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Sero-Diagnosis of Bacterial Pathogens Associated with Rotted Potato Tubers

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Abstract Studies were conducted in 2010-2011 on serological detection methods for identification of bacterial pathogens associated with rotted potato tubers. One hundred and two numbers of rotten potato tubers of variety Kufri Jyoti were collected from freshly harvested lot Tubers were out down to two equal halves and categorized into 6 groups, on the basis of internal symptoms exhibited. The association of two bacterial species were assayed following tube agglutination test using the known antiserum for each bacterium, *Ralstonia solanacearum* and *Pectobacterium carotovorum*. It was revealed that *Ralstonia solanacearum* could be found associated exclusively in 54.10% of diseased tubers of symptom showing brownish discoloration along the vascular region. Similarly, exclusive association of *P. carotovorum* could be detected in 87.50% of the rotten tubers of symptom showing soft rotten tissues extending towards center without brownish discoloration. In other categories both the test bacterium was found to be associated either singly or as mixture. Least bacterial infection due to *P. carotovorum* (12.5%) was observed in symptoms showing dry tissues with cavities surrounded with soft tissues around. It was very quicker detection methodology which could revealed the percentage of bacterial pathogen association within two to three hours after testing.

Keywords Sero-diagnosis, Bacterial pathogens, Rotted, Potato tubers.

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Introduction

Rotting of potato tubers is commonly noticed at the time of harvest, in storage at country stores and also at cold stores. The rotted tubers exhibit brown rot, soft rot and mixed symptoms. It is very much necessary about the accurate identification of the causal pathogen as management strategy is almost different for different organisms. The usual method of identification of the causal bacterial plant pathogen involves isolation, pathogenicity test followed by Gram staining, microscopic studies, growing them on selective medium, a series of biochemical tests and carbohydrate tests, and other tests like studies at molecular levels [1—3]. The characterization of soft rot bacterial strain of potato was done following physiological and biochemical tests such as (i) potato soft rot test, (ii) GRAM reaction test, (iii) glucose fermentation, oxidase reaction, (iv) catalase test and (v) gelatine liquefaction test, nitrite test, indole test and lecithinase test [4]. Similarly some workers isolated forty strains from macerated potato tubers and water soaked lesions of some ornamental plants in north parts of Iran, proved their pathogenicity in their respective horts and the causal organisms were identified as *Pectobacterium* spp. based on their physiological and biochemical assays and confirmed by

Table 1. Serological detection of *Ralstonia solanacearum* and *Pectobacterium carotovarum* associated with different categories of rotted tubers. A* = Cut tubers showing brownish discoloration along vascular region, B* = Cut tubers showing brownish discoloration along vascular region with soft rotten cavities filled with whitish ooze, C* = Cut tubers showing brownish - black discoloration along vascular region and soft rotting tissues extending towards center, D* = Cut tubers showing soft rotten tissues extending towards the center without brownish discoloration, E* = Cut tubers showing soft rotten tissues extending towards the center with brownish black discoloration surrounded by corky tissues, F* = Cut tubers showing dry tissues with cavities surrounded by corky with soft tissues around.

Sl. No.	Category	Percent of tubers under different category	*R. solana-cearum*	*P. caroto-vorum*	Mixed infection (%)	No test bacteria
1	A*	23.52	54.10	0.00	0.00	45.90
2	B*	15.68	56.20	31.20	12.60	0.00
3	C*	23.52	0.00	0.00	83.30	16.70
4	D*	7.84	0.00	87.50	0.00	12.60
5	E*	21.56	22.70	45.40	31.90	0.00
6	F*	7.84	0.00	12.50	0.00	87.50

species and subspecies following specific PCR and RFLP analysis of 16S–23S intergenic transcribed spacer region.

Both the bacteria (*Ralstonia solanacearum* and *Pectobacterium carotovorum*) cause rotting in harvest, and mostly *P. carotovorum* continue to enhance the rotting in transit and storage and spread easily by contact. Quick detection will help in adopting adequate management practices at earliest possible and the loss can be reduced subsequently. Among different methods the serological detect ion method is also a most accurate simple and quicker technique. By using specific antiserum the occurrence of particular race or strain of a particular plant pathogenic bacteria and virus can be studied easily and also at earliest time and further studies can be done ultimately. Hence in present study this methodology was followed for identification of bacteria associated with rotted potato tubers.

