J Krishi Vigyan 2023, 11 (2) : 115-119 DOI : 10.5958/2349-4433.2023.00025.9

Evaluation of Antagonistic Potential of Fructosphere-Associated Microflora Against Major Crown Rot Pathogen of Robusta variety Banana

Deepa R Chandran and Susha S Thara

Department of Plant Pathology, College of Agriculture Vellayani, Kerala Agricultural University, Thiruvananthapuram, 695522 (Kerala), India.

ABSTRACT

Banana is one of the tropical fruits that is exported in large quantities. Banana is an integral fruit component of most farming systems in Kerala and also an important commercial fruit crop of the country. Crown rot of dehanded banana is one of the most important and predominant postharvest diseases of banana which can lead to heavy losses for farmers as well as the wholesalers. A survey was conducted in five major banana growing districts of Kerala to identify the major pathogens associated with crown rot of banana in Kerala. *Lasiodiplodia theobromae* was found to be the major and most virulent pathogen associated with postharvest crown rot of Robusta variety banana in Kerala. Fructosphere microflora was isolated from healthy banana fruits to evaluate the antagonistic potential. 22 fungal isolates and 7 bacterial isolates were obtained from different locations of Kerala. Among the fructosphere isolates, the fungal isolates, W3B-BC and A3B-BC, showed highest inhibition on growth of the major pathogen with 44.44 and 40.00 per cent of inhibitions respectively. Based on the molecular studies, the best two effective biocontrol agents were identified as W3B-BC and A3B-BC as *Aspergillus aculeatus* (MN046330) and *Aspergillus niger* (MN04 6326) respectively using the universal inter transcriptional sequencing (ITS) primers.

Key Words: Antagonists, Banana, Crown rot, Robusta, *Aspergillus.*

INTRODUCTION

Postharvest losses of perishables in developing countries have been estimated to be in the range of 5-50 per cent or more of the harvest. In India, nearly 20-50 per cent of perishables are lost due to postharvest diseases, and for banana it accounts for 20-80 per cent (Yadav *et al*, 2013). The two primary postharvest rots of banana (*Musa* spp.) fruits are crown rot and anthracnose. The diseases usually appear on ripening fruits either at points of sale or later, after purchase. Crown rot of banana is a major threat causing losses during storage and marketing (Krauss and Johanson, 2000). Even though the infected fruits are safe for humans to consume, the infection reduces the fruit quality, shelf life and marketability. Since Robusta variety (Musa AAA) was most susceptible to crown rot diseases as reported by Raman *et al* (2007), it was selected for the study. From the study conducted at Department of Plant Pathology, College of Agriculture, Vellayani, *Lasiodiplodia theobromae* (T2C isolate) was identified as the major pathogen associated with crown rot of Robusta variety banana in Kerala. Although the use of synthetic chemical fungicides remains as the primary method of controlling postharvest diseases, the global trend appears to be shifting towards reduced use of fungicides, substituting them with alternative methods like bio-fungicides. It is very much essential to develop the stable biocontrol strategies efficient for the postharvest management. Hence, the study was formulated with the objective to identify the

Corresponding Author's Email:135deeparc@gmail.com

Table.1. Fructosphere microflora isolated from Robusta variety banana collected from major banana growing locations of Kerala

District	Locations	Isolates	Remarks
Thiruvananthapuram	Pallichal	T1 A-BC	Non-pathogenic fungi
	Pallichal	T1 B-BC	Pathogenic fungi
	Pallichal	T1 C-BC	Pathogenic fungi
	Pallichal	T1 D-BC	Pathogenic fungi
Alappuzha	Edathua	A1 A -BC	Non-pathogenic fungi
	Edathua	A1 B-BC	Pathogenic fungi
	Edathua	A1 C-BC	Non-pathogenic fungi
	Edathua	A1 D-BC	Pathogenic fungi
	Cherthala	A2A-BC	Pathogenic fungi
	Cherthala	A2B-BC	Pathogenic fungi
	Kayamkulam	A3 A-BC	Pathogenic fungi
	Kayamkulam	A3 B-BC	Non-pathogenic fungi
	Kayamkulam	A3 C-BC	Non-pathogenic fungi
	Kayamkulam	A3 D-BC	Non-pathogenic fungi
	Edathuva	A1E-BC	Non-pathogenic bacteria
	Cherthala	A2C-BC	Non-pathogenic bacteria
Pathanamthitta	Konni	Pt1A -BC	Non-pathogenic fungi
	Adoor	Pt2A-BC	Pathogenic fungi
	Adoor	Pt2B -BC	Non-pathogenic fungi
	Konni	Pt1B-BC	Non-pathogenic bacteria
	Konni	Pt1C-BC	Non-pathogenic bacteria
	Adoor	Pt2C-BC	Non-pathogenic bacteria
Palakkad	Mannarkkad	P1A-BC	Non-pathogenic fungi
	Palakkayam	P2A-BC	Pathogenic fungi
	Mannarkkad	P1B-BC	Non-pathogenic bacteria
Wayanad	Vaduvanchal	W3A-BC	Pathogenic fungi
	Vaduvanchal	W3B-BC	Non-pathogenic fungi
	Vaduvanchal	W3C-BC	Non-pathogenic fungi
	Karyambadi	W2A-BC	Non-pathogenic bacteria

potential biocontrol agents from the fructosphere and establish a stable biological strategy for the management of crown rot disease of banana.

