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Identification and Characterization of *Xanthomonas* spp. Affecting Fruit Crops Cultivated in Assam, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was carried out to identify the bacterial pathogens affecting different fruit crops and to consign them in proper taxonomic position following different morphological, biochemical and molecular protocols. Diseased samples exhibiting typical symptoms on pomegranate (*Punica granatum*), mango (*Mangifera indica*), peach (*Prunus persica*), and plum (*Prunus domestica*) were gathered from two locations in Assam (Jorhat and Sonitpur). Through morphological, biochemical, and molecular characterization, the bacterial isolates were identified within the genus Xanthomonas, displaying a broad host range encompassing pomegranate, mango, peach, and plum. Phylogenetic analysis of 16S rRNA gene sequences indicated the isolates as Xanthomonas citri pv. *Mangiferae indicae* (causing bacterial canker in mango), Xanthomonas axonopodis pv. punicae (responsible for bacterial blight in pomegranate), and

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Xanthomonas arboricola pv. pruni (associated with bacterial leaf spot in stone fruits). The findings underscore the importance of Xanthomonas in impacting diverse fruit crops, highlighting potential threats to their production.

Keywords: Bacterial diseases; fruit crops; Xanthomonas spp.; morphological and cultural; biochemical and molecular characterization.

1. INTRODUCTION

Cultivation of fruit crops contributes to the health, happiness and prosperity of the people. The standard of living of the people is often judged by production and consumption of fruits per capita. India, as the second-largest global producer of fruits, leads in the cultivation of various fruits, including mango, banana, sapota, pomegranate, and aonla. According to the latest data from the National Horticulture Database (3rd Advance Estimates) released by the National Horticulture Board, India achieved a remarkable output of 107.24 million metric tonnes of fruits and 204.84 million metric tonnes of vegetables during the 2021-22 period. The extensive land devoted to fruit cultivation spans 7.05 million hectares, while vegetables are grown across 11.35 million hectares. The state of Assam, situated in the Northeastern region of India, boasts diverse climates and soils conducive to the cultivation of numerous horticultural crops. These include fruits, vegetables, spices, potatoes, tropical tuber crops, mushrooms, ornamental, medicinal and aromatic plants, as well as plantation crops.Among the major fruit crops cultivated in the state are banana, pineapple, papaya, Assam lemon, orange, guava, litchi, jackfruit, and mango. This diverse cultivation not only enhances the agricultural landscape of Assam but also contributes significantly to the overall fruit production in India [1-3].

There is tremendous potential for large-scale expansion of fruit crops in Assam, which could significantly impact the state's economy. Bacterial pathogens causing plant diseases pose major constraints on crop production, leading to significant annual losses worldwide [4]. Despite the severe losses inflicted by bacterial diseases on various fruit crops in different districts of Assam, systematic research for the identification, characterization, and management of these bacterial pathogens is lacking in the region [5-7].

The identification and characterization of these pathogens are crucial for understanding specific disease problems and implementing effective management strategies. Unfortunately, comprehensive information regarding their complete characterization is currently unavailable. The present study aims to fill this gap by isolating, identifying, and characterizing bacterial pathogens associated with diseases in fruit crops.

2. MATERIALS AND METHODS

2.1 Sample Collection and Isolation of the Pathogens

Different parts of the plants like leaves and stem showing characteristic symptoms of bacterial infection were collected from two distantly located (about 235 km) places of Assam namely Jorhat and Sonitpur. The collected plant samples (*i.e*., mango, pomegranate, peach and plum) were brought to the Department of Plant Pathology, AAU, Jorhat for further studies. Observation of symptoms of blight on pomegranate (*Punica granatum*) was recorded on both leaves and fruits. Brown necrotic spots surrounded by yellow halo were observed on the leaves while brown to black raised oily spots were seen on the fruits (Fig.1a). The symptoms like brown angular to irregular lesions surrounded by yellow halo on diseased mango (*Mangifera indica*) leaves were seen (Fig.1b). The symptoms on peach (*Prunus persica*) (Fig.1c) and plum (*Prunus domestica*) were seen as brown or black spots surrounded with yellow halo while some leaves showed a typical shot-hole appearance. On the plum fruit small rough pit-like lesions were found (Fig.1d). The diseased tissues with a little portion of healthy tissues were cut into small pieces and surface sterilized with 0.1 per cent sodium hypochlorite solution for 60 sec. It was washed with sterilized distilled water for three times to remove traces of sodium hypochlorite solution. The cut pieces were placed onto sterile Petri plates containing Nutrient Agar (NA) medium. The plates were incubated for 2–3 days at 28°C. The pure culture of the isolates were obtained in nutrient agar medium as well as in yeast glucose chalk agar (YGCA) medium and kept in the incubator for 48 h at 28±2°C. The cultures were maintained in the refrigerator at

4°C which served as a stock culture for further studies. The pathogens isolated were designated as RC4 (mango), RC5 (pomegranate), RC6 (peach) and RC7 (plum) for convenience in study (Fig.2).

