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Biochemical and Molecular Characterization of Bacterial Wilt Disease of Banana and Evaluation of their Antibiotic Sensitivity

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ZFZ, SMZH, Md. Firose Hossain, Md. Faruk Hasan, MAI, MK and BS designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors ZFZ, SMZH and Md. Firose Hossain managed the analyses of the study. Author ZFZ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Isolation and characterization of bacteria from the bacterial wilt disease of banana and evaluation of their sensitivity.
Study Design: This study was an experimental laboratory design.
Place and Duration of Study: Disease infected banana plant leaves were collected from Rajshahi University Campus, Rajshahi, Bangladesh in 2015 and the experiment had been conducted from 2015 to 2016.
Methodology: Two bacterial colonies were isolated (W_1 and W_2) and characterized by some morphological, biochemical and molecular test. Antibiotic sensitivity pattern was determined by disc diffusion method against the isolated bacteria. Molecular analysis were observed through the

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amplification of 16s ribosomal RNA primers and *hrp* gene specific primers (BXW-1/RST65 and BXW-3/RST69) and were cross reacted with other pathovars of *Xanthomonas campestris*, including *X. campestris* pv. *musacearum* strains.

Results: Isolated both two bacterial colonies (W_1 and W_2) were rod-shape, gram negative and motile, but W_1 was yellow and W_2 was creamy in color. Biochemical tests showed that both of them were catalase positive with addition to isolate W_1 was MacConkey, citrate and TSI positive and SIM and urease negative whereas isolate W_2 was MacConkey, citrate and urease positive, and SIM and TSI negative. In antibiotic sensitivity test among the five antibiotic (chloramphenicol, gentamycin, erythromycin, penicillin and ciprofloxacin), isolate W_1 showed the highest susceptibility to penicillin and isolate W_2 at gentamycin. In the molecular analysis, DNA from the isolated bacteria W_1 showed approximately 1650bp band by using the 16s ribosomal primers 27-F and 1391-R. The presence of DNA sequence related to the *hrp* genes were successfully amplified by the isolate W_1 which was related to *X. campestris* pv. *musacearum*.

Conclusion: As there is no effective research work on bacterial wilt disease of banana in Bangladesh, this work will be helpful for further direction of this devastating disease management.

Keywords: Bacterial wilt of banana; Xanthomonas campestris; antibiotic sensitivity; PCR; hrp gene.

1. INTRODUCTION

Banana (*Musa paradisiaca*) is one of the most popular, nutritive and tasty fruit all over the world belonging to the family of Musaceae. Banana and plantain (*Musa* spp.) are the world's fifth most important food crops after maize, rice, wheat, and cassava [1].

Banana is one of the economically important fruit crops grown in Bangladesh in both homestead and commercial farms [2]. The country produces nearly 1.00 million tons of bananas annually [3].

Wilt is a common disease of banana of the whole world and caused by *Xanthomonas campestris* pv. *musacearum* (Xcm) [4] and it is known as BXW (Banana Xanthomonas Wilt). Bacterial wilt disease is also caused by *Ralstonia solanacearum* (redirected from *Pseudomonas solanacearum*) which is one of the most important pathogens on the banana plantation [5]. *R. solanacearum* (Smith) causes a vascular wilt disease [6] and has been ranked as the second most important bacterial pathogen. Both of the bacteria, *Xanthomonas campestris* and *Ralstonia solanacearum* are among the top ten bacterial species having been listed based on their scientific and economic importance in plant diseases [7].

Bacterial ooze is excreted from the plant organs and this is a mandatory sign that BXW may be present. Common symptoms on the fruit include yellow bacterial ooze in air spaces of leaf bases; premature ripening and internal discoloration of fruits [8]. The combination of disease symptoms can be effected by many factors. Some common visible symptoms are shown in vegetative parts of the enset, young leaves sometimes proceeded by yellowing and distortion, oldest leaves often develop a pronounced yellowing, necrosis and breakage of leaf, vascular bundles show a cream, yellow or pinkish discoloration that may extend throughout the plant. The BXW disease is spread by contaminated tools, sunbirds, insects such as bees and infected planting materials [9]. *R. solanacearum* shows the similar symptoms of wilting [10]. Among the numerous diseases infecting bananas, BXW along has been the most devastating diseases. Global concern arose over the livelihoods of African banana farmers and the millions relying on bananas as a staple food when the disease was at its worst between the years 2001 and 2005 [11]. It was estimated that there was a 30-52% decrease in banana yield due to BXW infection in Central Uganda from 2001-2004 [11]. The Pseudomonas wilt disease occurs in scattered plants or within group of plants [12].