MATERIALS AND METHODS

Isolation of fructosphere microflora

Collection was done from five banana growing districts of Kerala namely Thiruvananthapuram, Alappuzha, Pathanamthitta, Palakkad and Wayanad. Banana bunches were collected from fields or homesteads having reports of crown rot incidence. To identify the effective antagonists from the fructosphere of banana, total microflora was isolated from the fruit surface of healthy bananas taken from lots having diseased as well as healthy bunches. Healthy bananas from mixed lot

Table. 2. Evaluation of antagonistic effect of fructosphere microflora against major crown rot pathogen (T2C)

Sl.	Fructosphere isolate	Radial growth of pathogen (cm) *	Percent inhibition * (%)
1	T1A-BC	4.50	0 (1.351) [d]
2	A1A-BC	4.50	0 (1.351) [d]
3	A1C-BC	4.50	0 (1.351) [d]
4	A3B-BC	2.70 ± 0.08	40.00 (39.23) [a]
5	A3C-BC	4.50	0 (1.351) [d]
6	A3D-BC	4.50	0 (1.351) [d]
7	Pt1A-BC	3.50 ± 0.24	22.22 (27.99) [b]
8	Pt2B-BC	4.50	0 (1.351) [d]
9	W3B-BC	2.50 ± 0.20	44.44 (41.80) [a]
10	W3C-BC	4.50	0 (1.351) [d]
11	P1A-BC	4.50	0 (1.351) [d]
12	A1E-BC	4.50	0 (1.351) [d]
13	A2C-BC	4.50	0 (1.351) [d]
14	Pt1B-BC	4.00 ± 0.20	11.11 (19.16) [c]
15	Pt1C-BC	4.50	0 (1.351) [d]
16	Pt2C-BC	4.50	0 (1.351) [d]
17	W2A-BC	4.50	0 (1.351) [d]
18	P1B-BC	4.50	0 (1.351) [d]
CD (0.05)		0.355	3.872
SE (m) ±		0.162	1.843

* Mean of four replications and values in parenthesis are angular transformed data

were chosen for the experiment since the presence and activity of antagonists will be more prevalent in such conditions.

Bananas were dehanded (retaining the crown to each banana) from the mature harvested bunches. The fruits were subjected to serial dilution technique *in vitro* followed by plating, selection and pure culturing of the microflora. In the laminar airflow chamber, the fructosphere (fruit surface) of banana was washed using double distilled sterile water. The fructosphere wash was collected in to a sterile beaker (250 ml). The wash volume was made up to 100 ml using double distilled sterile water in a standard round bottom flask. This will serve as the stock solution with 10^{-2} dilution. The stock solution was gently vortexed for proper mixing. 1 ml of the stock was pipetted out and transferred into a test tube with 9 ml sterile water and vortexed thoroughly. This will give a solution of 10^{-3} dilution. Then 1 ml from this test tube was transferred in to another test tube with 9 ml sterile water and vortexed to give solution of 10^{-4} dilution. Similarly, dilutions up to 10^{-5} were made. The diluted stocks were respectively plated on media using spread plate method. For this, specific media like Rose Bengal Agar (for fungi), Nutrient Agar (for bacteria) and Kuster's media (for actinomycetes) were used. 15 - 20 ml of each media was poured in to sterile petri plates kept inside the laminar air flow chamber. The statistical design used was CRD and four replications of each treatment

were kept. The media were allowed to solidify. 0.1 ml of diluted stocks of fructosphere washes were pipetted out from test tubes with 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions respectively, poured on to the solidified media in petri plates and spread using an L - rod. The plates were kept for incubation at room temperature (28 ± 2 °C) after proper wrapping and labelling. The plates were observed for microbial colonies daily. When colonies started to appear, they were picked out before spreading and merging with other colonies and transferred on to respective growth media. Pure cultures of these colonies were separately maintained for further studies. This technique was performed for all the samples collected from the 15 locations. These isolated microbial colonies served as the total fructosphere microflora from banana. The pathogenic microflora was then screened out after artificial inoculation on healthy banana followed by verification of Koch's postulates.

