JOURNAL OF AGRICULTURE AND APPLIED BIOLOGY
2021, Vol. 2, No. 1, 53 - 60
http://dx.doi.org/10.11594/jaab.02.01.07

Short Communication

Evaluation of stored potato (*Solanum tuberosum* L.) for soft rot bacteria in Ibadan, Nigeria

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Article history:
Submitted 12 December 2020
Accepted 21 March 2021
Published 19 May 2021

Keywords:
Diseases
Isolation
Pectobacterium carotovorum
Pseudomonas syringae
Ralstonia solanacearum

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Abstract

Potato (*Solanum tuberosum* L.) is a tuberous crop from the *Solanaceae* family which is a source of starch and food to many in Nigeria, however, its production is being hampered in field and storage by bacterial pathogen which causes rot of the stored tubers. To this end, the study aimed at screening potatoes from different stores within Ibadan for the evaluation and diversity of bacteria pathogens responsible for rot in potatoes. Damaged stored potato samples were collected from different locations in Ibadan for studies, these samples were isolated for bacterial pathogen and characterized biochemically and observed under the microscope for identification. Three genera of bacteria were observed to be responsible for potato rot in the study and they include *Pectobacterium carotovorum*, *Pseudomonas syringae* and *Ralstonia solanacearum*. Of all the isolated bacteria, *Pectobacterium carotovorum* has the highest occurrence with a frequency of 60%, while *Pseudomonas syringae* has a frequency of 33% and *Ralstonia solanacearum* has a frequency of 27%. The pathogenicity of the isolates were tested and this revealed that *Pectobacterium carotovorum* is the most virulent with a severity score of 4.3, while *Ralstonia solanacearum* follows with a score of 3.3 and a score of 2.7 was recorded for *Pseudomonas syringae*, while the control had a score of 0. The diversity and differences shown in the isolated bacteria indicated that potato rot is a serious disease which is caused by different bacteria and need an integrated approach for its control from the field of harvesting to the storage house.

Introduction

Potato (*Solanum tuberosum* L.) is a starchy, tuberous crop from the perennial *Solanum tuberosum* of the Solanaceae family (Van Harsselaar et al., 2017) (also known as the Night shade). It is the world's fourth largest food crop following rice, wheat and maize (Liu et al., 2020). The crop is fairly new to sub-Saharan Africa (SSA) where it was introduced in the 19th century through activities of European Missionaries (McNeill, 1999). The United Nations Food and Agricultural organization reports that world production of potatoes in 2017 was 388 million tones with Africa

How to cite:
Abiodun, D.H. & Abisola, O.T. (2021) Evaluation of stored potato (*Solanum tuberosum* L.) for soft rot bacteria in Ibadan. *Journal of Agriculture and Applied Biology, 2*(1): 153 - 160. doi: 10.11594/jaab.02.01.07

producing a large part of it (El Sheika & Ray, 2017). Over two thirds of the global production is eaten directly by humans with the rest being fed to animals or used to produce starch.

Over the years, potato production in Nigeria has been on the increase as Nigeria has the largest area used in potato production in Africa but produces the lowest yield (output) compared to other African countries (Kakuhenzire et al., 2012).

In Nigeria, potato is eaten boiled, roasted or fried in vegetable oils. The chips are also packaged and sold as snacks and can also be processed into flakes (Kusur et al., 2020). The roots are peeled, chopped and boiled and then used as an ingredient in pie snacks. The tender leaves are also used as vegetables while vines are used as livestock feed especially during the dry season. Industrial uses of sweet potato roots in Nigeria are very limited. However, studies show starch content of 15.08 to 24.68 % from the high-yielding varieties developed by the National Root Crop Research Institute, Umudike, Nigeria (Tewe et al., 2003). These are therefore good sources of starch and amylose for food, alcohol, pharmaceuticals and the textile industries in Nigeria. (Tewe et al., 2003).

The production of potato in Nigeria is constrained by several factors (Adeyonu et al., 2019) among which storage rot is one of the most important (Echerenwa & Umechuruba, 2004). The principal species of microorganisms associated with potato rot in Nigeria include: *Fusarium oxysporum*, *Fusarim solani*, *Erwinia carotovora* (Clark & Hoy, 1994). *Penicillium* sp. *Cerocystis fimbriata, Diaporthe batatalis, Aspergillus flavus* and *Aspergillus niger* have also been recognized as potential organisms, but bacterial are the most important seed-borne pathogen of potato.