Materials and Methods

The apparently rotted tubers (102) were collected from the harvested lot of All India Coordinated Potato Research Project, Orissa University of Agriculture and Technology, Bhubaneswar and cut them into two equal halves. On the basis of internal rotting symptom, the tubers were categorized into six different types rotting groups. The association of two bacterial species were assayed following tube agglutination test using the known antiserum for each bacterium, *R. solanacearum* and *P. carotovorum.*

The rotting symptoms observed were categorized into 6 groups namely ; (A) cut tubers showing brownish discoloration along the vascular region (24 nos), (B) cut tubers showing brownish discoloration along the vascular region with soft rotten cavities filled with whitish ooze (16 nos), (C) cut tubers showing brownish blackish discoloration along the vascular region and soft rotten tissues extending towards center (in 24 numbers), (D) cut tubers showing soft rotten tissues extending towards center without brownish discoloration (8nos), (E) cut tubers showing soft rotten tissues extending towards center with brownish black discoloration (22 nos). (F) Cut tubers showing dry tissues with cavities surrounded by with soft tissues around (8 nos). Each tuber collected from experimental field was carefully washed in tap water using a soft brush. Care was taken not to damage the skin of tuber or disturb the disease symptom. Then it was blot dried and examined for characteristic symptom developed. Basing on the symptom, the tubers were separated out. Individual tubers in each symptom group were tested for association of test bacteria following modified tube agglutination test. Completely rotten portion of individual tuber in a group was separated out and the apparently healthy tissues adjacent to disease portion were cut into small

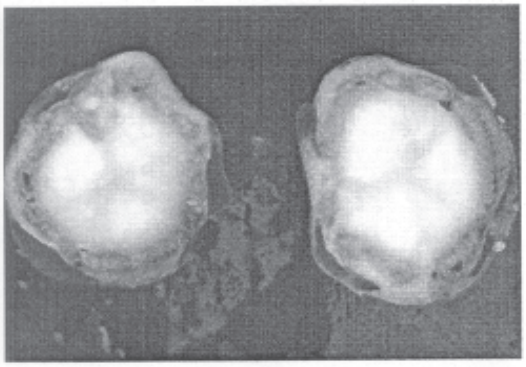

Fig. 1. Rotting symptoms of potatoes: Symptom–A.

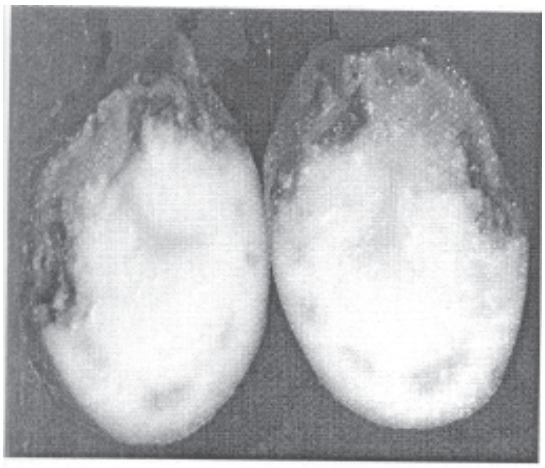

Fig. 2. Rotting symptoms of potatoes: Simptom–B.

pieces of about 1.0 cm size. Five cut pieces were transferred into 0.5 ml of sterile water taken in a culture tube. It was kept under laboratory condition for 30 minutes for bacterial oozing.

For detection of test bacterial species present in the bacterial suspension of each diseased tuber sample two agglutination tubes were taken for the two known anti-sera. About 0.5 ml of 10.0% diluted solution of each known antiserum was transferred into one tube and the other known anti serum to the second tube and leveled properly. To each of the two tubes 0.5 ml of bacterial suspension collected from one diseased sample was transferred and mixed thoroughly using Pasteur pipettes. The process was repeated for subsequent disease samples. The tubes were incubated in a hot water bath maintained at 37 ±1°C for 2 hours. Formation and deposition of precipitate at base of the tube indicated positive reaction on the basis of positive reaction against the known antiserum.

