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Characterization and identification of soft rot bacterial pathogens of different fruits in Bangladesh

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Abstract

The aim of the study was to characterize and identify the soft rot bacterial pathogens of different fruits in Bangladesh. Soft rotted fruit samples of mango, apple, banana, papaya and pineapple were collected from different areas of Bangladesh based on characteristic soft rot symptoms. From these samples, 50 isolates were isolated. Among the 50 isolates, only 17 isolates showed positive result in potato soft rot test. Among 17 potato soft rot positive bacterial isolates, 10 isolates were found as oxidative fermentative test (OF) positive. Biochemical and physiological tests were performed for characterization of 10 'OF' positive and 07 OF negative bacterial isolates. Eight 'OF' positive isolates namely Mango 01, Mango 10, Mango 13, Mango 16, Apple 03, Banana 01, Pineapple 01 and Papaya 01 were identified as *Erwinia carotovora* subsp. *carotovora*. Remaining two 'OF' positive isolates namely Mango 11 and Mango 12 were identified as *Dickeya dadantii* (formerly *Erwinia chrysanthemi)*. The 07 'OF' negative bacterial isolates, namely Mango 02, Mango 03, Mango 05, Mango 08, Apple 01, Apple 02 and Pineapple 02 were identified as *Pseudomonas marginalis*.

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Introduction

Fruits are a very important component of a healthy diet. They are good source of vitamins, minerals, starch, protein, water etc. Fruits protect humans from various types of major diseases. Dietary fiber from fruits, as part of an overall healthy diet, helps reduce blood cholesterol levels and may lower risk of heart disease (Anon., 2009). Eckert and Ogawa (1985) stated that because of high moisture content, fresh fruits are very susceptible to attack by diverse pathogens during the period between harvest and consumption. Worldwide post-harvest fruit loss per year is estimated to be as high as 30-40% and may be even higher in some developing countries (Panhwar, 2006). Among the causes of post-harvest losses of fruits, bacterial soft rot is one of the major causes. Different pectolytic bacteria cause the soft rot of different fruits (Anon., 2013). The bacterial soft rot disease is commonly found in pineapple, banana, mango, grape, apple, jackfruit, avocado, papaya, citrus etc. Soft rot can occur in plants cultivated in the field and also in harvested crops. In Bangladesh, fruit loss due to soft rot attack is approximately 25-50% (Miaruddin and Shahjahan, 2008). Several bacterial species of different genera can enzymatically macerate paranchymatous tissue of a wide range of plants. Once in the plant tissue, these bacteria produce increasing amounts of pectolytic enzymes that break down the pectic substances of the middle lamella causing the maceration and collapse of the tissues (Gupta and Thind, 2006). Although many bacteria possess the ability to produce tissue-macerating enzymes, only a few have been associated with rotting of living plant tissue. These include *Erwinia* spp., *Bacillus subtilis, B. polymixa, Pseudomonas marginalis* and pectolytic strains of *Pseudomonas* and *Flavobacterium* spp. (Dowson, 1957). A common characteristic of soft rots and associated disorders is the lack of specificity of the host pathogen interaction. Certain bacterial species can infect a wide range of crops and vegetables and conversely one crop can be infected by several species or pathovars. Species of *Erwinia* belonging to the carotovora group (Lelliot and Dickey, 1974) are usually referred as the soft rot bacteria. They are *E. carotovora* subsp. *carotovora, E. carotovora* subsp. *atroseptica* and *E. chrysanthemi*. They have a worldwide distribution. *E. chrysanthemi* (presently *Dickeya dadantii*) is a pathogen of a wide range of tropical and subtropical crops. It is common in greenhouse crops and some field crops (Hopper and Kelman, 1969). *E. carotovora* subsp. *carotovora* strains have a wide distribution in both the temperate and tropical zones and are pathogenic to a much wider range of plants than *E. chrysanthemi* (Dickey, 1979). In Bangladesh, little is known about the characteristics of soft rot bacterial strains of different fruits. In fact, research reports on soft rot bacteria of fruits are scarce in Bangladesh. Identification of causative bacterial strains may significantly help to take appropriate control measures and develop detection systems in field as well as storage conditions. Considering the above facts the present study was undertaken to characterize and identify soft rot causing bacterial pathogens of different fruits in Bangladesh.