The genus *Pectobacterium* is an important Gram-negative plant pathogen that belongs to the family Enterobacteriaceae (Waleron et al., 2019). *Pectobacterium atrosepticum* (*Erwinia carotovora* ssp. *atroseptica*) and *Pectobacterium carotovorum* ssp. *carotovorum* (*Erwinia carotovora* ssp. *carotovora*) are the most important species and subspecies in this genus that cause severe losses of many commercial crops in the field and during storage (Pérombelon, 2002; Toth et al., 2003; Gnanamanickam, 2006). Among them, *Pectobacterium carotovorum* ssp. *carotovorum* is the most diverse and has the broadest host range and geographic distribution (Toth et al., 2003; Gnanamanickam, 2006).

Therefore, specific and sensitive methods for detecting and isolating pathogenic microorganisms of farm produce are required to improve the diagnosis and investigate the epidemiology of the diseases caused by these pathogens. The study aimed to evaluate the bacterial pathogens associated with tuber rot of potato in three locations in Ibadan, Oyo state, Nigeria.

Materials and methods

Experimental location

This research study was conducted at the pathology research laboratory of the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria.

Collection of samples

Samples of potato showing rot disease symptoms were obtained from fruit stores in Ojoo, Bodija and Moniya in Ibadan, Oyo State, respectively. The samples were collected into separate, well labelled polythene bags and immediately transferred to the laboratory.

Media preparation

Nutrient agar (NA) was prepared following the manufacturer's instructions (i.e. 28 grams of nutrient agar powder to 1 litre of water). The mixture was then sterilized by autoclaving at 121 ^{0}C for 15 minutes at a pressure of 1.05 kg cm$^{-1.}$ The medium was then allowed to cool to room temperature and poured into sterile Petri-dishes using aseptic technique. The same amount of nutrient agar medium was prepared 24 hours later for sub-culturing following the same procedure explained above.

Isolation and identification

The potato samples were rinsed in water to remove traces of soil from the samples, then they were surface- sterilized with 1% sodium hypochlorite for 1 minute to remove saprophytes present on the sample surface and rinsed in three changes of sterile distilled water

before they were blot-dried with sterile tissue paper and then macerated in different sterile Petri-dishes containing sterile distilled water to free up the bacterial ooze. A loopful each of the resulting ooze from each location was streaked on sterile freshly prepared plates of nutrient agar using a modified method of (Durojaye et al., 2019). The plates were labelled with the name of the market source and the date of inoculation. The plates were incubated at 32^{0}C for 24 hours to obtain bacterial isolates.

Sterile wire loop was used to introduce a colony from 24 hours–old culture into a freshly prepared nutrient agar plate for sub-culturing to obtain pure isolates, and the colony was streaked out appropriately. The same procedure was repeated for the remaining plates. The plates were labelled as in the original culture with the name of the market source and the new date of inoculation. The freshly sub-cultured plates were incubated at 32^{0}C for 24 hours.

Morphological and biochemical tests

The colonies on the sub-cultured plates were observed for the following cultural characteristics: shape, colour, appearance, and elevation.

Oxidase test

One ml of distilled water was added to make 1% solution of 0.01 gram tetramethyl phenylene diamine dihydrochloride. A few drops of the reagent were pipetted onto a filter paper. Using an inoculating loop, a loopful culture of the test organism was rubbed across the impregnated filter paper. The observation was recorded as positive with purple colour or negative with no purple colour after 60 seconds (Khatri-Chhetri et al., 2003).

Catalase test

Few drops of hydrogen peroxide were added to the surface of 48 hours - old culture of each isolate on nutrient agar medium. Bubble formation was recorded as positive for catalase activity (Khatri-Chhetri et al., 2003).

Potassium hydroxide test (KOH)

A drop of sterile distilled water was placed on a clean, grease-free slide. A flamed and cooled loop was used to transfer a colony of bacteria to the drop of water on the slide. A tincture of potassium hydroxide is added to the colony on the slide. The colony was mixed with the KOH using the sterile loop. A positive result is indicated if the KOH-colony mixture turns slimy or mucoidal. Otherwise, a negative result is indicated.

Gram staining

A drop of sterile distilled water was placed on a clean slide. A flamed, sterile inoculating loop was used to pick a colony of the bacteria and this was used to make a smear on the slide. The slide was heat-fixed by passing it lightly over a lighted spirit lamp. The slide was flooded with crystal violet for 1 min (as the primary dye) and gently washed under a running tap. Afterwards, the slide was flooded with Lugol's iodine (mordant) for 30 seconds. The Lugol's iodine was rinsed off with tap water. Next, ethanol was added to the smear to decolourize it. Thereafter, the slide was counter-stained with Safranin (the secondary dye) for 30 seconds. The slide was rinsed under tap water, drained, blotted dry and examined under the microscope using the oil immersion objective. Gram positive organisms appear purple while gram negative ones appear pink (Vauterin et al., 1991).