Pathogenicity test: Detached leaf inoculation technique (Randhawa and Civerolo,1985) was followed for proving pathogenicity of the isolates obtained from the diseased samples. Three middle aged leaves were selected and detached from the plants. They were washed well in tap water, swabbed with 70 per cent ethanol and allowed to dry. Then injuries were made at several points by pricking with sterilized needle laid with 10^9 cfu/ml inoculums of the isolates and also smeared on both sides with culture soaked sterilized cotton swab. The leaves were kept in plates which were lined with sterilized moist filter paper to maintain humidity and incubated at 30°C. One plate was taken as control and the leaves were inoculated with sterile water. The disease symptoms were recorded at different time interval.

Cultural, morphological and biochemical characterization of the isolates: Morphological and cultural characteristics, encompassing attributes like shape, size, colony shape, colony color, colony elevation, gram staining, KOH test, pigment production, oxygen requirement, and growth on different media, were thoroughly examined. The morphological features of the isolates were investigated using the Carl Zeiss Sigma Field Emission Scanning Electron Microscope. Additionally, the bacterial isolates underwent various biochemical tests, including citrate utilization, starch hydrolysis, lysine utilization, urease production, phenylalanine deamination, nitrate reduction, H2S production, and carbohydrate utilization. These biochemical tests were performed using the KB002 and KB009 biochemical test kit from Himedia, India.

Molecular characterization and phylogenetic analysis: Genomic DNA of the bacterial isolates was extracted by following the modified method of Cardinal et al. [8]. The extracted

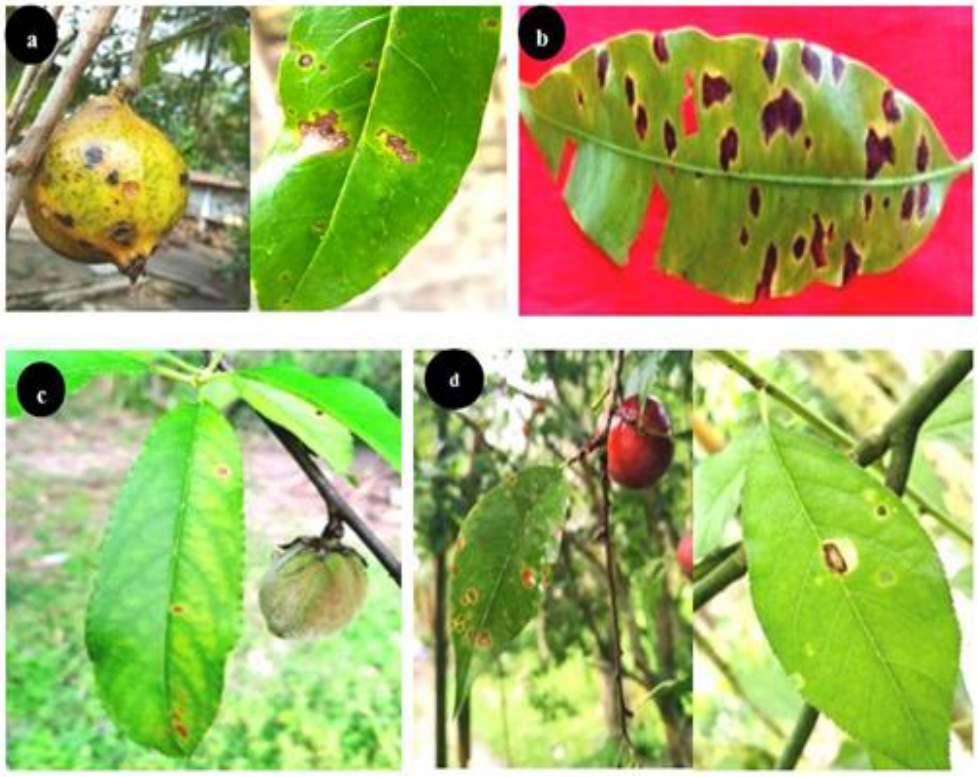

Fig. 1. (a) Brown necrotic spots on diseased Pomegranate (*Punica granatum*) leaves surrounded by yellow halo; brown to black raised oily spots on fruits. (b) Brown angular to irregular lesions surrounded by yellow halo on diseased mango (*Mangifera indica*) leaves. (c) Small brown spots with yellow halo in diseased Peach (*Prunus persica)* leaves. (d) Small brown spots surrounded by yellow halo in diseased Plum (*Prunus domestica*) leaves; Shot-hole symptoms appearance in the diseased leaves