In Bangladesh, the wilt disease of banana is a serious disease causing for several economic harms. But there is no suitable report on this disease.

Hence, the present study was carried out for the isolation, characterization and molecular analysis of bacterial wilt disease of banana with some antibiotic sensitivity test.

2. MATERIALS AND METHODS

2.1 Plant Materials Collection

Disease infected banana plant leaves were collected from Rajshahi University Campus,

Rajshahi, Bangladesh and identified as wilt disease of banana. Wilt disease infected leaves of banana plant were used as plant material for the present investigation.

2.2 Isolation of Bacteria

Wilt disease infected leaves were surface-sterilized with 10% bleach for 5 min, followed by seven rinses in sterile distilled water. Infected portion of leaves were aseptically excised with a sterile scalpel and placed onto the sterile petri dishes. Then the sample was crushed with sterile mortar and pestle. The crushed tissue was placed in LB liquid medium and was incubated at 37℃ for overnight. The liquid medium consists of peptone; NaCl and yeast extract containing respectively 1g, 1g and 0.5 g per 100mL. The pH of the constitution was generally adjusted to 7.3-7.5 using 0.1 N NaOH and 0.1 N HCl where necessary with the help of pH meter. The composition of 100mL LB agar medium is same as liquid medium with the addition of agar at 1.5g only. The pH of the constitution was adjusted to 7.3-7.5 and autoclaved at 121^0C and poured in each petri dish and was kept in the air flow cabinet for solidification. Then the isolated samples were plated in the petri dish for colony formation of bacteria in a sterilized condition and incubated them at 37°C for overnight. There were many single colonies found on the plates. From mixed culture, single colonies were taken by a loop of inoculation needle and touched on LB agar medium on the laminar flow bench for sub culturing. Then the sample was incubated for 16-18 hours at 37^0C temperatures. This process was repeated for 6 times until the pure cultures of isolated bacteria were obtained [13].

2.3 Characterization of Isolated Bacteria

Morphological and biochemical test were conducted for the characterization of the isolated bacteria. Two types of bacteria were identified as isolate W_1 (forming yellow color colony) and isolate W_2 (forming cream color colony). Some morphological and biochemical test were performed by selective media method followed by [14] and [15]. Colony morphology (size, shape, color and growth pattern) was recorded after overnight growth on LB agar plate at 37°C. Cell size was observed by light microscope. Gram staining test and a series of biochemical tests were performed for the characterization of the isolated bacteria using the criteria of Bergey's Manual of systematic bacteriology [16].

2.3.1 Gram staining

A loop full of the bacteria was spread on a glass slide and fixed by heating on a very low flame. Whole procedure of gram staining was maintained following by Chaudhry and Rashid (2011) [17].

2.3.2 SIM-medium test (Sulphide-indole-motility medium)

Semisolid SIM (Sulfide-Indole-Motility) medium was used for this experiment (ex. SIM medium, 0.35% agar), which was jellylike consistency, and was used for motility or certain chemical tests like gelatin-hydrolysis testing of bacteria. Bacteria were inoculated on SIM medium to test for hydrogen sulfide, indole and motility of the microorganism. The bacteria was inoculated by a swab and stab type method (rubs some bacteria on the surface of the medium and stabs a straight hole through the medium using a straight wire with the bacteria on it) and incubated for about 24 hours. The motility of bacteria was observed through the media, if they were motile. The indole portion of the test was performed by adding Kovac's reagent to the inoculated medium.

2.3.3 TSI (triple sugar iron) test

The TSI slant is a test tube that contains agar, a pH-sensitive dye (phenol red), 1% lactose, 1% sucrose, 0.1% glucose, (www.mbgbio.com) as well as sodium thiosulfate and ferrous sulfate or ferrous ammonium sulfate. With a sterilized straight inoculation needle, the top of a well isolated colony was touched. Bacteria was inoculated on TSI agar by first stabbing through the center of the medium to the bottom of the tube and then streaked on the surface of the agar slant. The test tube cap was kept loosely and incubated the tube at 35°C in ambient air for 18 to 24 hours.

