Biochemical Characteristics and Pathogenicity of *Xanthomonas campestris* pv. *musacearum* Isolates Associated with Enset (*Ensete ventricosum*) Bacterial Wilt from Southwestern Ethiopia

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Abstract

Bacterial wilt [*Xanthomonas campestris* pv. *musacearum* (Xcm)] is a very destructive disease that attacks *enset* (*Ensete ventricosum*). The objective of this study was to characterize Xcm isolates morphologically, biochemically, physiologically and pathogenically. The objective of this study was to determine the morphological, biochemical, physiological and pathogenic characteristics of Xcm isolates collected from Southwestern Ethiopia. One hundred and twenty Xcm isolates were collected from 120 infected *enset* fields in 10 major *enset* growing districts, namely Andiracha, Bita, Chena, Decha, Gimbo, Maji, Masha, Semen-Bench, She-Bench and Yeki, in southwestern Ethiopia during 12 June to 08 August 2017, and were isolated on yeast peptone sucrose agar (YPSA) medium in the laboratory. From each sample, a loopful of the suspension was streaked on yeast peptone sucrose agar (YPSA) medium. The plates were arranged in complete randomized design and incubated at 28 °C for 48–72 hours. The isolates were characterized on the bases of their colony growth, morphological, physiological and pathogenic features, and their responses to various biochemical tests. Morphologically, variability among isolates was observed in appearances in colony color that varied from light to deep yellow, and growth as its' growth type that ranged from low to highly mucoid colony. Moreover, variability in tolerance abilities within isolates was observed under different NaCl concentrations (0–5%) and temperature variation (26, 28, 30 and 32 °C). On the contrary, all the 14 biochemical tests showed similarities amongst the isolates. Thus, potassium hydroxide solubility test revealed that all the isolates were Gram-negative. All the isolates were catalase and oxidase positive, could hydrolyze casein and gelatin, produce H_2S, and utilized citrate and malate while the isolates could not reduce nitrate to nitrite, could not hydrolyze Tween 80 (about 92.5% of the isolates) and starch, and could not produce indole. Morphological and biochemical tests in combination with the pathogenicity test confirmed the isolates were Xcm, and suggested the presence of population diversity among Xcm isolates tested. However, further studies that target the genetic diversity of the pathogen using large number of isolates are essential to draw a meaningful inference.

Keywords: Characterization, Colony, Inoculation, Morphological and physiological tests, Variability, Xcm isolates.

Introduction

Enset (Ensete ventricosum) is one of the major crops that can significantly help to ensure food security in Ethiopia (Brandt *et al.*, 1997). The corm and the pseudostem are the most important sources of food. The types of food from these parts are locally known as '*Kocho*', '*Bulla*' and '*Amicho*' (Spring *et al.*, 1996). Currently, Bacterial wilt [*Xanthomonas campestris* pv. *musacearum* (Xcm)] of *enset* is a severe constraint to the production of *enset* plant in the country. Enset bacterial wilt (EBW) has developed a broad host range, by infecting cultivated and wild *enset*, banana, *Canna indica, Canna orchoides,* maize, sorghum and finger millet (Alemayehu *et al.*, 2016). It invades its host through wounds, colonizes the xylem vessels, and systemically spreads rapidly throughout the plant. The pathogenesis of Xcm is mainly due to the gradual mechanical blocking of plant vesicular vessels by the bacterium itself and effects of toxins or pectolytic enzymes produced by the bacterium too (Manners, 1993).

Infected *enset* plants show wilting of the heart-leaf, followed by progressive yellowing and necrosis of the neighbouring overlapping leaves and pseudostem, and eventual death of the whole plant. During opening of wilted petioles, yellow or cream colored bacterial oozes are clearly observed (Gizachew, 2000). The losses of *enset* production due to this pathogen were previously reported to reach up to 70% (Dereje, 1985). Recently, many researchers reported even losses of up to 100% in some *enset* fields in southwestern Ethiopia, leading to a continuous declining of the production area and productivity of the crop (Desalegn and Addis, 2015; Tariku *et al.*, 2015; Mekuria *et al.*, 2016).

The International Committee on Systematic Bacteriology suggested several species of the genus *Xanthomonas* to be reduced to the pathovar level under the type species *Xanthomonas campestris* and the pathovars were more distinguishable with only host range (Dye *et al.*, 1980) rather than by the usual biochemical tests used in Bergey's Manual of Systematic Bacteriology (Schaad and Stall, 1988). Accordingly, the genus *Xanthomonas* is classified under the kingdom: Bacteria, phylum: Proteobacteria, class: Gamma proteobacteria, order: Xanthomonadales, family: Xanthomonadaceae, genus: *Xanthomonas*, species: *Xanthomonas campestris* and pathovar: *musacearum*; and the yellow-pigmented colony plant pathogens of this family have been unified in this genus (Bradbury, 1984). Recent work based primarily on DNA sequence and fatty acid analyses data has shown that strains of Xcm have very close homology to strains of *Xanthomonas vasicola* and most likely belong to this species. Accordingly, the name *X. vasicola* has been proposed for Xcm (Aritua *et al.*, 2008).

Cells of Xcm are straight rods usually within the range 0.4–0.7 µm wide X 0.7–1.8 µm long. They are Gram-negative, aerobic and motile by a single polar flagellum. Gram-negative nature of the bacterium and its motility was quite evident for all Xcm isolates. The optimum temperature for the bacterium growth is usually within 28–30 °C. Colonies are yellow, smooth textured and butyrous or viscid (Kidist, 2003). Phenotypic and genetic variations were observed in different *X. campestris* pathovars. For example, biochemical and pathogenic variation were observed in strains of Xcm collected from southern regions of Ethiopia (Kidist, 2003). Gizachew (2000) also indicated that yellowish colony with mucoid growth was exhibited by all isolates of Xcm. Pathogenicity tests with Xcm isolates obtained from different locations on

different *enset* clones showed no significant variation and no clone by isolate interaction; but mean incidences ranged from 95.7-100% (Quimio and Mesfin, 1996).