Fig. 2. Pure culture of the bacterial isolates in nutrient agar (NA) slants
RC4= Mango Isolate; RC5= Pomegranate Isolate; RC6= Peach Isolate and RC7= Plum Isolate

genomic DNA was amplified using universal primer pairs universal primers U16SF (5-AGAGTTTGATCMTGGCTCAG-3) and U16SR (5-TACGGYTACCTTGTTACGACTT-3) (Goswami et al., 2017). PCR assay was performed in 10 µl reaction mixture containing 0.5 µl of template DNA; 5 µl Emerald Amp GT PCR Master Mix (2X Premix) (Takara); 0.5 µl Forward primer (10 pmol/µl); 0.5 µl Reverse primer (10 pmol/µl) and 3.5 µl sterile distilled water. The PCR was carried out at an initial denaturation of 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 1 min, extension at 72°C for 1.30 min, with the final extension at 72°C for 7 min. PCR products were separated in 1.5 per cent agarose gel and results were observed in Gel Doc. The PCR products of the samples were sent to Bioserve Biotechnologies Private Limited, Hyderabad respectively for 16S rRNA sequencing. The 16S rDNA sequence reads obtained after sequencing were assembled into contig using CodonCode Aligner (CodonCode Corporation, USA). Further, sequence similarity tool BLAST was employed to find the similarity of the sequences with known 16S rDNA sequences available in the NCBI GenBank databases. Phylogenetic analysis of the 16S rRNA sequences were carried out using the neighbor-joining tree by MEGA-X (Molecular Evolutionary Genetics Analysis) software with Kimura-2 parameter model method with 500-step bootstrap [9].

3. RESULTS AND DISCUSSION

3.1 Results

The survey was conducted in two distinct locations in Assam, India, where samples suspected to be infected with bacterial pathogens causing diseases were collected. The disease symptoms induced by the bacterial genus *Xanthomonas*, observed on the collected samples, were briefly described in the preceding section. Morphological and cultural studies revealed that the bacterial isolates possessed rod-shaped cells. The bacterial cells were gram-negative, aerobic, rod-shaped, with sizes ranging from 0.3 to 1.8 µm (Fig. 4), and were non-spore formers. All isolates developed colonies that were smooth, round, entire, deep yellow, convex, mucoid, glistening, opaque, and had colony sizes ranging from 2 to 3 mm in diameter. The observed results were consistent with those characteristic of the genus *Xanthomonas* (Table

1). The findings regarding various biochemical characteristics of the bacterial isolates are presented in Table 2. The results indicated that isolates from mango, pomegranate, peach, and plum exhibited positive results for the KOH test, citrate utilization, gelatin liquefaction, oxidase, indole production, levan production, and arginine dihydrolase. Additionally, all isolates showed positive results for the catalase test, indicating the aerobic nature of the bacteria. However, isolates from peach and plum demonstrated a positive result for the nitrate reduction test, evidenced by the formation of gas bubbles in the Durham tubes of the broth, while isolates from mango and pomegranate showed negative results. Furthermore, isolates from peach and plum did not hydrolyze starch, whereas isolates from mango and pomegranate exhibited a positive result for starch hydrolysis. Results for different carbohydrate utilization tests conducted using the biochemical test kit (KB002 and KB009; Himedia, India) are also presented in Table 2. In the pathogenicity test, typical symptoms were observed on the inoculated hosts. Mango leaves exhibited water-soaked lesions that later transformed into brown to black angular leaf spots after three days of inoculation. The pathogen was re-isolated from the observed symptoms on nutrient agar, displaying the same growth characteristics as observed earlier. On pomegranate leaves, symptoms appeared as small water-soaked lesions three days after inoculation, turning into brown to black-colored lesions after six days. The re-isolated pathogen was found identical to the original culture. Peach leaves displayed water-soaked lesions that later developed necrotic brown spots, expanding to produce a shot-hole. Re-isolation of bacterial strains from the diseased peach leaves confirmed their identity with the original isolates. Plum leaves exhibited symptoms three days after inoculation as small water-soaked lesions, which later turned brown to black in color. Re-isolation of the pathogen from the symptomatic leaves showed similarity with the original culture (Fig. 3). However, no symptoms developed in the control cases.

Sequencing of 16S rRNA was performed to identify the bacterial isolates. The nucleic acid of the isolates subjected to agarose gel (1.5%) electrophoresis yielded one distinct amplicon of 1200-1400 bp in size (Fig. 5). The nucleotide BLAST search revealed the highest nucleotide similarity of the bacterial isolates with 10 different strains of the respective genera. Sequence comparison of the 16S rRNA gene of the isolate from mango with GenBank entries further confirmed the identity as the similarity percentage was 100% to that of *Xanthomonas citri* pv. *mangiferaeindicae.* The BLAST results showed highest nucleotide similarity (100%) of the pomegranate bacterial isolate with *Xanthomonas axonopodis* pv. *punicae*. Similarly, the BLAST results for isolates from peach and plum exhibited highest homology with the strains of *Xanthomonas arboricola* pv. *pruni* with 100% similarity (Fig. 6). Further, the phylogenetic tree

Table 1. Morphological characters of the Xanthomonas spp. isolated from four fruit plants

