 10	1 : 20
10	−	n.a.	< 1 : 10	< 1 : 10
11	−	n.a.	−[3]	−
12	−	n.a.	−	1 : 20
13	−	n.a.	1 : 40	−
14	−	n.a.	−	−
15	−	n.a.	1 : 20	−
C. sepedonicum (NCPPB 2140)	+	coryneform	1 : 1280	1 : 1280
C. sepedonicum (PD 37)	+	coryneform	1 : 1280	1 : 1280

[1] Gram stain reaction: + = positive reaction; − = negative reaction.
[2] Cell morphology: n.a. = not applicable.
[3] Titre: − = no detectable reaction at an antiserum dilution of 1 : 10.

potatoes produced symptoms typical for ringrot within 10 days and *C. sepedonicum* was isolated.

Cross-reactions with different *Corynebacterium* species, isolates of bacteria known to be pathogenic to potato and isolates of soil coryneform bacteria found either adhering to potato tubers or within potatoes are given in Table 2.

Bacteria isolated from potatoes in Norway and reported to produce positive reactions with IF (personal communication) were sent by Dr Bræke for further study. Gram positive isolates were examined for their cell morphology and all were tested with the IF technique using serial dilutions of Dutch and French antisera (Table 3). The Norwegian isolates were subjected to the eggplant test but they were all found to be non-pathogenic.

Discussion

Cross-reacting coryneform bacteria from healthy potato extracts have been reported by Miller (1984) who demonstrated that a mean (from 48 extracts) of c. $2.5\text{-}5.10^3$ fluorescent coryneform cells/ml at antiserum dilutions 1:640 - 1:1280 constitute a normal level of IF background tolerance. Miller also showed that a high correlation between antiserum dilution and numbers of cross-reacting bacteria exists and that at a

dilution of 1 : 40 the numbers of cross-reacting bacteria from healthy potato extracts have a mean value of c. 2.10^4 cells/ml. In one healthy potato lot a mean of c. 2.10^5 cells/ml extract was found at a dilution of 1 : 40 but at a dilution of 1 : 1280 fluorescing cells were no longer found. This supports the Dutch conclusion that not only were all the potato heel-end extracts from numbers N12 1-2192 to Bo15 1-0494 (Table 1) free of *C. sepedonicum* but remarkably low in numbers of cross-reacting fluorescent coryneform bacteria. The conclusion of Mainolfi et al. (1982) that numbers N12 1-2192 and N23 1-2020 were positive for *C. sepedonicum* is therefore totally irresponsible. Mainolfi et al. made no mention of antisera titres with homologous isolates, other isolates of *C. sepedonicum* or positive control extracts; neither were they able to provide any evidence with regards to the specificity of antisera. These authors failed to support their claim, as pathogenicity tests which they carried out in eggplants were also reported to be negative. Our own pathogenicity tests on these extracts confirmed this fact.

A serological relationship between members of the genus *Corynebacterium* is not surprising even though the IF titres were at al low level (Table 2). This has already been demonstrated by Lazar (1968) who studied *Corynebacterium* species with the aid of pricipitin, double gel-diffusion and immuno-electrophoretic techniques. More alarming are the results obtained with cross-reacting soil coryneform bacteria, but compared to the positive controls used, they usually reacted at much lower dilutions as has been demonstrated in this paper and by Miller (1984). Even the isolates received from Norway (Table 3) reacted at relatively low titres with both Dutch and French antisera. Calzolari et al. (1982), however, reported cross-reactions of *C. sepedonicum* antiserum with *Arthrobacter polychromogenes* at a dilution of 1 : 1000 and stated that related organisms in the soil could be responsible for the detection of cross-reacting bacteria in or on potatoes even at high antiserum dilutions. Similar findings have also been reported by Crowley & De Boer (1982).

The relatively small differences between antisera (Tables 1, 2 and 3) can be explained by the variations in methods used to prepare antisera as well as differences in antibody production patterns found among rabbits. This has been reported by De Boer (1982) who also found that the isolated immunoglobulin G component of antisera gave greater specificity when obtained from rabbits at earlier dates after immunization. The increase of antibody specificity by adsorption does not yet seem to be practicable according to Crowley & De Boer (1982) who were unable to remove all cross-reacting antibodies in their experiments. In this situation, therefore, the dilution method would appear to be a suitable method in cross-reaction studies.

Samenvatting

Kruisreacties van antisera tegen Corynebacterium sepedonicum met bodembacteriën afkomstig van aardappelknollen

Het optreden van lage aantallen fluorescerende, coryneforme bacteriën gevonden met behulp van immunofluorescentiemicroscopie in extracten van naveleinden van gezonde aardappelen, bleek een normaal verschijnsel te zijn. Het aantal gevonden bacteriën was sterk afhankelijk van de verdunning van het antiserum. Bacteriën die geïsoleerd werden van ander gezond aardappelmateriaal vertoonden ook kruisreac-

ties, meestal bij lage verdunningen van het antiserum in vergelijking met homologe isolaten of bacteriën van dezelfde soort, die beide nog reageerden bij een verdunning van 1 : 1280. Tussen de getoetste antisera bleken slechts geringe onderlinge verschillen te bestaan. De gegevens in dit artikel onderstrepen het gevaar van een onjuiste interpretatie van IF-resultaten, wanneer het voorkomen van kruisreagerende, onbekende bodembacteriën over het hoofd wordt gezien.

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