2.3.4 Citrate test

Citrate is utilized as carbon and energy source in the citrate test of bacterial isolate [18]. This test was done to determine whether the isolated bacteria were better suited to aerobic or anaerobic environments, using Simmons citrate agar medium [19]. A single pure isolated colony of both the isolate W_1 and isolate W_2 was picked with a needle and the slant surface was lightly streaked. The culture was incubated at 35^0C for 18 to 48 hours.

2.3.5 Catalase test

Bacteria were incubated (18-24 h) on LB agar plates and 3% hydrogen peroxide (H_2O_2) was used to observe production of gas bubbles. A loop full of bacterial culture was mixed with a drop of H_2O_2 on a glass slide and observed for the production of gas bubbles with the naked eye and under a dissecting magnification of 25X [20]. Some bacteria contain flavoproteins that reduce oxygen (O_2), resulting in the production of hydrogen peroxide (H_2O_2) and in some cases, an extremely toxic superoxide (O_2–). This test is used to identify microorganism that produces the enzyme, catalase.

2.3.6 MacConkey test

To differentiate lactose fermenting from lactose non-fermenting gram-negative bacteria, MacConkey agar test was conducted [21]. Bacteria was inoculated on MacConkey agar media using streak plate technique and incubates the plate in incubator at 37°C for 24 hours. MacConkey agar plates were prepared using the ready-made kit of the Difco (1998) [18] instructions. MacConkey agar contains bile salts which selectively facilitate the growth of gram negative bacteria. Strongly lactose fermenting bacteria were grown on MacConkey agar but did not ferment lactose on the medium and agar surrounding the bacteria remained relatively transparent.

2.3.7 Urease test

A loopful bacterium was inoculated on broth medium from a pure culture; the surface of the agar slant was streaked with the test organism. The test tube cap was kept loosely and incubated bacteria within the test tube at 35°C in ambient air for 18 to 24 hours; unless specified for longer incubation.

2.4 DNA Extraction

Genomic DNA from bacterial cultures was extracted by heat lysis and selective precipitation of cell debris and polysaccharides with CTAB (cetyltrimethylammonium bromide) and the procedure was maintained as similar as described by DNA extraction method. The DNA was suspended in TE buffer and quantified using a spectrophotometer then electrophoresed on 1% agarose gel by comparison with DNA samples of known concentration.

2.5 PCR Analysis

The 16S rRNA gene was amplified by PCR for both of the isolates using the primers: 27F forward primer (5'-TGGTAGTCCACGCCCTAAAC-3') and 1391R reverse primer (5'-GACGGGCGGTGTGTRCA-3'), PCR was performed in a volume of 25mL, containing nuclease free ddH_2O 15µL, dNTP mix 1.0µL, forward primer 1.0µL, reverse primer 1.0µL, DNA template 1.5µL, $MgCl_2$ 2.5µL, Taq buffer B 2.5µL and Taq polymerase 0.5µL. The procedure was followed as: initial denaturation at 95°C for 5 min; 35 cycles of denaturation for 40s at 95°C, annealing for 1 min at 65°C, and extension for 2 min at 72°C; the final extension was at 72°C for 10 min, followed by cooling to 4ºC until the sample was recovered. Gel electrophoresis was used to visualize and purify PCR products. For the preparation of 1.2% agarose gel 0.5x TBE buffer was used at 70V for 50 min and visualized on a UV transilluminator.

2.6 Amplification of Specific '*hrp*' Gene

For the identification of pathogenic xanthomonads, the *hrp* gene (hypersensitive reaction and pathogenicity) specific amplification of DNA was used as a defined molecular detection of pathogenicity [22]. The hypersensitive (HR) response in plants in resistant and causation of disease in susceptible plants were detected by *hrp* gene. In this study, *hrpB* gene cluster was amplified by *X. campestris* pv. *musacearum* specific PCR primers BXW-1/RST65 : 5′GTGTTGGCACCATGCTCA3′ and BXW-3/ RST69 : 5′TCCGACCGATACGGCT3′ [23].