Some studies indicated variations in the occurrence and incidence of EBW in major *enset* growing areas in Ethiopia (Desalegn and Addis, 2015; Tariku *et al.*, 2015; Mekuria, 2016). This could be caused by variability within Xcm populations in addition to variation in environmental conditions and the host plants. Therefore, detection of this variation may contribute towards the development of technologies to manage EBW mainly thorough selection and deployment of resistant clones (Gizachew, 2000). In a study conducted at Hawassa, for example, some *enset* clones that are tolerant to some isolates of the causal agent became susceptible to other strains of the bacterium, while some *enset* clones showed consistently better reaction to *enset* wilt pathogen in different experiments and at varying times than other clones (Fikre and Gizachew, 2007). Nevertheless, diversity in strains within the bacterial pathogen populations is not well studied in southwestern Ethiopia. Therefore, the objective of this research was to determine the morphological, biochemical, physiological and pathogenic characteristics of Xcm isolates associated with *enset* from Southwestern Ethiopia.

Materials and Methods

Sample specimen collection

One hundred and twenty infected *enset* leaf samples were collected during June 12 to August 8, 2017 from 30 Farmers' Associations (FAs) of 10 districts in three zones (Kefa, Bench-Maji and Sheka) in southwestern Ethiopia (Table 1). Only leaf petioles, which showed early stage of the disease symptom, were collected to avoid some saprophytic microorganisms that grow in tissues killed by the primary pathogen. Bacteria cells oozing out of the vascular tissues of each of the 120 samples were picked using sterile toothpick and then suspended in steril distilled water (SDW) in half filled screw capped vials according to Quimio (1994). Each sample was labeled with location (zone, district and FAs), and altitude of the sample collection site was also recorded in meters above sea level (m.a.s.l.) using geographic positioning system (GPS). Isolates were coded Xcm-1 to Xcm-120.

Isolation, identification and preservation of the pathogen

From each of the 120 bacterial samples, a loopful of the suspension was streaked on yeast peptone sucrose agar (YPSA) medium (yeast extract, 5 g; peptone, 10 g; sucrose, 20g; and agar, 12-15g in 1L distilled water with pH 7.4 and autoclaved at 121 °C for 15 min) in 9cm petri-plates. The plates were incubated at 28 °C for 48-72 hours according to Schaad and Stall (1988). Bacterial colonies from each plate were further sub-cultured and transferred to YPSA slants incubated at 28 °C for 48-72 hours, and preserved at 4 °C for further work in Plant Pathology Laboratory of Haramaya University, Ethiopia.

Table 1. Geographic origin of *Xanthomonas campestris* pv. *musacearum* isolates from 30 FAs and ten districts of three zones (Kefa, Bench-Maji and Sheka) in southwestern Ethiopia during the 2017 cropping year.

Isolate code [a]	Zone	Districts	Farmer association	Altitude (m.a.s.l.)	Isolate code [a]	Zone	District	Farmer association	Altitude (m.a.s.l.)
Xcm-1	Kefa	Decha	Awurada	1954	Xcm-32	Kefa	Chena	Boba-Bela	2003
Xcm-2				1956	Xcm-33			Boba-Wodity	2209
Xcm-3				1965	Xcm-34				2207
Xcm-4				2023	Xcm-35				2157
Xcm-5			Aerimo	1957	Xcm-36				2140
Xcm-6				1948	Xcm-37		Bita	Yeda	1894
Xcm-7				1938	Xcm-38				1891
Xcm-8				1982	Xcm-39				1922
Xcm-9			Shapa	1946	Xcm-40				1913
Xcm-10				1943	Xcm-41			Sheda	1893
Xcm-11				1935	Xcm-42				1902
Xcm-12				1987	Xcm-43				1913
Xcm-13		Gimbo	Dakity	2010	Xcm-44				1884
Xcm-14				2047	Xcm-45			Gawatiy	1868
Xcm-15				2030	Xcm-46				1875
Xcm-16				2077	Xcm-47				1874
Xcm-17			Key-Kelo	1637	Xcm-48				1881
Xcm-18				1659	Xcm-49	Bench-Maji	Maji	Gebariku	2360
Xcm-19				1652	Xcm-50				2370
Xcm-20				1644	Xcm-51				2374
Xcm-21			Keja-Araba	1692	Xcm-52				2360
Xcm-22				1664	Xcm-53			Chayit	2360
Xcm-23				1685	Xcm-54				2353
Xcm-24				1689	Xcm-55				2346

Table 1. Continued.