Materials and methods

Collection of disease sample

Diseased fruit samples were selected based on visible symptoms of soft rot and characteristic odor as described by Agrios (1997) and Singh (2001). The soft rot causing bacteria were isolated from five kinds of rotted fruits such as mango (*Mangifera indica*), apple (*Malus domestica*), banana (*Musa sapientum*), papaya (*Carica papaya*) and pineapple (*Ananus comosus*). The infected fruits were collected from various markets and storage of Gazipur, Dhaka, Rajshahi, Dinajpur and Naogaon districts of Bangladesh. Altogether 50 rotting fruit samples were collected of which 32 were mangoes, 10 apples, 3 papaya, 3 pineapple and 2 were banana. The collected samples were brought to the microbiology laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) and soft rot bacterial strains were extracted within 24 to 48 h.

Isolation of bacterial pathogens

Bacterial organisms were isolated from different fruit samples by the "streak plate" technique as described by Mortensen (1997) and Kim *et al.* (2002).

Yeast Peptone Dextrose Agar (YPDA) was used for isolation of soft rot bacteria. Firstly, a small part from the margin of rotted tissues of the infected fruits was cut and then surface disinfected with 1% sodium hypochlorite (NaOCl) for 2-3 min. Sterilized samples were washed several times in distilled water to remove the residual hypochlorite. The samples were placed in petridishes containing distilled water and were crushed with a sterile scalpel. After crushing, the petridishes were kept undisturbed for 10-15 min to release the bacteria associated with the rotted tissues. One loop-full of resulting suspension was streaked on the solidified YPDA medium in each plate. The plates were incubated at 30°C for 48 h. Characteristic individual bacterial colonies that appeared on YPDA medium were sampled using a wire loop and transferred to another plate. Purification of bacterial colonies was done by re-streaking of a single colony on a fresh plate.

Potato soft rot test

All of the bacterial isolates originated from single colonies were tested for their ability to cause soft rot on potato tubers following standard procedure (Lelliot *et al.,* 1966). The bacterial cultures that produced characteristic symptoms of soft rot on potato slices were selected and preserved at 4°C for further studies in test tubes containing YPDA media overlaid with sterile liquid paraffin.

Characterization of the pathogenic bacterial isolates

For characterization of the isolated pathogenic bacterial isolates, a series of physiological and biochemical tests were performed. The tests were a. fermentation of glucose (OF test) (Hugh and Leifson, 1953) b. Gram reaction (Suslow *et al.,* 1982), c. catalase production, d. gelatin liquefaction test (Schaad, 1988), e. urease activity (Schaad, 1988), f. nitrate reduction test (Lelliot and Dickey, 1974), g. indole test (Lelliot and Dickey, 1974), h. acetoin production (Dye, 1968), i. methyl red test (Dye, 1968), j. gas formation (Hugh and Leifson, 1953), k. growth at 41°C temperature l. growth in 5% NaCl and m. utilization of diverse carbon sources (Ayers *et al.,* 1919).

Two strains *E. carotovora* subsp. *carotovora* P 138 and *Dickeya dadantii* Ura-2 (formerly *Erwinia chrysanthemi*) were used as reference strains in this experiment.

Results

Isolation of bacteria

A total of 50 bacterial isolates isolated from mango, apple, banana, pineapple and papaya were collected from different locations of Bangladesh. Colony morphology of most of the isolates on YPDA was white, creamy white or grayish creamy white, smooth, round, glistening and slightly raised. Some isolates were flat to slightly raised, margins undulated to feathery and visible on isolation plates after about 24 hrs. (Fig. 1).

Fig. 1. Bacterial isolate isolated from rotted fruit sample.

Potato soft rot test

Based on the potato soft rot test result, the pathogenic isolates were selected from isolated 50 isolates. Among 50 bacterial isolates, 17 isolates produced soft rot on potato slices (Fig. 2). A list of potato soft rot positive isolates with their host and sampling locations are given in Table 1. All soft rot positive isolates were selected for characterization and identification.

Characterization of isolated soft rot bacteria

Oxidative fermentative (OF) test

Among the 17 isolates, 10 isolates viz. Mango 01, Mango 10, Mango 11, Mango 12, Mango 13, Mango 16, Apple 03, Banana 01, Pineapple 01, Papaya 01, reference strain *E. carotovora* subsp. *carotovora* P 138 and *Ddadantii* Ura-2 produced positive of test results (Table 2).

They produced yellow color in both liquid paraffin covered and uncovered tubes (Fig 3). The remaining 7 isolates namely Mango 02, Mango 03, Mango 05, Mango 08, Apple 01, Apple 02 and Pineapple 02 were 'OF' negative (Table 4). They produced yellow color only in uncovered tubes (Fig. 3).

Table 1. List of potato soft rot positive isolates isolated from soft rotted fruits from different locations of Bangladesh.