2.7 Antibiotic Sensitivity Test

Susceptibility of isolates to different antibacterial agents was determined *in-vitro* by employing a modified disc diffusion method. The isolated bacterial strains were incubated overnight in nutrient broths that were placed in the shaker at 37°C temperature and 150 rpm for the antibiotic sensitivity test. A serial dilution technique was made for the respective test. At first we prepared LB agar medium, then for making culture plates, the sterile liquid medium was distributed in sterile conical flasks when the temperature cooled down at 40-50°C. Approximately 15-20 mL of the medium was poured in each petri dish and left on the airflow cabinet for solidification. Using a loop the colony was streaked on LB agar culture. Commercially available and frequently prescribed

standard antibiotics namely, penicillin, ciprofloxacin, erythromycin, gentamycin, and chloramphenicol were used to test antibiotic sensitivity of the two isolated bacteria. Antibiotic discs were placed centrally on the respective plates and incubate overnight at 37°C. After overnight incubation the diameter of zone of inhibition was observed and measured with the help of mm scale.

2.8 Statistical Analysis

All the above assays of the present investigation were conducted in triplicate and repeated threes for consistency of results and statistical purpose. The data were expressed as mean±SE and analyzed by one way analysis of variance (ANOVA) followed by Dunnett 't' test using SPSS software of 10 version. $P<0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Results

After liquid culture of the bacteria from the disease affected plants, two bacterial strains were found in pure culture; those were indicated as isolate W_1 and isolate W_2. Maintaining the suitable staining method, the following result was observed for both isolated bacterial strains.

3.1.1 Characterization of isolated bacteria

Both of the isolates W_1 and W_2 showed pink color in gram staining test, which indicated gram negative, rod shape bacteria. The two samples (Isolate W_1, Isolate W_2) were grown on MacConkey agar plates for the identification of gram-negative enteric bacteria. Both of the isolated strains can grow on MacConkey agar plates. The lactose fermenting capability of these strains was also detected from the MacConkey agar test. The isolate W_1 didn't grow on the media, it was positive but lactose non-fermenting. On the other hand, isolate W_2 grew on the media; it was positive and lactose fermenting as it changed the color.

Motility in bacteria has long been recognized as an important taxonomic tool and biological characteristic of microorganisms. In the Motility test, both strains (isolate W_1 and isolate W_2) grew only along inoculation line and indicated that they were motile. SIM test was done to determine if bacteria can breakdown the amino acid tryptophan into indole. In SIM media or TSB (tryptic soy broth), bacteria was inoculated using a transfer needle. After incubating both of the isolates for at least 48 hours, Kovac's reagent was added to the SIM media to detect if indole had been produced by the bacteria. After 28 hours of incubation isolate W_1 and W_2 did not produce red/pink layer on top of the media. So, both isolates were negative (the bacteria could not breakdown tryptophan to indole).

Citrate test was performed based on the citrate metabolism ability of the strains. The uninoculated medium had the deep forest green color. The test tube containing citrate media, inoculated with isolate W_1 was citrate negative as it showed no color change (green) and isolate W_2 was citrate positive as it showed the Prussian blue color.

Catalase activity is very useful in differentiating between groups of bacteria. For example, the morphologically similar *Enterococcus* (catalase negative) and *Staphylococcus* (catalase positive) can be differentiated using the catalase test. All gram negative bacteria produce gas bubbles when these were mixed with a drop on peroxide glass slide. In the present study, both of the isolates were positive for catalase test.

The two samples (isolate W_1 & W_2) were grown in TSI agar media in test tubes. The isolate W_1 produced yellow color both of the slant and butt i.e. lactose (or sucrose) was fermented, a large amount of acid was produced, which turned the phenol red indicator yellow in both butt and slant, so it was positive. For isolate $W_{2,}$ both slant and butt were remained red in color i.e. neither lactose/sucrose nor glucose was fermented, so it was TSI negative.

The two samples isolate W_1 and W_2 were grown into the urease media and the results were observed after 48 h of incubation at 37℃. The sample W_1 was turned into yellow color as no urease enzyme was formed, so sample W_1 was urease negative. Isolate W_2 was turned into magenta color as the urease enzyme was formed, so the sample W_2 was urease positive (Table 1, Image were not given).