Xcm-25		Chena	Boba-Kocha	2151	Xcm-56			2344
Xcm-26				2147	Xcm-57		Maji-01	2368
Xcm-27				2139	Xcm-58			2366
Xcm-28				2128	Xcm-59			2392
Xcm-29			Boba-Bela	1969	Xcm-60			2393
Xcm-30				1980	Xcm-61	She-Bench	Tikimiteshet	2121
Xcm-31				1983	Xcm-62			2054
Xcm-63	Bench-Maji	She-Bench	Tikimiteshet	2091	Xcm-92	Sheka	Masha	Abelo 2207
Xcm-64				2080	Xcm-93			Keja 2121
Xcm-65			Kuka	2071	Xcm-94			2128
Xcm-66				2083	Xcm-95			2134
Xcm-67				2097	Xcm-96			2142
Xcm-68				2087	Xcm-97		Andiracha	Chicha 2134
Xcm-69			Ziyagin	2029	Xcm-98			2165
Xcm-70				2031	Xcm-99			2146
Xcm-71				2027	Xcm-100			2160
Xcm-72				2023	Xcm-101			Tugiri 2092
Xcm-73		Semen-Bench	Kasha	2167	Xcm-102			1958
Xcm-74				2173	Xcm-103			1949
Xcm-75				2184	Xcm-104			1932
Xcm-76				2178	Xcm-105			Gebina 2353
Xcm-77			Muya-Kela	2161	Xcm-106			2423
Xcm-78				2152	Xcm-107			2363
Xcm-79				2198	Xcm-108			2411
Xcm-80				2239	Xcm-109		Yeki	Erimichi 1886
Xcm-81			Wacha-Maji	2212	Xcm-110			1881
Xcm-82				2166	Xcm-111			1741
Xcm-83				2118	Xcm-112			1680
Xcm-84				2125	Xcm-113			Kubito 1493

Table 1. Continued.

Xcm-85	Sheka	Masha	Gatimo	2309	Xcm-114		1522
Xcm-86				2299	Xcm-115		1553
Xcm-87				2286	Xcm-116		1577
Xcm-88				2293	Xcm-117	Achani	1470
Xcm-89			Abelo	2212	Xcm-118		1581
Xcm-90				2211	Xcm-119		1618
Xcm-91				2227	Xcm-120		1482

[a] *Xcm* = *Xanthomonas campestris* pv. *musacearum* isolates designated from 1–120.

Characterization of *Xanthomonas campestris* pv. *musacearum*

Colony morphology on YPSA

Colony morphology (shape, color and growth pattern) was recorded on YPSA agar plate at 28 °C after overnight growth.

Solubility on KOH solution

The KOH solubility test was performed using 48 hour old cultures following the method of Fahy & Hayward (1983). A drop of 3% KOH (w\v) was placed on a microscope slide and part of a single colony from YPSA was removed using a sterile loop and mixed with a drop of KOH solution on the slide until an even suspension was obtained. When mucoid thread was lifted with the loop from the slide, it was designated as a Gram-negative bacterium; but when a completely dissolved suspension was produced, it was designated as a Gram-positive bacterium.

Colony growth on asparagine medium

All Gram-negative isolates were allowed to grow on Asparagine medium [Asparagine, 0.5g; KH_2PO_4, 0.1g; $MgSO_4.7H_2O$, 0.2g; KNO_3, 0.5g; $CaCl_2$, 0.1g; NaCl, 0.1g and agar, 12-15g (for plates) in 1L distilled water with pH 7 and autoclaved at 121 °C for 15 minutes] at 28 °C for 48-72 hours without any other carbon and nitrogen sources (Dye *et al.*, 1980). This is used as a diagnostic test for *Xanthomonas* because they were not able to grow on asparagine medium, while others like yellow *Enterobacteriaceae* and many *Pseudomonads* can grow on it. The growth of the bacteria on Asparagine agar plates was recorded and those isolates that are unable to grow on the medium were used for further tests. In all cases, un-inoculated medium was included as negative control.

Colony growth on nutrient agar with 5% glucose

All isolates, that were not able to grow on asparagine medium, were streaked on nutrient agar with 5% glucose (nutrient agar, 23g; 5% glucose in 1L distilled water with pH 7 and autoclaved at 121 °C for 15 minutes) and were incubated at 28 °C for 48–72 hours. Mucoid and yellow colony growth on this medium is one of the characteristics that differentiate *Xanthomonas campestris* from other *Xanthomonas* species (Bradbury, 1984). Therefore, the growth pattern and colony color of each isolate was recorded on this medium after overnight growth.

Catalase test on pure colonies

The presence of catalase activity in the isolates, the ability to detoxify the toxin compound hydrogen peroxide (H_2O_2) by changing it into non-toxic compounds H_2O and O_2, was checked by flooding pure colonies of 48 hours old culture on the slide with a drop of 3% hydrogen peroxide (H_2O_2) solution. Immediate effervescence of gas bubble was recorded as a positive result (Sands, 1990).

Nitrate reduction ability by Xcm isolates

Based on the procedures employed by Dickey and Kelman (1988), the ability of the isolates to reduce nitrate to nitrite was evaluated in a test tube medium that contains NO_3, 1 g; peptone, 5 g; yeast extract, 3 g and agar, 3 g in 1L distilled water, sterilized at 120 °C for 15 minutes. Each isolate was inoculated by stabbing and sealing with 3 ml sterilized molten agar to avoid false positives and incubated at 28 °C. The growth of each bacterial isolate and bubble formation beneath the upper agar layer was observed and recorded as positive

result for nitrate reduction within seven days after inoculation and incubation.

Bacterial activity vs. Tween 80 hydrolysis

Fatty acid esterase activity, to detect the ability of bacteria to hydrolyze the lipid, was tested by streaking the bacterial cell mass of each isolate onto a fresh nutrient agar medium containing calcium chloride and Tween 80, a polymer consisting of polyoxyethylene sorbitan monooleate (Sands, 1990). The medium was prepared from peptone, 10 g; $CaCl_2$ dihydrochloride, 0.1 g; NaCl, 5 g; agar, 15 g; and distilled water, 1L; with the pH adjusted to 7.4. Tween 80 was autoclaved separately and added with 10 mL L^{-1} and mixed before plating. The Petri-plates were incubated at 28 °C for up to 7 days (Fahy and Hayward, 1983). An opaque zone of crystals around a colony was recorded as positive reaction for hydrolysis of Tween 80.