Character	Feature	Mango	Pomegranate	Peach	Plum
Morphological	Size (µm)	0.3x1.7	0.3x1.8	0.3x1.8	0.4x1.9
	Shape	Rod	Rod	Rod	Rod
Colony on NA plate	Colony shape	Circular	Circular	Circular	Circular
	Surface	Smooth, mucoid, glistening	Smooth, glistening	Smooth, mucoid, glistening	Smooth, mucoid, glistening
	Edge	Entire	Entire	Entire	Entire
	Colour	Deep yellow	Yellow	Bright yellow	Yellow
	Elevation	Convex	Convex	Convex	Convex
	Opacity	Opaque	Opaque	Opaque	Opaque
	Size (mm)	2-3	2.5-3	2-2.5	2-3
Cultural	Gram reaction	(-)	(-)	(-)	(-)
	Pigment production YGCA	(+)	(+)	(+)	(+)
	Pigment production King's B	(-)	(-)	(-)	(-)
	KOH test	(+)	(+)	(+)	(+)
	Oxygen requirement	Aerobic	Aerobic	Aerobic	Aerobic
Pathological test	On host crop	(+)	(+)	(+)	(+)

(+) - positive reaction, (-) - negative reaction

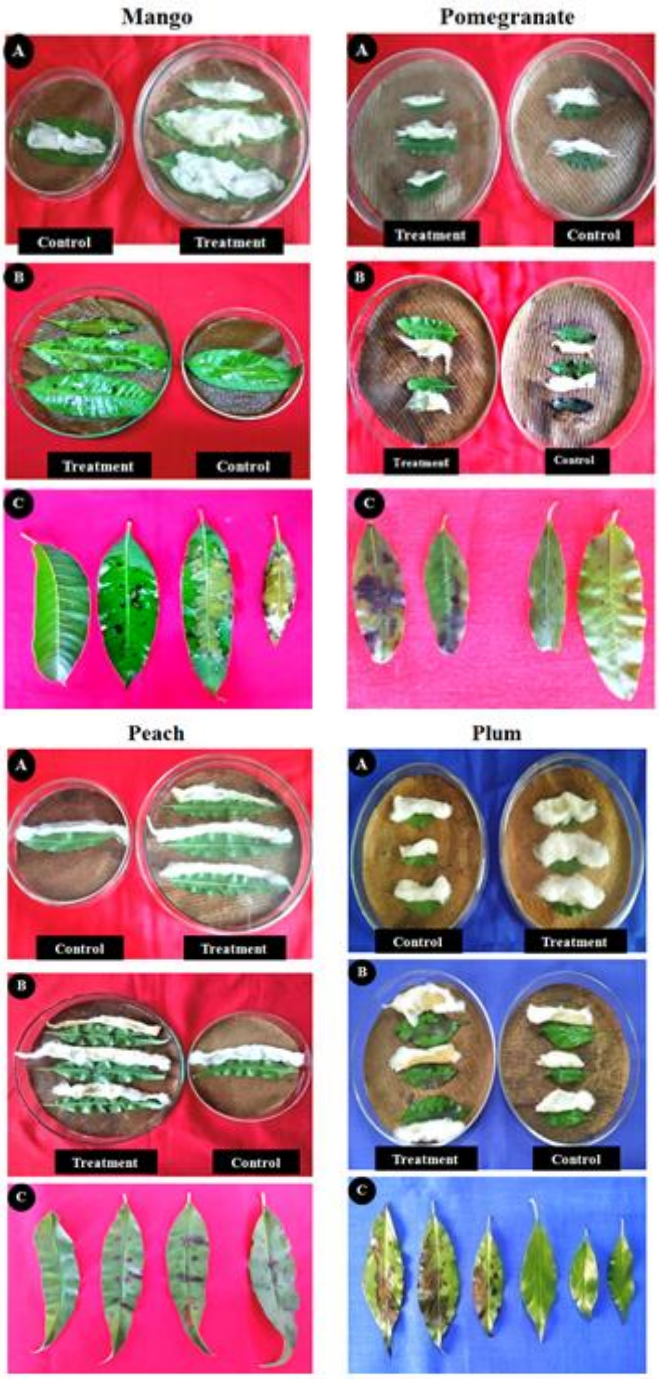

Fig. 3. (A) Inoculation of the fruit plant leaves of mango, pomegranate, peach and plum with the bacterial isolates by detached leaf technique; (B) Appearance of symptoms on the inoculated leaves; (C) Comparison of healthy and inoculated leaves

was constructed using 16S rRNA sequences of the isolates along with the sequences retrieved from NCBI GenBank databases. The results from the phylogenetic analysis indicated that each bacterial isolate was clustered to its corresponding strain from GenBank based on their sequence homology which was reflected by the bootstrap value in the node. These results also indicated the isolates to be *X. citri* pv. *mangiferaeindicae, X. axonopodis* pv. *punicae,* and *X. arboricola* pv. *pruni* causing bacterial canker in mango, bacterial blight disease in pomegranate and bacterial leaf spot of peach and plum, respectively.