3.1.2 PCR amplification

The genomic DNA of both isolates was amplified by PCR. Under UV light there was a clear DNA band of isolate W_1 in gel electrophoresis. On the other hand, isolate W_2 did not show any band. A sensitive and specific assay was developed to

detect bacterial wilt caused by the isolated bacteria "W_1" in leaves of banana. One pair of the universal 16s ribosomal RNA gene mapping primers for bacteria (27F and 1391R) was used to amplify and 1650 bp DNA fragment was visible through the gel electrophoresis (Fig. 2a). The *hrp* gene amplification of the isolate W_1 showed around 412bp product with approximately additional 250bp amplicon using the specific primers BXW-1 and BXW-3. The base pair was measured using 100bp ladder (Fig. 2b).

3.1.3 Antibiotic sensitivity test

In this study, five different types of standard antibiotic discs were used. The highest antibiotic activity with 38.0mm and 28.0mm diameter of zone inhibition were showed by penicillin and erythromycin against the isolate W_1 bacteria. On the other hand, penicillin did not showed any inhibition zone against the isolated W_2 bacteria. Gentamycin, ciprofloxacin and chloramphenicol showed moderate antibiotic sensitivity against both of the isolates W_1 and W_2 (Table 2, Fig. 3).

Table 1. Morphological and biochemical characteristics of the isolated bacteria

Test name	Results	
	Isolate W_1	**Isolate W_2**
Gram test	Gram negative	Gram negative
Motility test	+ (ve)	+ (ve)
SIM-medium test	Indole – (ve)	Indole –(ve)
TSI test	+(ve)	-(ve)
Citrate test	+(ve)	+(ve)
Catalase test	+(ve)	+(ve)
MacConky Test	+(ve)	+(ve)
Urease test	-(ve)	+(ve)

Note: +=positive (presence), - =negative (absence)

Fig. 1.Showing the plant sample and causal organism isolation (A) Wilt disease infected banana leaf (B) Isolated W_1 (yellow) and (C) Isolated W_2 (cream) bacteria

Table 2. Effect of some standard antibiotics against the isolated bacteria

Name of antibiotic	Disc potency	Diameter of zone of inhibition (in mm)		Response	
		Isolate W_1	**Isolate W_2**	**Isolate W_1**	**Isolate W_2**
Chloramphenicol	30mcg	25	11	S	I
Ciprofloxacin	15µg	24	22	S	S
Erythromycin	15µg	28	15	S	I
Gentamycin	10mcg	21	23	S	S
Penicillin	10units	38	0	S	R

Note: R=Resistant=<10 mm; I=Intermediate =10-15 mm; S=Susceptible=>15 mm

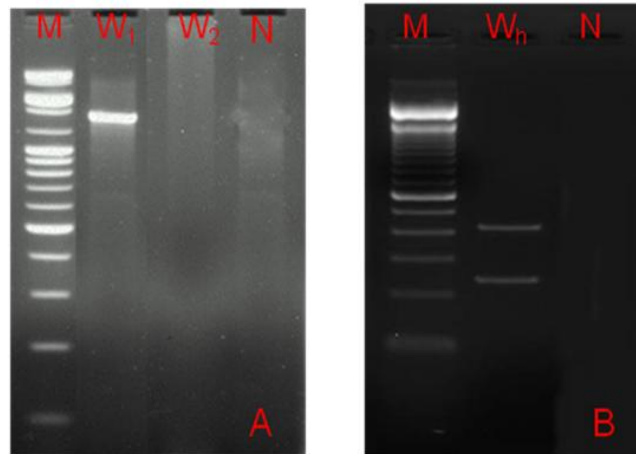

Fig. 2. PCR analysis and *hrp* related gene amplification (A) PCR of isolated bacteria (B) *hrp* gene of *Xanthomonas*, M=Marker, W_1=Isolate W_1, W_2=Isolate W_2, W_h= *hrp*, N= negative control