Sl No.	Isolate No.	Locations	Isolation time
1	Mango 01	Storehouse	2013
2	Mango 02	Gazipur	,,
3	Mango 03	,,	,,
4	Mango 05	,,	,,
5	Mango 08	Rajshahi	,,
6	Mango 10	,,	,,
7	Mango 11	Salna	,,
8	Mango 12	Storehouse	,,
9	Mango 13	Naogaon	,,
10	Mango 16	,,	,,
11	Apple 01	Storehouse	,,
12	Apple 02	,,	,,
13	Apple 03	,,	,,
14	Banana 01	Gazipur	,,
15	Pineapple 01	,,	,,
16	Pineapple 02	,,	,,
17	Papaya 01	Gazipur	,,

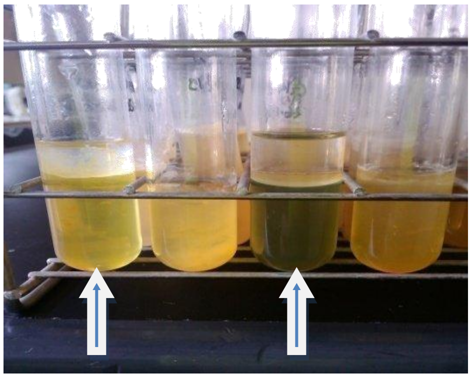
A. Soft rot positive B. Un-inoculated control

Fig. 2. Soft rot test on potato slice.

Characterization of 'OF' positive soft rot bacterial isolates

All the 10 'OF' positive bacterial isolates and reference strain *E. carotovora* subsp. *carotovora* P 138 and *Ddadantii* Ura 2 produced positive results in the catalase (Fig. 4), gelatin liquefaction (Fig. 5), nitrate reduction (Fig. 6) and acetoin tests (not shown) and also were able to grow at 41°C temperature (not shown). The isolates gave a negative Gram reaction and also tested negative for urease (Table 2).

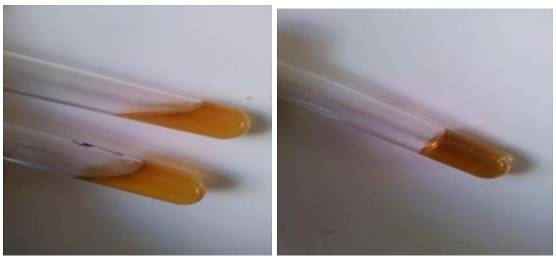
However, they differed in the gas formation, indole test (not shown), methyl red test (Fig. 7) and in their ability to grow in 5% NaCl.



A B

Fig. 3. Oxidative fermentative test; A showing positive reaction and B showing negative reaction.

Fig. 4. Catalase test (Bubble formation; which denotes positive reaction).



A **B**

Fig. 5. Gelatin liquefaction test. A. Positive B. Negative.

Fig. 6. Nitrate reduction test (positive).

All the of 'OF' positive isolates coincided with reference strain *E. carotovora* subsp. *carotovora* P 138 by producing positive results in the methyl red test, growth in 5% NaCl and gas production test. This was in contrast to isolates Mango 11, Mango 12 and *Ddadantii* Ura 2 which produced negative results (Table 2, Fig 7).

A B C

Fig. 7. Methyl red test: A & B showing positive and C showing negative result.

Furthermore, all 'OF' positive isolates together with reference strain *E. carotovora* subsp. *carotovora* P 138 gave negative results in the indole test, in contrast to isolates but Mango 11, Mango 12 and reference strain *Ddadantii* Ura 2, which produced a positive result (Table 2).

Carbon sources utilization of 'OF' positive bacterial isolates

All the 'OF' positive bacterial isolates, together with the two reference strains *E. carotovora* subsp. *carotovora* P 138 and *D. dadantii* Ura 2 utilized arabinose, xylose, galactose, raffinose and manitol as sole source of carbon (Table 3). They did not use maltose, sorbitol and dulcitol as sole source of carbon (Table 3). Lactose and inositol utilization was not observed in two isolates, namely Mango 11 and Mango 12 and in reference strain with reference strain *D. dadantii* Ura 2,while all other 'OF' positive isolates and reference strain *E. carotovora* subsp. *carotovora* P 138 utilized these compounds as sole sources of carbon (Table 3).

Table 2. Physiological and biochemical characteristics of 'OF' positive soft rot bacterial isolates sampled from different fruits.