Table 2. Biochemical characters of the *Xanthomonas* spp. isolated from four fruit plants

Tests	Mango	Pomegranate	Peach	Plum	Tests	Mango	Pomegranate	Peach	Plum
KOH	+	+	+	+	Cellobiose	+	-	-	-
Catalase	+	+	+	+	Melezitose	-	-	-	-
Oxidase	+	+	+	+	α-Methyl-D-mannoside	-	-	-	-
Starch hydrolysis	+	+	+	+	Xylitol	**-**	**-**	-	-
Levan production	+	+	+	+	ONPG	**+**	**-**	-	-
Citrate utilization	+	+	+	+	Esculin hydrolysis	+	+	+	+
Nitrate reduction	-	-	+	+	D-Arabinose	**+**	-	-	-
Gelatin	+	+	+	+	Sorbose	-	-	-	-
Arginine dihydrolase test	+	+	+	+	Malonate utilization	-	+	+	+
Indole production	+	+	+	+	Inulin	**+**	**-**	+	**+**
Lactose	**-**	**+**	+	**+**	Sodium gluconate	+	-	-	-
Xylose	+	-	-	-	Glycerol	-	-	+	+
Maltose	+	-	+	+	Salicin	+	-	+	+
Fructose	+	-	+	+	Dulcitol	**-**	**-**	-	-
Dextrose	**+**	**-**	+	+	Inositol	**-**	**-**	+	+
Galactose	**+**	**-**	+	+	Sorbitol	-	-	+	+
Raffinose	-	-	-	-	Mannitol	**+**	-	-	-
Trehalose	**+**	+	-	-	Adonitol	-	+	-	-
Melibiose	-	-	-	-	Arabitol	-	-	-	-
Sucrose	+	+	-	-	Erythritol	**-**	**-**	-	-
L-Arabinose	**+**	**-**	+	+	α-Methyl-D-glucoside	-	**-**	-	-
Mannose	+	**-**	+	+	Ornithine utilization	-	-	+	-
Urease	-	-	-	-	Phenylalanine deamination	**+**	**+**	+	+
H_2S production	-	-	-	-	Rhamnose	**-**	**-**	+	**+**

(+) - positive reaction, (-) - negative reaction

3.2 Discussion

The agri-based Indian economy is heavily depended on production and export of fruit crops and thus has helped in enhancing the economic status of the country. Diseases of bacterial origin cause severe loss to these fruit crops in different states of India. However, among the bacterial diseases of plants, diseases caused by the genus *Xanthomonas* is of great economic importance because of its wide host range [10]. In our study, the nature of the symptoms caused by the genus *Xanthomonas* in pomegranate, mango, peach and plum were similar to what has been reported for by Jami et al. [11], Pruvost et al. [12], Bora and Kataki [13], Jadalla and Saad [14] and Robe et al. [15]. All the biochemical characters under the present study were co-related with the characters for all four isolated pathogens as described in the Bergey's Manual of Systematic Bacteriology Buchanan and Gibbons, [16]. The identification of the bacterial isolates in the present investigation with respect to symptoms, morphological, biochemical characters and pathological study are in agreement with the previous descriptions (Hingorani and Mehta [17], Mondal and Singh [18], Icoz et al., [19], Ofoe et al. [20] Chowdappa et al [21]. The study of these characteristics played an important role in identifying the genus as *Xanthomonas*. Classical methods