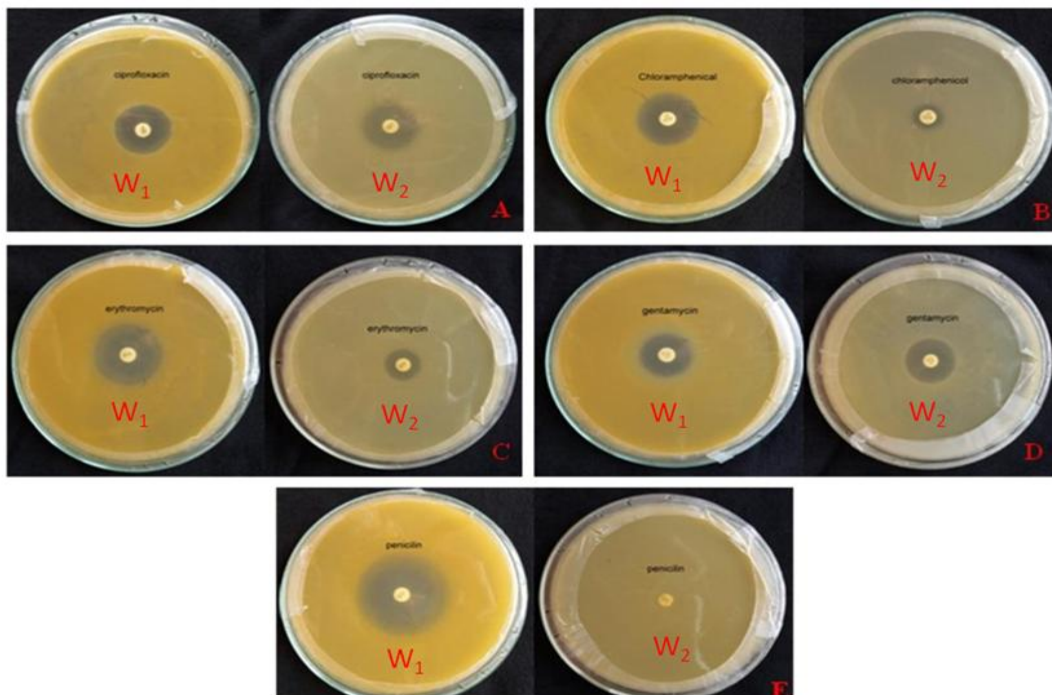

Fig. 3. Effect of some standard antibiotics against the isolated W_1 and W_2 bacteria (A) ciprofloxacin (B) chloramphenicol (C) erythromycin (D) gentamycin (E) penicillin

3.2 Discussion

From the mixed culture two bacterial single colonies (isolate W_1 and isolate W_2) were isolated on LB agar solidified medium from banana wilt disease infected plant. Morphological test indicates that both of the isolates W_1 and W_2 bacteria were rod shaped, gram negative and motile. Isolate W_1 was yellow in color that was similar to *Xanthomonas campestris* described earlier [4], and isolate W_2 was cream color which was similar to *Ralstonia solanacearum.*

Biochemical test showed that isolate W_1 was TSI, MacConkey and catalase test positive while SIM-medium, citrate and urease test negative.