Activity of isolate in gelatin liquefaction

This test was performed to determine the ability of an isolate to produce extracellular proteolytic enzymes (gelatinases that hydrolyze gelatin) based on methods described by Dickey and Kelman (1988) by employing gelatin medium that contains beef extract, 3 g; peptone, 5 g and gelatin, 120 g in 1 L distilled water, poured into test tubes and autoclaved at 121 °C for 15 minutes and cooled without slanting. The medium was stab-inoculated separately with each isolate grown on YPSA medium for 48 hours and was incubated at 28 °C for seven days. After incubation, each isolate was evaluated for gelatin liquefaction. The isolates in test tubes were kept at 4 °C for 30 minutes and gently tipped over immediately. A medium that flows readily as the tube is gently tipped was considered as positive for gelatin liquefaction.

Isolate activity vs. starch hydrolysis

The isolates were streaked on starch agar medium (starch soluble, 20 g; peptone, 5 g; beef extract, 3 g; agar, 15 g in 1L distilled water with pH 7 and autoclaved at 121 °C for 15 minutes) to evaluate their ability to hydrolyze starch (amylase production). The plates were incubated at 28 °C for 2–3 days, and starch hydrolyses were observed by flooding the plates with Gram's iodine solution for 30 seconds. The appearance of clear zone around the line of growth of each isolate considered as indicates positive starch hydrolysis (Aneja, 1996).

Isolate activity vs. protein casein hydrolysis

The ability of the isolates to degrade the protein casein by producing proteolytic exo-enzymes was tested by growing the isolates on milk agar plates (skim milk powder, 100 g; peptone, 5 g; agar, 15 g in 1L distilled water with pH 7.2 autoclaved at 121 °C for 15 minutes). Clear zone around the growth of the isolates was recorded as positive for casein hydrolysis (Aneja, 1996).

Isolate activity vs. hydrogen sulfide (H_2S) production

H_2S production was detected according to Aneja (1996) by using sulphide indole motility (SIM) agar medium (peptone, 30 g; beef extract, 3 g; ferrous ammonium sulfate, 0.2 g; sodium thiosulphate, 0.025 g and agar, 3 g in 1L distilled water autoclaved at 121 °C for 15 minutes). The isolates were inoculated by stabbing and by incubating at 28 °C for 48–72 hours. The presence of black coloration along the line of stab inoculation was recorded as positive for H_2S production (Aneja, 1996).

Isolate activity on indole production

Indole production due to isolate activity was tested with a medium composed of tryptone, 10 g; $CaCl_2$, 0.03 M; NaCl, 5 g in 1 L distilled water that was autoclaved at 121 °C for 15 minutes. Each isolate was inoculated into the tryptone broth and incubated at 28 °C for two to five days. After incubation, 0.5 mL of Kovak's Reagent (P-dimethyl amino benzaldehyde 5 g, butanol 75 mL, and concentrated HCl 25 mL) was added into each tube and shaken gently. Dark red coloration on the surface layer was taken as positive for indole production (Aneja, 1996).

Isolate activity vs. Kovac's oxidase reaction

A sterile toothpick tip of each bacterial isolate was rubbed onto a filter paper with drops of 1% aqueous N, N, N, N-tetra methyl-p-phenylenediamine dihydrochloride solution. Isolates that develop purple color within 60 seconds were taken as positive and with no color for more than 60 seconds were recorded as negative for oxidase reaction (Dickey and Kelman, 1988).

Isolate activity vs. citrate utilization

This test was performed following the methods employed by Aneja (1996) by using Simon's citrate agar slants [$NH_4H_2PO_4$, 1 g; K_2HPO_4, 1 g; NaCl, 5 g; $MgSO_4.7H_2O$, 0.2 g; sodium citrate, 2 g; agar, 15 g; bromothymol blue, 0.08 g in 1 L distilled water (pH 6.9)] and autoclaved at 121 °C for 15 minutes). A loopful from each isolate was streaked on the slant and incubated at 28 °C for 48–72 hours. A change of color from green to blue was taken as positive for citrate utilization and uninoculated tubes were used as negative control.

Isolate activity towards malate utilization

Malate utilization of the isolates was tested using a broth medium containing malic acid, 2 g; $NH_4H_2PO_4$, 1 g; K_2HPO_4, 1 g; $MgSO_4.7H_2O$, 0.2 g; yeast extract, 1 g; bromothymol blue, 12.5 ml of 0.2% solution in 1L distilled water (pH 6.9) and autoclaved at 121 °C for 15 minutes. Forty-eight hours old bacterial isolates were inoculated and incubated at 28 °C for 48–72 hours. Color change from green to blue was taken as positive for malate utilization and un-inoculated tube was used as a negative control (Aneja, 1996).

Isolate variation in salt tolerance

By following the methods of Hayward (1964), bacterial isolates were inoculated into nutrient broth with 0, 1, 2, 3, 4 and 5% NaCl concentration to evaluate their salt tolerance. The nutrient broth without salt (0%) was positive control and the presence or absence of bacterial growth was recorded on the inoculated broth with different salt concentrations.

Temperature sensitivity test

The ability of Xcm isolates to grow at different selected temperature regimes was tested by separately growing isolates on YPSA medium. A loopful of each bacterial suspension was streaked on YPSA medium (yeast extract, 5 g; peptone, 10 g; sucrose, 20 g; agar, 12–15 g in 1 L distilled water) with pH 7.4 and autoclaved at 121 °C for 15 min. The inoculated YPSA Petri-plates were incubated at four temperature (26, 28, 30 and 32 °C) levels. The presence or absence of colony growth was observed and recorded 72 hrs after incubation.