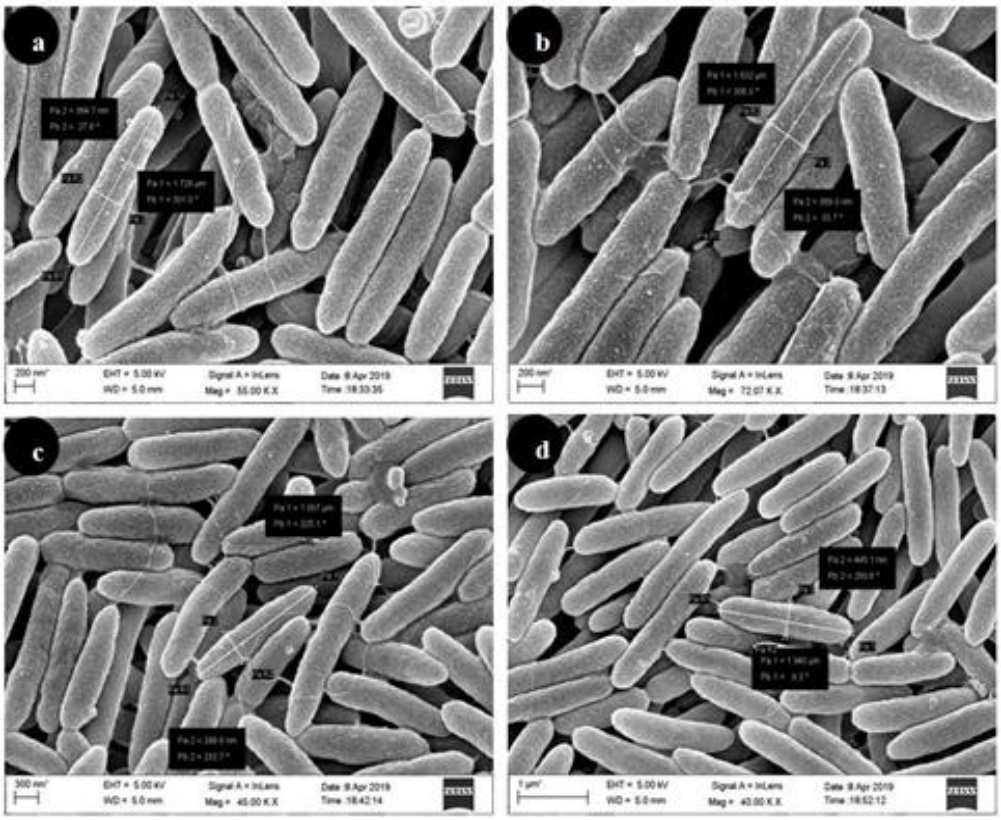

Fig. 4. Scanning electron micrograph of *Xanthomonas* spp
isolated from four fruit plants. (a) X. citri pv. mangiferaeindicae from mango, (b) X. axonopodis pv. punicae from pomegranate, (c) X. arboricola pv. pruni from peach and (d) X. arboricola pv. pruni from plum

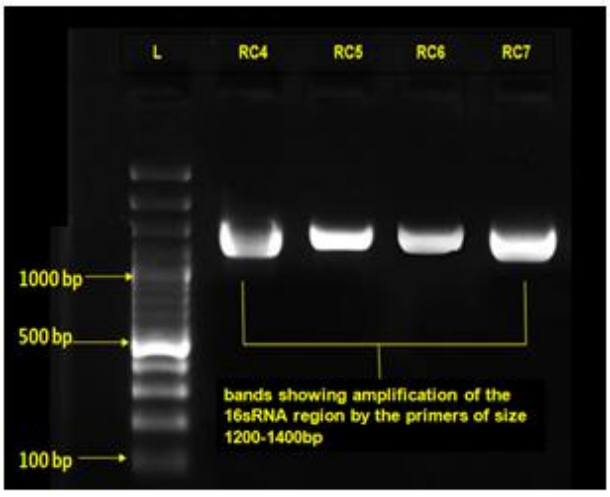

Fig. 5. Agarose gel electrophoresis showing amplification of the DNA products of bacterial pathogen Xanthomonas species
L-100bp ladder. RC4 -Mango isolate X. citri pv. mangiferaeindicae, RC5 -Pomegranate isolate X. axonopodis pv. punicae, RC6 -Peach isolate X. arboricola pv. pruni, RC7 -Plum isolate X. arboricola pv. Pruni

like biochemical and carbohydrate utilization tests were also used for identification and differentiation of bacteria; however, these tests cannot distinguish among the closely related species [22]. The 16S rDNA sequencing has been widely used as a reliable tool for identification and establishing phylogenetic relationships among bacteria [23]. Various