The causal agent of BXW is a motile, gram-negative, rod-shaped bacterium possessing a single polar flagellum, for which the name *Xanthomonas campestris* was adopted in the 1960s and 1970s [24]. *Xanthomonas campestris* pv. *musacearum* is motile, possess a single polar flagellum, gram-negative, oxidase negative, obligate aerobic, rod shaped. It produces typical yellow, convex, mucoid colonies on nutrient agar and other media, forms xanthomonadin pigment and reduces nitrate [25,26,24]. The biochemical characteristics of SIM, indole and urease test was negative as reported by Akhtar (1989) [27] for *X. campestris* which supports our findings. The isolate W_2 was TSI and SIM-medium test negative and citrate, catalase, MacConkey, and urease test positive. *R. solanacearum* was also gram negative, catalase and urease test positive as described by Dhital et al., (2000/2001) [28]. Motility in bacteria can be provided by a variety of mechanisms, but this is because of the most common involvedness of flagella [29,30]. The presence of flagella occurs primarily in bacilli but there are few flagellated cocci, thus motility is a very important means of identification in the family *Enterobacteriaceae* [31]. In the present work, both of the bacteria were motile and also SIM indole test negative. The Triple Sugar Iron (TSI) test is a microbiological test roughly named for its ability to test microorganism's ability to ferment sugars and to produce hydrogen sulfide. It is often used in the selective identification of enteric bacteria including *Salmonella* and *Shigella.* In the study, isolate W_1 was TSI positive but isolate W_2 was negative. Development of purple color on the isolates was observed within 10 seconds, 60 seconds and more than 60 seconds that was similar result, described by [32] and the result was recorded as negative for oxidase reaction. The citrate test is often part of a battery of tests used to identify gram-negative pathogens and environmental isolates [33]. In catalase test, production of gas bubbles gives a clue for presence of aerobic and facultative anaerobic bacteria [13,20]. In the MacKonkey agar test, the inclusion of crystal violet and bile salts in the media prevent the growth of gram-positive bacteria and fastidious gram-negative bacteria. The tolerance of gram-negative enteric bacteria to bile is partly a result of the relatively bile-resistant outer membrane, which hides the bile-sensitive cytoplasmic membrane [34]. In the study, isolate W_1 was gram negative and lactose fermenting and B was gram negative but lactose non-fermenting. Urease test can be used as part of the identification of several genera and species of Enterobacteriaceae including *Proteus, Klebsiella*, and *Yersinia* species. It is also useful to identify *Cryptococcus* species, *Brucella*, *Helicobacter pylori*. In this study, isolate W_1 was urease negative that was similar with [27] for *Xanthomonas Campestris* but isolate W_2 was positive and it was similar to [28] for *Ralstonia solanacearum* which can produce urease enzyme.

In antibiotic sensitivity test, isolate W_1 was found to be susceptible to chloramphenicol, gentamycin, erythromycin, penicillin and ciprofloxacin. On the other hand, isolate W_2 was found to be susceptible to gentamycin and ciprofloxacin but resistant to penicillin and intermediate to the chloramphenicol and erythromycin. This test possesses to detect the effective bactericide against two bacteria and their comparative approach.

Strategic management and control of the BXW is likely to benefit from specific and reliable diagnostic laboratory tools to detect the *X. campestis* pv. *musacearum* (Xcm) pathogen. Recently, a PCR protocol detecting Xcm as well as closely related *Xanthomonas* pathovars, *X. axonopodis* pv. *vasculorum* (Xav) and *X. vasicola* pv. *holcicola* (Xvh) was reported by [23]. Providing a simple PCR method with complete specificity was regarded to Xcm detection. In the molecular analysis of the isolated bacteria W_1 using PCR amplification showed a 1650bp DNA of the bacteria by using 27-F and 1391-R primers. The sequencing of the DNA band will be helpful for the identification of the bacterial strain.

The *hrp* gene amplification of the isolate W_1 was observed with 412bp product and a smaller 250bp band amplicon. The presence of this additional amplicon was visibly unique and detected the *hrpB* gene region as RST primers were designed for the gene. Here, BXW-3 (/RST69) reverse primer was for the analysis of 412bp sequence analysis which had two binding sites of *X. campestris* pv. *musacearum.* The primer was internally designed for additional smaller amplicon and that specified by BXW-1 (/RST65). The finding was closely similar to the finding of [23] in banana plant. That was about the proof of the pathogenicity and hypersensitivity reactions of *X. campestris* pv. *musacearum* (Xcm) pathogen.

4. CONCLUSION

The economic importance of pathogenic bacteria is increasing day by day. In the present study,

different experiments were carried out to observe the morphological, physiological, biochemical and molecular characteristics of both isolate W_1 and isolate W_2 bacteria. Morphological test indicated that isolate W_1 and W_2 both of them were found as rod-shaped and gram negative, yellow and creamy in color and where both isolates were indole negative and motile. Biochemical test showed that the isolated W_1 was TSI, MacConkey and Catalase positive and Citrate and Urease negative. The isolated W_2 was TSI negative and Citrate, Catalase, MacConkey, Urease positive. The antibiotic sensitivity of the both isolate W_1 and W_2 were screened using some standard antibiotics disc. So, from the present findings it can be concluded that the investigation will help for the detection of isolated bacteria and biological control managements of this devastating bacterial disease.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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