Identification of effective antagonists

The non-pathogenic microflora isolated from fructosphere of healthy banana was selected for studying the antagonistic effect on the major crown rot pathogen (T2C isolate, *L. theobromae*) under *in vitro* condition by dual culture technique. In the dual culture technique, five-day old cultures of the pathogen and the non-pathogenic microflora were used for inoculation. For testing the antagonistic effect of fungal microflora against the pathogen, culture disc of fungal microflora was taken using a cork-borer (5mm diameter) and placed at 2 cm from the periphery of petri plate containing solidified Potato Dextrose Agar (PDA) medium. Similarly, culture disc of pathogen was placed carefully at 2 cm away from periphery on the opposite side. In addition, culture disc of pathogen kept at the center of petri plate containing solidified PDA medium served as the control. This method was followed for all fungal microflora. In case of bacterial microflora, the media used was a 1:1 medium of PDA and NA. Moreover, instead of placing culture disc, the bacterium was streaked 2 cm away from the periphery and pathogen culture disc was placed at the opposite side 2 cm away from periphery of the petri plate. For each treatment four replications were maintained. The plates were kept for incubation at room temperature (28 ± 2 °C) after proper labelling and wrapping. Growth in the control plates were regularly monitored. When growth of pathogen (T2C) in the control plate was full, observations were taken. In order to identify the effective antagonist, the radial growth of pathogen in control plate and treated plate were recorded to find the percent inhibition using the following formula described by Vincent (1927) originally and modified by Girish and Sushma (2018).

$$\text{Per cent Inhibition} = \frac{(C - T)}{C} \times 100$$

C - radial growth of mycelia of the pathogen in control plate

T - radial growth of mycelia of the pathogen in the presence of respective fructosphere microflora

RESULTS AND DISCUSSION

Fructosphere microflora from healthy banana fruits were isolated by serial dilution technique followed by plating and incubation. Colonies of bacteria and fungi appeared on NA and RBA media, two and three days after incubation, respectively. No actinomycetes were obtained. From the fructosphere washes of 15 different samples, 22 fungal isolates and seven bacterial isolates were obtained in total (Table 1) but not from all the locations surveyed. The absence of microflora from some locations could be from the over use of fungicides on banana. On contrary, more than one isolate has been obtained even from a single banana fruit.

All the isolates were then subjected to artificial inoculation on healthy banana fruits to verify Koch's postulates and screen out pathogenic isolates. Out of the 29 fructosphere microflora isolated, 11 fungi and 7 bacteria were found to be non-pathogenic (Table 1). The non-pathogenic fungal and bacterial

isolates were then subjected to dual culture with the pathogen for studying their antagonism. Smilanick (1994) reported that the antagonists to the crown rot pathogen could be isolated from the surface of plants like from fructosphere or phyllosphere. Among the isolates, Pt1A-BC, Pt1B-BC, A3B-BC and W3B-BC showed different levels of inhibition of growth of pathogen in PDA whereas others showed zero inhibition. The fungal isolates namely W3B-BC (*Aspergillus* sp. from Vaduvanchal, Wayanad) and A3B-BC (*Aspergillus* sp. from Kayamkulam, Alappuzha) showed maximum antagonistic effect on growth of the major pathogen (T2C isolate, *L. theobromae*) with 44.44 and 40.00 per cent of inhibition respectively (Table 2).

Based on the molecular studies, effective biocontrol agents namely W3B-BC and A3B-BC were identified as *Aspergillus aculeatus* (GenBank Accession: MN046330) and *Aspergillus niger* (GenBank Accession: MN046326) respectively using the universal Inter Transcriptional Sequencing (ITS) primers.

CONCLUSION

Among the fructosphere microflora isolated from healthy banana samples collected from different locations from five banana growing districts of Kerala, W3B-BC and A3B-BC recorded maximum inhibition of mycelial growth of the major crown rot pathogen. If the active principle inhibiting the pathogen's growth could be identified in the further studies, it could lead to the development of novel biocontrol strategies for postharvest disease management. In this era of increasing promotion of biocontrol strategies for management of plant diseases, isolation and development of innovative formulations of biocontrol agents, with due respect to all the safety concerns, for management of postharvest diseases can unlock a great opening.

ACKNOWLEDGEMENT

I thankfully acknowledge Kerala Agricultural University for the facilities; and Kerala State Council for Science, Technology and Environment (KSCSTE) for the funding and fellowship.

REFERENCES

Girish K and Sushma G (2018). Screening of bacteria for antifungal activity against phytopathogenic fungi of sugarcane (*Saccharum officinarum* L.). *Eurasia J Biosci* **12**(2): 195-203.

Krauss U and Johanson A (2000). Recent advances in the control of crown rot of banana in the Windward Islands. *Crop Prot* **19** (3): 151-159.

Raman T, Sangeetha G and Mustaffa M M (2007). Cross-infection potential of crown rot pathogen (*Lasiodiplodia theobromae*) isolates and their management using potential native bioagents in banana. *Australasian Pl Pathol* **36**: 595–605.

Smilanick J L (1994). *Strategies for the isolation and testing of biocontrol agents*. in: Wilson, C. L. and Wisniewski, M. E. *Biol. Control Postharvest Dis* pp. 25-41.

Vincent J M (1927). Distortion of fungal hyphae in the presence of certain inhibitors. *Nat* **159**: 800.

Yadav S K, Babu S, Yadav M K, Singh K, Yadav G S and Pal S A (2013). Review of Organic Farming for Sustainable Agriculture in Northern India. *Int J Agron* 8p.

Received on 11/1/2023 *Accepted on 18/4/2023*