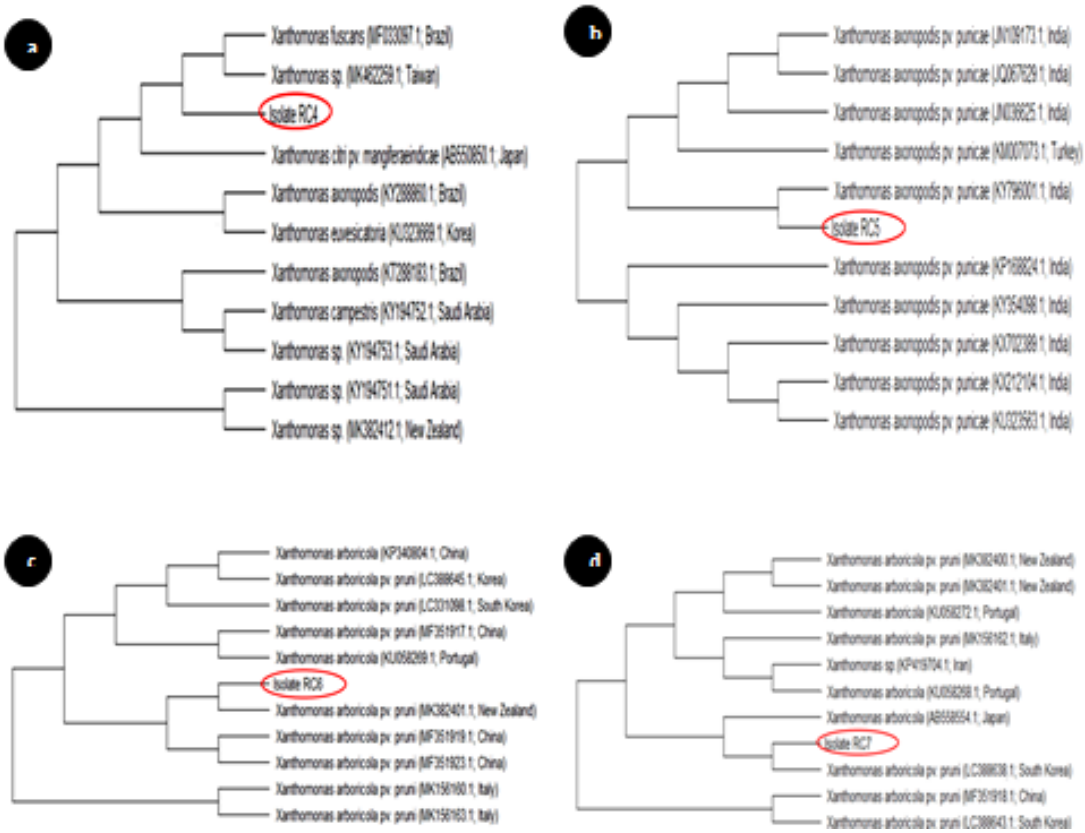

Fig. 6. Phylogenetic tree showing the genetic relationship of the bacterial isolates (*Xanthomonas* spp.) with other strains by using maximum likelihood method with 500 bootstrap replicates

RC4 -Mango isolate X. citri pv. mangiferaeindicae, RC5 -Pomegranate isolate X. axonopodis pv. punicae, RC6 -Peach isolate X. arboricola pv. *pruni*, *RC7 -Plum isolate X. arboricola pv. pruni*

studies also indicated 16S rDNA sequence analysis as an authenticated technique to review bacterial isolates at species level Garrity and Holt [24], Alam et al [25]. Hence the identity of the bacterial pathogen was confirmed in present study as Xanthomonas spp. based on the nucleotide sequencing. In addition to the identification of bacterial pathogens, confirmation of the disease could be done by matching the characteristics described by Pruvost et al. [26] and Ofoe et al. [20] for X. citri pv. mangiferaeindicae causing bacterial canker in mango. In case of bacterial blight of pomegranate, earlier reports Chowdappa et al. [21] Sharma et al. [26] helped in ascertaining the disease caused by *X. axonopodis* pv. *punicae*. Previously, the occurrence of bacterial leaf spot of peach caused by *X. arboricola* pv. *pruni* was reported from other countries like Iran Jami et al. [11] and China Robe et al [15]. On the other hand, Shen et al. [27] reported bacterial leaf spot disease on Japanese plum caused by *X. arboricola* pv. *pruni* from Taiwan. Ritchie [28] viewed bacterial spot of stone fruit (BSSF) as the most economically important disease of peach, nectarine, Japanese plum, apricot and almond which motivated to carry out the present investigation on bacterial disease of peach and plum along with two most important fruit crops (cash crops) of India *viz.*, mango and pomegranate. To our knowledge these bacterial diseases were not reported earlier from Assam although Bora and Kataki [13] first reported bacterial blight disease on pomegranate from this state.

4. CONCLUSION

The current study sheds light on the prevalence of bacterial diseases attributed to the genus Xanthomonas and underscores its significance in fruit crops. A comprehensive investigation encompassing all fruit crop diseases in the North Eastern Region (NER), including Assam, is imperative to comprehend the biodiversity of these plant pathogenic microflora. This understanding is essential for devising effective management strategies. The favorable climate in