Pathogenicity assay

Growing enset clone

A recommended local susceptible *enset* clone *Yeko* was used for pathogenicity test (Befekadu *et al.*, 2014). One year old suckers of the clone from disease free fields were transplanted in pots (30×30cm) filled with sterilized mixture of topsoil, composted manure and sand at 3:2:1 ratio (Quimio, 1992). Then the suckers were allowed to establish for three months in screen house at 25–30 °C day and 15–18 °C night temperature levels at Tepi National Spice Research Center (TNSRC). Plants were watered uniformly (to maintain the relative humidity within 60 to 80%) every day both in the morning and afternoon up to the end of the assessment periods.

Inoculum preparation and inoculation

Among the 120 isolates, 30 Xcm isolates (Xcm-6, Xcm-7, Xcm-10, Xcm-16, Xcm-20, Xcm-22, Xcm-26, Xcm-34, Xcm-39, Xcm-43, Xcm-46, Xcm-49, Xcm-55, Xcm-59, Xcm-65, Xcm-69, Xcm-73, Xcm-75, Xcm-77, Xcm-81, Xcm-86, Xcm-90, Xcm-93, Xcm-97, Xcm-99, Xcm-103, Xcm-108, Xcm-114, Xcm-117 and Xcm-120) were randomly picked (after isolates were clustered to their respective farmer associations for ease of identification) for pathogenicity assay. The selected isolates were grown on YPSA medium by incubating at 28 °C for 48 hours to prepare sufficient amount of inocula. Following mass culturing, bacterial cells were independently harvested by scratching and suspending the culture in sterilized distilled water in a sterile beaker and adjusted to a concentration of $1x10^8$ cfu ml^{-1} using spectrophotometer (Fikre and Gizachew, 2007). A 3 mL of the bacterial cell suspension was inoculated using a sterile hypodermic syringe and needle to the second innermost leaf petiole of a three-month-old (well-established) *enset* plant. Sterile distilled water inoculated plants were included as negative controls. Data on EBW typical symptom developments were observed and recorded starting from first date of symptom development and continued at an interval of 7 days.

Data analysis

The required parameters were measured and the relevant data were collected under each test. All of the collected data were qualitative. Hence, they were analyzed based on consistency of results from the repeated colony growth, morphological and biochemical tests.

Results

Colony growth characteristics

The isolates showed variation from low to highly mucoid growth with light to deep yellow and creamy colony color, when grown on YPSA medium. The colonies were also dome-shaped; mucoid; circular and shiny that confirms positive reaction to *Xanthomonas campestris* pv. *musacearum* (Figure 1). The growth of the bacterial isolates on nutrient agar with 5% glucose medium showed slight variation in color and growth character of colonies. And, all the bacterial isolates did not grow on asparagines medium (Table 2).

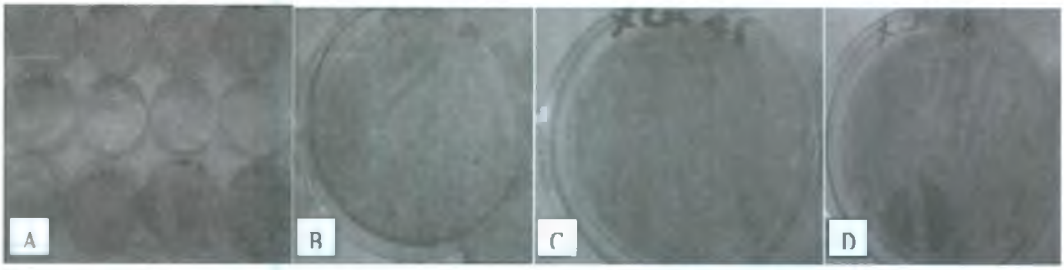

Figure 1. Cultural characteristics of *Xanthomonas campestris* pv. *musacearum* isolates grown on yeast peptone sucrose agar (YSPA) medium after incubation at 28 °C for 48 hrs. Light colony (A and B) and deep yellow colony (C and D) color.

Physiological characteristics

Variation among Xcm isolates was observed in physiological tests. In NaCl tolerance test, all isolates tolerated 1 and 2% NaCl except the isolates Xcm-18, Xcm-65, Xcm-85, Xcm-91, Xcm-94 and Xcm-105, which did not grow on 2% NaCl. In addition to isolates that failed to grow on 2% NaCl, seven isolates, namely Xcm-53, Xcm-55, Xcm-71, Xcm-81, Xcm-83, Xcm-87 and Xcm-92 failed to grow on 3% NaCl. The growths of most isolates were retarded by 4% NaCl concentration and were suppressed by 5% NaCl concentration (Figure 2 and Table 2). Isolates of *Xanthomonas campestris* pv. *musacearum* also varied in their sensitivity to different temperature levels (Table 2). About 94 and 91% of the isolates of Xcm grew better at 28 and 30 °C, respectively, than the rest; but about 36 and 30% of the test isolates failed to grow at 26 and 32 °C, respectively.