Starch hydrolysis

The medium used for this test is nutrient starch agar and contains nutrient agar (28 g), starch soluble (2.0 g), water (1000 ml) and pH (7.0). The medium was sterilized at 121^{0}C for 15 minutes at a pressure of 0.12 MPa and poured into sterilized Petri plates. The medium was allowed to solidify and inoculated with the test organism. The plates were incubated and tested for starch hydrolysis. The agar surface was flooded with Lugol's iodine and allowed to act for two minutes for development of colourless zone around the bacterial growth.

Gelatin liquefaction

Nutrient gelatin medium was prepared by weighing 28 g of nutrient agar and 20 g of gelatin, which was made to 1 L with pH 7.0 and heated over a water bath until the gelatin was dissolved and sterilized at 121^{0}C for 15 minutes at a pressure of 0.12 MPa. The media was cooled and poured to the Petri plates and allowed to solidify before being inoculated with 48 hours growth of the test bacterium and the plates were incubated at 35^{0}C. Then the surface of agar was flooded with 0.2% mercuric chloride solution of dilute HCl (20%) and observed for formation of white precipitation.

Pathogenicity test

For the pathogenicity test, clean tubers of potato were disinfected with 1% sodium hypochlorite for a minute and rinsed 3 times with sterilized distilled water. Two holes of 12 mm diameter were bored in each potato tuber and each of the bacterial inocula that has been adjusted turbidimetrically using a spectrophotometer to a concentration approximating to an optical density of 600 nm which is corresponding to 2.4 x 10^8 colony forming units per mL (Durojaye et al., 2019) were carefully dispensed using a sterile pipette into each of the bored holes while the control potato was inoculated with sterile water. The inoculated holes of each potato tuber were covered with sterile cotton wetted with sterile water and kept in polythene bags in order to maintain humid condition during incubation. The test was done in three replicates. Development of rot symptoms on potatoes was observed after 24 h of incubation at 25 °C. Final evaluation of pathogen to induce soft rot in potato was done 5 days after inoculation.

Disease severity rating

The soft rot severity was rated on a scale of 0 to 5 according to Awoyemi et al. (2016).

0= no visible symptoms (non-virulence).
1= visible rot lesions on 15% root area (resistant)
2= 16 – 30% of root area affected by rot (moderately resistant).
3= severe rot symptoms 31 – 45% of root area affected (moderately susceptible)
4= severe rot symptoms 46 – 60% root area by rot (susceptible) and
5= severely affected with about 60 - 100% root area infected by rot (highly susceptible)

Statistical analysis

The data collected were subjected to analysis of variance (ANOVA) of SAS, and significant means were separated using LSD at 5% level of probability.

Results and discussion

A total of fifteen bacteria pathogen belonging to 3 genera were isolated from the collected samples with Moniya having the greatest number of rot inducing pathogens (6), which was closely followed by Bodija with 5 isolates and Ojoo with 4 isolates.

The shape, size, elevation, appearance and colour on nutrient agar of each bacterium was used in morphological identification. *Pectobacterium carotovorum* was observed to be rod shape, large in size with convex elevation and shiny white colour whereas *Pseudomonas syringae* was also rod shape, large in size with convex elevation and whitish yellow colour on nutrient agar, while *Ralstonia solanacearum* was rod shape, large in size with raised elevation and creamy colouration (Table 1).

These isolated bacteria were characterised using the basic conventional methods, from these methods the bacteria isolated were identified as *Pectobacterium carotovorum*, *Pseudomonas syringae* and *Ralstonia solanacearum*. This was confirmed by some biochemical tests which include oxidase, catalase, Gram staining, starch hydrolysis, gelatin liquefaction and KOH test (Table 2).

All these bacterial isolates were confirmed to be Gram negative following these biochemical tests and they were all able to induce soft rot in potato during the pathogenicity test except the control which was not showing any symptom of infection because it was inoculated with only water.

From the pathogenicity test, *Pectobacterium carotovorum* had the highest virulent rating with a severity score of 4.3, while *Ralstonia solanacearum* had a score of 3.3 and a score of 2.7 was recorded for *Pseudomonas syringae*

with the control maintaining a score of 0 throughout the experiment. There were significant differences among the severity scores recorded in the various isolates as shown in Table 3.