Results and Discussion

Results revealed that out of total diseased tubers 23.52% of tubers exhibited symptom categorized with brownish discoloration along the vascular region without any soft rotting of tissues (Category–A) (Table 1, Fig. 1). About 15.68% of tubers exhibited symptom with brownish discoloration along the vascular region with soft rotten cavities filled with whitish ooze (Category–B, Fig. 2). It was closely followed by the Category–C (23.52% Fig. 3) with brownish black discoloration along the vascular region with soft rotting of tissues extending towards the center. About 7.84% tubers exhibited symptom Category–D (Fig. 4) and F each. In Category–D (Fig. 4) the tubers showed soft rotten tissues extending towards the center without brownish discoloration and in Category–F the tuber exhibited dry cavities surrounded by corky layer with soft tissues around. In Category E about 21.56% the tubers showed soft rotten tissues extending towards center with brownish black discoloration.

Ralstonia solanacearum could be found associated exclusively in 54.10% of diseased tubers of symptom Category–A. No other bacteria could be detected from the rest of the sample belonging to the said category. Similarly exclusive association of *P. carotovorum* could be detected in 87.50% of the rotten tubers of symptom Category–D. In the symptom Category B and E both the test bacterium was found to be associated either singly or as mixture. However maximum infection of 56.20% was caused by *R. solanacearum* in symptom Category B followed by *P. carotovorum*. Similarly *P. carotovorum* was found to be associated with maximum per cent of tuber infection (45.40% of Symptom Category–E. Mixed infection of both the bacteria could be detected in 31.9% of diseased Category-E. Only 12.50% of tubers found

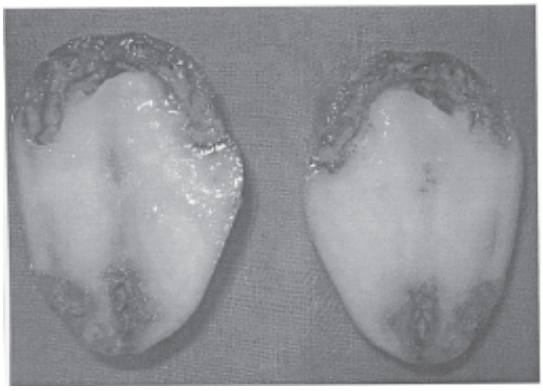

Fig. 3. Rotting symptoms of potatoes: Symptom–C.

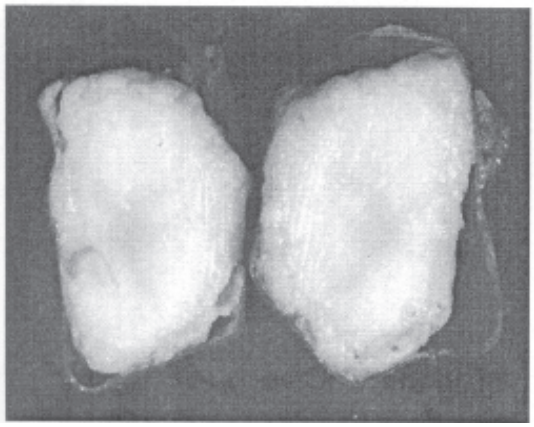

Fig. 4. Rotting symptoms of potatoes: Symptom–D.

associated with *P. carotovorum* in symptom Category-F and no bacterial infection in rest of 87.50% of tubers. Serological detection methods followed by different workers around the globe in different crops in addition to potato. Studies in detail on the serological characteristics of *Erwnia carotovora* isolated from fields was carried out in Egypt [5]. Several workers were also adopted serological methods in studies of viral and fungal diseases. There was report on biological and serological properties of Potato Virus Y isolates in Northeastern United States potato in New York [6]. Serological methods followed for detection of *Phytophthora infestans* in infected symptomatic, asymptomatic potato tissues (leaves and tubers) and could provide important interaction and disease development [7]. Hence by using specific antiserum the occurrence of particular race or strain of a particular plant pathogenic bacteria and virus in potato can be studied easily.

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