Sample name	OF test	Gram reaction	Catalase test	Gelatin liquefaction	Nitrate reduction	Indole test	Methyl red test	Acetoin test	Urease test	Growth at 41°C	Growth in 5% Nacl	Gas production
Mango 01	+	-	+	+	+	-	+	+	-	+	+	-
Mango 10	+	-	+	+	+	-	+	+	-	+	+	-
Mango 11	+	-	+	+	+	+	-	+	-	+	-	+
Mango 12	+	-	+	+	+	+	-	+	-	+	-	+
Mango 13	+	-	+	+	+	-	+	+	-	+	+	-
Mango 16	+	-	+	+	+	-	+	+	-	+	+	-
Apple 03	+	-	+	+	+	-	+	+	-	+	+	-
Banana 01	+	-	+	+	+	-	+	+	-	+	+	-
Pineapple 01	+	-	+	+	+	-	+	+	-	+	+	-
Papaya 01	+	-	+	+	+	-	+	+	-	+	+	-
Ecc P 138	+	-	+	+	+	-	+	+	-	+	+	-
Ddad Ura-2	+	-	+	+	+	+	-	+	-	+	-	+

Reference isolates: *Ecc* P 138 (*Ecc* = *Erwinia carotovora* subsp. *carotovora*), *Ddad* Ura-2 (*Ddad*= *Dickeya dadantii*), (+) = growth positive, (-) = negative result.

Table 3. Utilization of different sugars and alcohols by OF positive soft rot bacterial isolates.

Sample name	Maltose	Lactose	Arabi-nose	Raffinose	Xylose	Galactose	Inositol	Dulcitol	Sorbitol	Manitol	Control
Mango 01	-	+	+	+	+	+	+	-	-	+	-
Mango 10	-	+	+	+	+	+	+	-	-	+	-
Mango 11	-	-	+	+	+	+	-	-	-	+	-
Mango 12	-	-	+	+	+	+	-	-	-	+	-
Mango 13	-	+	+	+	+	+	+	-	-	+	-
Mango 16	-	+	+	+	+	+	+	-	-	+	-
Apple 03	-	+	+	+	+	+	+	-	-	+	-
Banana 01	-	+	+	+	+	+	+	-	-	+	-
Pineapple 01	-	+	+	+	+	+	+	-	-	+	-
Papaya 01	-	+	+	+	+	+	+	-	-	+	-
Ecc P 138	-	+	+	+	+	+	+	-	-	+	-
Ddad Ura-2	-	-	+	+	+	+	-	-	-	+	-

(+) = growth positive (-) = negative result.

Characterization of 'OF' negative soft rot bacterial isolates

All the 7 'OF' negative bacterial isolates, namely Mango 02, Mango 03, Mango 05, Mango 08, Apple 01, Apple 02 and Pineapple 02 yielded a Gram negative reaction test and were gave negative results in the indole, methyl red and urease test and were unable to grow at 41ºC (Table 4). Conversely, the isolates yielded positive catalase, gelatin liquefaction, nitrate reduction, acetoin test and were able to grow in 5% NaCl.

Carbon sources utilization of 'OF' negative bacterial isolates

All 'OF' negative bacterial isolates, namely Mango 02, Mango 03, Mango 05, Mango 08, Apple 01, Apple 02 and Pineapple 02 utilized maltose, lactose, arabinose, xylose, galactose, inositol, sorbitol and manitol as sole source of carbon (Table 5). On the other hand, they did not utilize raffinose and dulcitol (Table 5).

Table 4. Physiological and biochemical characteristics OF negative of soft rot bacterial isolates.

Sample name	OF test	Gram reaction	Catalase test	Gelatin liquefaction	Nitrate reduction	Indole test	Methyl red test	Acetoin test	Urease test	Growth at 41° C	Growth in 5% Nacl	Gas production
Mango 02	-	-	+	+	+	-	-	+	-	-	+	-
Mango 03	-	-	+	+	+	-	-	+	-	-	+	-
Mango 05	-	-	+	+	+	-	-	+	-	-	+	-
Mango 08	-	-	+	+	+	-	-	+	-	-	+	-
Apple 01	-	-	+	+	+	-	-	+	-	-	+	-
Apple 02	-	-	+	+	+	-	-	+	-	-	+	-
Pineapple 02	-	-	+	+	+	-	-	+	-	-	+	-
*P mar**	-	-	+	+	+	-	-	+	-	-	+	-

* =Results given from according to Kreigh and Holt (1984) from Burgey's Manual of Systematic Bacteriology, *P mar* = *Pseudomonas marginalis*.

Table 5. Utilization of different sugars and alcohols by OF negative soft rot bacterial isolates.