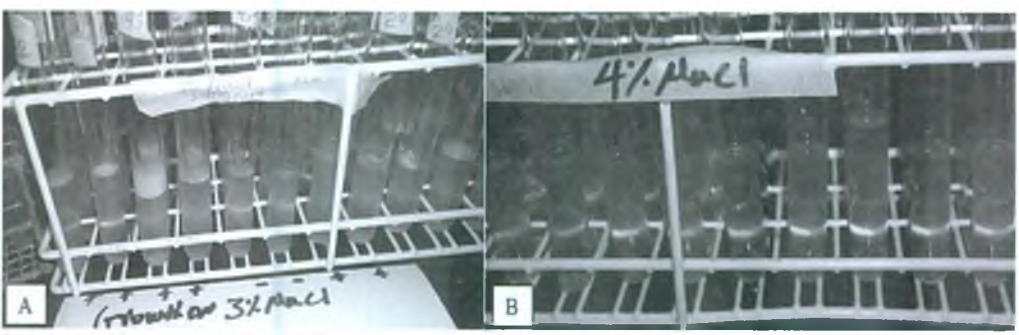

Figure 2. Growing ability of *Xanthomonas campestris* pv. *musacearum* isolates at different levels of NaCl concentration. Growth on 3% NaCl (A) and growth on 4% NaCl (B) concentrations.

Biochemical reactions

The various biochemical characters of the Xcm isolates are presented below (Figure 3 and Table 2). KOH tests on most Xcm isolates indicated that the bacterium was Gram negative as it did not dissolve in 3% KOH solution. The isolates rather showed a thin strand of slime when the mixed bacterial culture in the solution was lifted with the inoculating loop. Most (87.5%) of the isolates were not able to reduce nitrate to nitrite. They were all catalase positive; and also released gas upon addition of hydrogen peroxide. Oxidase reaction test was positive to all isolates; and a purple color appeared during addition of culture to the oxidase reagent, except for Xcm-45.

About 92.5% of the Xcm isolates did not hydrolyze Tween 80. Only 7.5% of isolates hydrolyzed starch, as clear zone was observed surrounding the bacterial growth when the plates were flooded with Gram's iodine solution for 30 seconds. In casein hydrolysis ability, all isolates were found positive. Similarly, almost all isolates (with the exception of Xcm-102) hydrolyzed gelatin, as gelatin medium flowed readily as soon as the isolates in test tubes were gently tipped after they were kept at 4 °C for 30 minutes. More than 96% of isolates produced black coloration along the line of stab-inoculated test tubes, indicating production of H_2S. Only 3 isolates, namely Xcm-16, Xcm-16 and Xcm-16, were found positive to indole production. With regard to citrate and malate utilization of the isolates, about 89% of the isolates utilized citrate and 88% of the isolates utilized malate.

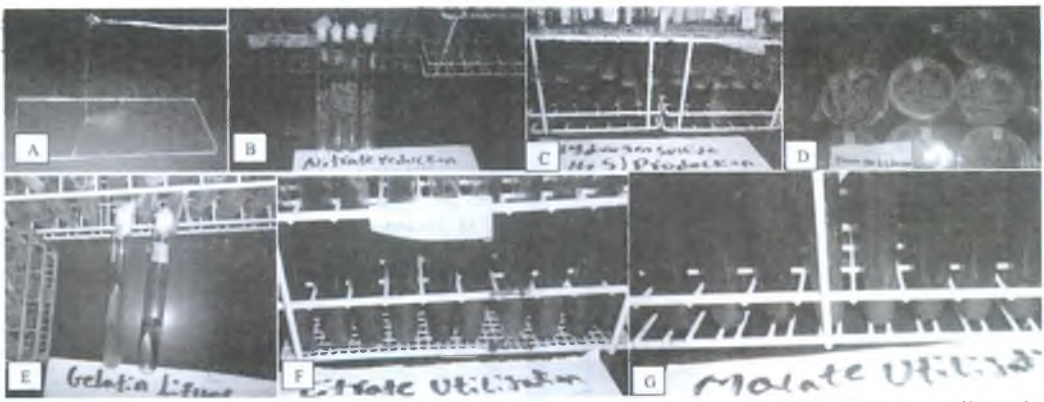

Figure 3. Biochemical reaction of *Xanthomonas campestris* pv. *musacearum* isolates collected from southwestern Ethiopia. KOH solubility test where isolates showing formation of thin strand when lifted with inoculating loop (A). Nitrate reduction reaction (B). Hydrogen sulfide production (C). Tween 80 hydrolysis (D). Gelatin liquefaction (E). Citrate utilization (F). Malate utilization test (G).

Table 2. Reaction of *Xanthomonas campestris* pv. *musacearum* isolates collected from southwest Ethiopia to different biochemical testes, during the 2017 cropping year.

Isolate code [a]	Biochemical test types [b]														NaCl concentration (%)						Temperature (°C)			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	0	1	2	3	4	5	26	28	30	32
Xcm-1	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	–
Xcm-2	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	+	–	–	+	+	+
Xcm-3	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+w	–	–	–	+	+	+
Xcm-4	+	–	+	+	+	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+w	+	+	+
Xcm-5	+	–	+	+	–	+	–	+	+	+	+	–	+	+	+	+	+	+	–	–	–	+	–	–
Xcm-6	+	–	+	+	–	+	–	–	+	+	+	–	–	–	+	+	+	+	–	–	+	+	+	+w
Xcm-7	+	–	+	+	–	+	–	–	+	–	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-8	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-9	+	–	+	+	–	+	–	–	+	+	+	–	+	–	+	+	+	+	–	–	–	+	+	+
Xcm-10	+	–	+	+	+	+	–	–	+	+	+	–	+	+	+	+	+	+	+w	+w	+	+	+	+
Xcm-11	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+	+	–
Xcm-12	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+w
Xcm-13	+	–	+	+	+	+	–	–	+	+	+	–	+	+	+	+	+	+	+	+w	+	+	+	+
Xcm-14	+	–	+	+	–	+	–	–	+	+	+	–	–	+	+	+	+	+w	–	–	–	–	+	–
Xcm-15	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-16	+	–	+	+	–	+	–	–	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+
Xcm-17	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+	+	–
Xcm-18	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	–	–	–	–	–	+	+	+
Xcm-19	+	–	+	+	+	+	–	–	+	–	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-20	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	–	–
Xcm-21	+	–	+	+	–	+	–	–	+	+	+	–	–	+	+	+	+	+	–	–	–	+	–	–
Xcm-22	+	–	+	+	–	+	–	+	+	+	+	–	+	+	+	+	+	+	–	–	+w	+	+	+
Xcm-23	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	–
Xcm-24	+	–	+	+	–	+	–	+	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-25	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+w	+	+
Xcm-26	+	–	+	+	–	+	+	–	+	+	–	–	–	–	+	+	+	+	–	–	+w	+	+	+

Table 2. Continued.