Table 1. Morphological features of bacteria isolated from potatoes showing symptoms of potato soft rot in Ibadan on nutrient agar (NA)

Isolate	Shape	Size	Elevation	Appearance	Colour on NA
Pectobacterium carotovorum	Rod	Large	Convex	Shinny	White
Ralstonia solanacearum	Rod	Large	Convex	Shinny	Whitish Yellow
Pseudomonas spp.	Rod	Large	Raised	Shinny	Creamy

Table 2. Results of biochemical tests carried out on bacteria isolated from potatoes showing symptoms of potato soft rot in Ibadan

Sample code	Oxidase test	Catalase test	KOH test	Gram reaction	Starch hydrolysis	Gelatin liquefaction	Suspected organism
B1	-	+	+	-	+	+	*Pseudomonas syringae*
B2	-	+	+	-	+	+	*Pseudomonas syringae*
B3	-	+	+	-	+	+	*Pseudomonas syringae*
B4	-	+	+	-	+	+	*Pseudomonas syringae*
B5	-	+	+	-	+	+	*Pseudomonas syringae*
O1	+	+	+	-	+	+	*Ralstonia solanacearum*
O2	+	+	+	-	+	+	*Ralstonia solanacearum*
O3	+	+	+	-	+	+	*Ralstonia solanacearum*
O4	+	+	+	-	+	+	*Ralstonia solanacearum*
M1	-	-	+	-	-	-	*P. carotovorum*
M2	-	-	+	-	-	-	*P. carotovorum*
M3	-	-	+	-	-	-	*P. carotovorum*
M4	-	-	+	-	-	-	*P. carotovorum*
M5	-	-	+	-	-	-	*P. carotovorum*
M6	-	-	+	-	-	-	*P. carotovorum*

Keys: + = positive reaction, - = negative or no reaction

Table 3. Pathogen virulence rating for bacterial soft rot

Isolates	Mean severity
Pectobacterium carotovorum	4.3[a]
Ralstonia solanacearum	3.3[b]
Pseudomonas syringae	2.7[b]
Control	0.0[c]
LSD value	0.94

The results of the biochemical tests carried out indicated that the bacteria isolates were *Pectobacterium carotovorum*, *Pseudomonas syringae* and *Ralstonia solanacearum*. This was in agreement with the study carried out by Frampton et al. (2012) and Tanni et al. (2019) that suggested that *Pectobacterium carotovorum* is the most dominant of all the isolated bacteria from rot infected potato where the frequency of *P. carotovorum* was 46% in their study and 60% was observed in this study.

The diversity of the causative agents of potato soft rot has been reported to be complex due to the fact that it is caused by several organisms aside *P. carotovorum*, in this study where other organisms like *P. syringae* and *R. solanacearum* were isolated and reported to be associated with potato soft rot, similar occurrence has also been reported by El-habbak and Refaat (2019) where specie of *Pseudomonas* was isolated in their study. The pathogenicity of the bacterium was established by inoculating bacterial suspension to potato tubers where it was discovered that the inoculum of *P. carotovorum* is more virulent than the other isolated pathogen and this is showing a relationship with the report of El-habbak and Refaat (2019) that reported *P. carotovorum* as the most severe and virulent pathogen associated with potato soft rot. The three bacterial pathogen isolated from this study were confirmed to be responsible for potato soft rot and this study is in harmony with the study of Wakil and Oyinlola (2011) , the high severity recorded in *P. carotovorum* may be due to the ability of the bacterium in producing some certain cell-wall degrading enzymes such pectinases, cellulases, proteases and xylanases as reported by Vreugdenhil et al. (2011).

Also *P. carotovorum* have been reported to cause severe rot in stored produce due to the presence of the pathogen from the field which later thrive and multiply rapidly in the store as suggested by Wakil and Oyinlola (2011) and this leads to a great loss to potato producers and marketers (Elhalag et al., 2020). The severity pattern observed in the pathogens with respect to their collection area can be linked to the variability in virulence reported by Al-lami et al. (2020) or environmental factor coupled with the management practices of the sellers, pathogen virulence and the potato seed source described by Elhalag et al. (2020).

From the work carried out in the past as well as results obtained from this work, it will be suggested that potato soft rot is a field to store disease (Czajkowski et al., 2010) caused by several pathogenic agents (Elhalag et al., 2020). However, it can be prevented in the field through various control methods which include sanitation, application of chemicals (Yaganza et al., 2014) like acetic acid, boric acid and bleaching powder (Rahman et al., 2017) and proper handling during transit etc.. Reducing wounds during harvest and handling, promoting wound healing after harvesting and proper ventilation during storage (Vreugdenhil et al., 2011).

Conclusion

The result shows that potato soft rot is caused by several pathogenic bacteria which accounted for the loss and shortage recorded in potato production during storage in Nigeria. The pathogenic bacteria include *Pectobacterium carotovorum*, *Pseudomonas syringae* and *Ralstonia solanacearum*. Among the isolated bacteria, *Pectobacterium carotovorum* happens to be the most dominant and virulent bacterial agent responsible for potato soft rot in Ibadan.

Acknowledgments

Special thanks goes to the pathology unit of the department of Crop Protection and Environmental Biology, University of Ibadan.

Author declaration

DHA designed the study, assisted with laboratory work, edited and reviewed the manuscript write-up, while OTA collected samples, carried out lab work and drafted the manuscript.

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