the entire North Eastern region, especially Assam, provides ideal conditions for the commercial cultivation of fruit crops. Consequently, there is a pressing need for more extensive studies on bacterial diseases, coupled with an in-depth exploration of pathogen taxonomy, to harness the full potential of fruit crop cultivation in this region.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Opina N, Tavner F, Hollway G, Wang JF, Li TH, Maghirang R, Fegan M, Hayward AC, Krishnapillai V, Hong WF, Holloway BW, Timmis JN. A novel method for development of species and strain-specific DNA probes and PCR primers for identifying *Burkholderia solanacearum* (formerly *Pseudomonas solanacearum*). Asia-Pacific Journal of Moleculer Biology and Biotechnology. 1997;5:19-30.
2. Patel MK, Kulkarni YS, Moniz L. *Pseudomonas Mangifera eindicae* pathogenic on mango. Indian Phytopathology. 1948;1:147-152.
3. Prakash O, Misra A, Raoof M. Studies on mango bacterial canker disease. Bioogical Memoirs. 1994;20:95-107.
4. Randhawa PS, Civerolo EL. A detached-leaf bioassay for Xanthomonas campestris pv. pruni. Phytopathology. 1985;75(9): 1060-3.
5. Rohrbach KG, Johnson MW. Pests, diseases and weeds. In: The Pineapple: Botany, Production and Uses. CABI Publishing, Wallingford, UK. 2003;203-251.
6. Sundin GW, Castiblanco LF, Yuan X, Zeng Q, Yang CH. Bacterial disease management: challenges, experience, innovation and future prospects: challenges I bacterial molecular plant pathology. Molecular Plant Pathology. 2016;17(9):1506-1518.
7. Cardinal MJ, Meghrous J, Lacroix C, Simard RE. Isolation of *Lactococcus lactis* strains producing inhibitory activity against Listeria. Food Biotechnology. 1997; 11(2):129-146.
8. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution. 2018;35:1547-1549.
9. Sharma A, Gautam S, Wadhawan S. Xanthomonas. In: Encyclopedia of Food Microbiology, 2nd edn. Academic Press. 2014;811-817.
10. Jami F, Kazempour MN, Elahinia SA, Khodakaramian G. First report of *Xanthomonas arboricola* pv. *pruni* on stone fruit trees from Iran. Journal of Phytopathology. 2005;153(6):371-372.
11. Pruvost O, Boyer C, Vital K, Gagnevin L, De Bruno Austin L; Rey JY. First report in Ghana of *Xanthomonas citri* pv. *mangiferaeindicae* causing mango bacterial canker on *Mangifera indica*. Plant Disease. 2011;95(6):774-774.
12. Bora LC, Kataki L. *Xanthomonas axonopodis* pv. *punicae*-A new threat to pomegranate plants in Assam. *Indian* Journal of Hill Farming. 2014;27(1):100-101.
13. Jadalla NM, Saad GIF. Morphological and biochemical characterization of *Xanthomonas arboricola* pv. *pruni*. Libyan Journal of Plant Protection. 2018; 8: 108-121.
14. Robe BL, Wang CA, Zhang Z, Li S. Bacterial leaf spot of peach caused by *Xanthomonas arboricola* pv. *pruni* in China. Canadian Journal of Plant Pathology. 2018;40(2):299-305.
15. Buchanan RE, Gibbons NE. Bergey's Manual of Systematic Bacteriology. 8th edn. Williams & Wilkins. 1984.
16. Hingorani MK, Mehta, PP. Bacterial leaf spot of pomegranate. Indian Phytopathology. 1952;5: 55-56.
17. Mondal KK, Singh D. Bacterial blight - an emerging disease of pomegranate. In Souvenir and abstracts 5th international conference on plant pathology in the globalized era, IARI, New Delhi. 2009; 10-13.
18. Icoz SM, Polat I, Sulu G, Yilmaz M, Unlu A, Soylu S, Baysal Ö. First report of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* in Turkey. Plant Disease. 2014;8(10):1427-1427.
19. Ofoe R, Dzidzienyo D, Honger JO, Coffie SA, Out B. Morphological and molecular characterization of *Xanthomonas campestris* that infect mango in Ghana. PhD. Thesis. University of Ghana, Ghana; 2016.

20. Chowdappa A, Kamalakannan A, Kousalya S, Gopalakrishnan C, Venkatesan K, Raju GS. Isolation and characterization of *Xanthomonas axonopodis* pv. *punicae* from bacterial blight of pomegranate. Journal of Pharmacognosy and Phytochememistry. 2018; 7(4): 3485-3489.
21. Goswami G, Deka P, Das P, Bora SS, Samanta R, Boro RC, Barooah M. Diversity and functional properties of acid-tolerant bacteria isolated from tea plantation soil of Assam. 3 Biotech. 2017;7(3), 1-16.
22. Borsodi AK, Kiss RI, Cech G, Vajna B, To´th EM, Ma´rialigeti K. Diversity and activity of cultivable aerobic planktonic bacteria of a saline lake located in Sovata, Romania. Folia Microbiologica. 2010;55 (5):461–466.
23. Garrity GM, Holt JG. The road map to the Manual. In: Boone DR, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology; 2nd edn. Springer, New York. 2001;119–166
24. Alam SI, Bansod S, Goel AK, Singh L. Characterization of an environmental strain of *Bacillus thuringiensis* from a hotspring in western Himalayas. Current Microbiology. 2011;62:547–556.
25. Sharma J, Sharma KK, Kumar A, Mondal KK, Thalor S, Maity A, Jadhav VT. Pomegranate bacterial blight: symptomatology and rapid inoculation technique for *Xanthomonas axonopodis* pv. *punicae*. Journal of Plant Pathology. 2017; 99(1):109-119.
26. Shen YM, Huang TC, Chao CH, Liu H. First report of bacterial spot caused by *Xanthomonas arboricola* pv. *pruni* on Japanese plum in Taiwan. Plant Disease. 2013;97(6):835-835.
27. Ritchie DF. Bacterial spot. In: Compendium of stone fruit diseases. St. Paul: The American Phytopathological Society Press. 1995;50-52.

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