Xcm-27	+	–	+	+	+	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+w	+	+
Xcm-28	+	–	+	+	+	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	–
Xcm-29	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
Xcm-30	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+w	–	–	–	+	+	+w
Xcm-31	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-32	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	–
Xcm-33	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+w	–
Xcm-34	+	–	+	+	+	+	–	–	+	+	+	–	+	+	+	+	+	+	+	+w	–	+	+	+
Xcm-35	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+	+	+
Xcm-36	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-37	+	–	+	+	–	+	–	–	+	+	+	+	+	+	+	+	+	+	–	–	+w	+	+	+
Xcm-38	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	–	+	–
Xcm-39	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-40	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+	+	+w
Xcm-41	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-42	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-43	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+w	–	–	–	+	+	+
Xcm-44	+	–	+	+	–	–	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	–
Xcm-45	+	–	+	+	–	+	–	–	+	+	+	–	+	–	+	+	+	+	+	–	+	+	+w	+
Xcm-46	+	–	+	+	–	+	–	–	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+
Xcm-47	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-48	+	–	+	+	–	+	–	–	+	+	+	–	–	+	+	+	+	+	–	–	+	+	+	–
Xcm-49	+	–	+	+	–	+	–	–	+	+	-	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-50	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+	+	+
Xcm-51	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-52	+	–	+	+	–	+	–	+	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	–
Xcm-53	+	–	+	+	+	+	–	–	+	+	+	–	+	+	+	+	+	–	–	–	–	+	+	+
Xcm-54	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+w	+	+	+w
Xcm-55	+	–	+	+	–	+	–	–	+	+	+	–	+	–	+	+	+	–	–	–	+	+	+	+

Table 2. Continued.

Xcm-56	+	–	+	+	+	+	–	–	+	+	+	–	–	+	+	+	+	+w	–	–	+	+	+	+
Xcm-57	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	–
Xcm-58	+	–	+	+	–	+	+	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+	+	+
Xcm-59	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-60	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+	–	–
Xcm-61	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	+	–	–	+	+	+
Xcm-62	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	–
Xcm-63	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	+	–	–	+	+	+
Xcm-64	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	+	–	+w	+	+	+
Xcm-65	+	–	+	+	–	+	–	–	+	+	+	–	–	–	+	+	–	–	–	–	+	+	+	+
Xcm-66	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+	+	+
Xcm-67	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	–
Xcm-68	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+	–	–
Xcm-69	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	–	+	+
Xcm-70	+	–	+	+	–	+	–	–	+	–	+	–	+	+	+	+	+	+	–	–	–	+w	+	–
Xcm-71	+	–	+	+	–	+	+	–	+	+	+	–	+	+	+	+	+	–	–	–	+	+	+	+
Xcm-72	+	–	+	+	–	+	–	+	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-73	+	–	+	+	–	+	–	–	+	+	+	–	–	–	+	+	+	+	+w	–	+	+	+	+w
Xcm-74	+	–	+	+	+	+	–	–	+	+	+	–	–	–	+	+	+	+	–	–	–	+	–	–
Xcm-75	+	–	+	+	–	+	–	–	+	+	+	–	+	–	+	+	+	+	–	–	–	–	–	+
Xcm-76	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-77	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+w	–	–	–	+	+	+w
Xcm-78	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-79	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	–
Xcm-80	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+
Xcm-81	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	–	–	–	–	+	+	+	+
Xcm-82	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	+	–	–	+	–	–
Xcm-83	+	–	+	+	–	+	–	+	+	+	+	–	+	+	+	+	+	–	–	–	+	+	+	+
Xcm-84	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+

Table 2. Continued.

Xcm-85	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	–	–	–	–	–	+	+	+
Xcm-86	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	–	+	+
Xcm-87	+	–	+	+	–	+	–	–	+	+	+	–	–	+	+	+	+	–	–	–	–	+	+	–
Xcm-88	+	–	+	+	–	+	–	–	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-89	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+w	+	+	+
Xcm-90	+	–	+	+	–	+	+	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+	+	+
Xcm-91	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	–	–	–	–	+	+	+	+
Xcm-92	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	–	–	–	–	+	+	–
Xcm-93	+	–	+	+	–	+	–	–	+	+	+	–	–	–	+	+	+	–	–	–	–	+	+	+
Xcm-94	+	–	+	+	–	+	–	+	+	+	+	–	+	+	+	+	–	–	–	–	+	+	+	–
Xcm-95	+	–	+	+	–	+	–	+	+	+	+	–	+	+	+	+	+	+	+	–	+	+	+	+
Xcm-96	+	–	+	+	–	+	–	–	–	+	+	–	+	+	+	+	+	+	–	–	–	+	+	+w
Xcm-97	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
Xcm-98	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	–
Xcm-99	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+	+	–
Xcm-100	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-101	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	+	–	–	+	+	+
Xcm-102	+	–	+	+	–	+	–	–	+	–	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-103	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+	–	–
Xcm-104	+	–	+	+	+	+	–	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
Xcm-105	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	–	–	–	–	+	+	+	+
Xcm-106	+	–	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
Xcm-107	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+	+	+
Xcm-108	+	–	+	+	–	+	–	–	+	+	+	–	+	–	+	+	+	+w	–	–	–	–	+	–
Xcm-109	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-110	+	–	+	+	–	+	–	–	+	+	+	–	+	–	+	+	+	+	–	–	+	+	+	+
Xcm-111	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+	+	–
Xcm-112	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+w	+w	+	+
Xcm-113	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+	–	–

Table 2. Continued.