Sample name	Maltose	Lactose	Arabinose	Raffinose	Xylose	Galactose	Manitol	Inositol	Dulcitol	Sorbitol	Control
Mango 02	+	+	+	-	+	+	+	+	-	+	-
Mango 03	+	+	+	-	+	+	+	+	-	+	-
Mango 05	+	+	+	-	+	+	+	+	-	+	-
Mango 08	+	+	+	-	+	+	+	+	-	+	-
Apple 01	+	+	+	-	+	+	+	+	-	+	-
Apple 02	+	+	+	-	+	+	+	+	-	+	-
Apple 04	+	+	+	-	+	+	+	+	-	+	-
Pineapple 02	+	+	+	-	+	+	+	+	-	+	-
*P mar**	+	+	+	-	+	+	+	+	-	+	-

* =Results given from according to Kreigh and Holt (1984) from Burgey's Manual of Systematic Bacteriology, (+) = growth positive, (-) = negative result.

Table 6. List of identified bacterial isolates isolated from different fruits according to growth, physiological and biochemical characteristics.

Name of Isolates	Host	Identified as
Mango 01	Mango	*Erwinia carotovora* subsp. *carotovora*
Mango 02	,,	*Pseudomonas marginalis*
Mango 03	,,	*P. marginalis*
Mango 05	,,	*P. marginalis*
Mango 08	,,	*P. marginalis*
Mango 10	,,	*E. carotovora* subsp. *carotovora*
Mango 11	,,	*Dickeya dadantii*
Mango 12	,,	*D. dadantii*
Mango 13	,,	*E. carotovora* subsp. *carotovora*
Mango 16	,,	*E. carotovora* subsp. *carotovora*
Apple 01	Apple	*P. marginalis*
Apple 02	,,	*P. marginalis*
Apple 03	,,	*E. carotovora* subsp. *carotovora*
Banana 01	Banana	*E. carotovora* subsp. *carotovora*
Pineapple 01	Pineapple	*E. carotovora* subsp. *carotovora*
Pineapple 02	,,	*P. marginalis*
Papaya 01	Papaya	*E. carotovora* subsp. *carotovora*

Discussion

The results obtained from physiological and biochemical tests, in addition to the carbon source utilization tests of 8 'OF' positive fruit soft rot bacterial isolates (Mango 01, Mango 10, Mango 13, Mango16, Apple 03, Banana 01, Pineapple 01 and Papaya 01) were identical to those produced by reference strain of *E. carotovora* subsp. *carotovora* P 138. Thus, they were identified as *Erwinia carotovora* subsp. *carotovora* (Table 6). Two other 'OF' positive isolates, namely Mango 11 and Mango 12 were identical with reference strain of *Dickeya dadantii* Ura-2 and were, therefore, identified as the member of *Ddadantii* (formerly *E. chrysanthemi*) (Table 6). All remaining 'OF' negative bacterial isolates, namely Mango 02, Mango 03, Mango 05, Mango 08, Apple 01 and Pineapple 02 were identified as *Pseudomonas marginalis* (Table 6). The similar test patterns yielded by results *E. carotovora* subsp. *carotovora, Ddadantii* and *P. marginalis* have been described in Burgey's Manual of Systematic Bacteriology (Kreigh and Holt, 1984). The findings are also similar to the findings of Alam *et al.* (1999) and Khan *et al.* (2000) for *E. carotovora* subsp. *carotovora* and *E. chrysanthemi. E. carotovora* subsp. *carotovora* bacteria were isolated from mango, apple, banana, pineapple and papaya. *Ddadantii* was isolated from mango and *P. marginalis* was isolated from mango, apple and pineapple.

These bacterial pathogens were also reported in earlier studies in different fruits, including mango, apple, pineapple, banana and papaya (Sundararaj *et al.,* 1972; Guzman and Wang, 1998; Gardan *et al.,* 2003; Cole, 2008). Usually *E. carotovora* subsp. *carotovora* and *D. dadantii* are the causative agents of soft rot in the tropical regions (>25ºC) (Perombolen and Kelman, 1980). Since the climate of Bangladesh is moderately tropical and humid, the findings of *E. carotovora* subsp. *carotovora, D. dadantii* and *P. marginalis* as major soft rot bacterial pathogens were logically acceptable. The biochemical and physiological techniques were used as the characterization tools. However, the utilization of molecular characterization techniques would provide more reliable results. So, the results of the present study may be validated by molecular characterization methods.

Place of Work

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Conclusion

Three bacterial pathogen namely *Erwinia carotovora* subsp. *carotovora, Dickeya dadantii* (formerly *Erwinia chrysanthemi*) and *Pseudomonas marginalis* were identified from soft rot disease of mango, banana, apple, pineapple and papaya in Bangladesh. These findings were based on conventional biochemical and physiological analysis. However, molecular analysis will be needed to draw a solid conclusion about these findings.

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