Xcm-114	+	–	+	+	+	+	–	–	+	+	–	–	+	+	+	+	+	+	–	–	+	+	+	+
Xcm-115	+	–	+	+	–	+	–	–	+	+	+	–	–	–	+	+	+	+	–	–	+	+	+	+
Xcm-116	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	–
Xcm-117	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	+	–	+	+	+	+
Xcm-118	+	–	+	+	+	+	–	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+	–	–
Xcm-119	+	–	+	+	–	+	–	–	+	+	+	–	+	+	+	+	+	+	+w	+	+	+	+	+
Xcm-120	+	–	+	+	–	+	–	–	+	+	+	–	–	–	+	+	+	+	+	–	–	+	+	–

[a] *Xcm = Xanthomonas campestris pv. musacearum isolates designated from 1–120 code.* [b] *1 = KOH test; 2 = growth on asparagines medium; 3 = growth on nutrient agar with 5% glucose; 4 = catalase test; 5 = nitrate reduction reaction; 6 = Kovac's oxidase reaction; 7 = Tween 80 hydrolysis; 8 = starch hydrolysis; 9 = casein hydrolysis; 10 = gelatin liquefaction; 11 = H_2S production; 12 = indole production; 13 = citrate utilization; 14 = malate utilization; + = positive reaction or growth; +w = weak positive reaction or growth; and – = negative reaction or no growth.*

Pathogenicity assay

The results of pathogenicity test revealed that all the tested isolates of Xcm were able to cause bacterial wilt symptoms on the susceptible *enset* clone *Yeko* (Figure 4A). The inoculated leaves of the *enset* plants showed light yellow to dark brown necrosis around the inoculated areas of the leaves and those leaves then became yellowish and finally dried from apex end till the petiole collapsed (Figure 4B). There was typical oozing of bacterial cells in dissected petioles of symptomatic leaves (Figure 4C). These typical symptoms started on the inoculated leaf and spread gradually to the remaining leaves of the plant leading to systemic complete death of *enset* (Figure 4D). These symptoms were consistent with bacterial wilt of *enset* observed under the field conditions during the disease assessment periods. Re-isolation and identification confirmed their similarities with the parent isolates. Those leaf parts inoculated with sterilized distilled water remained healthy until end of the study period (Figure 4A).

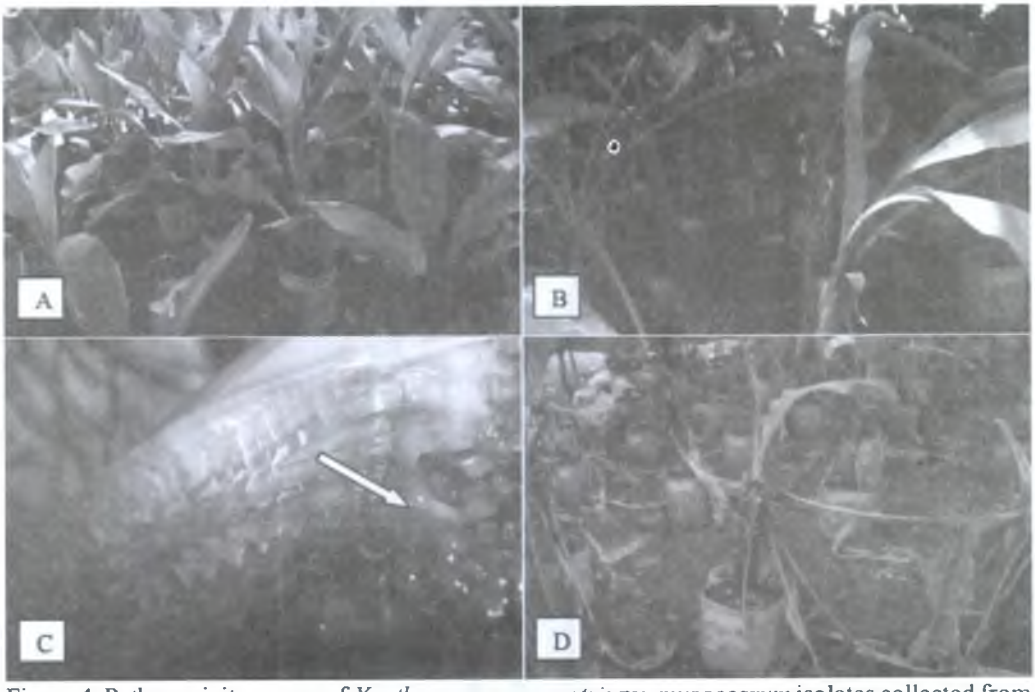

Figure 4. Pathogenicity assays of *Xanthomonas campestris* pv. *musacearum* isolates collected from southwestern Ethiopia. Un-inoculated susceptible *enset* clone *Yeko* (A). Yellowing and necrosis of infected leaves (B). The bacterial cells were oozing out from leaf petioles while opening the infected *enset* plant as indicated by the arrow (C). Completely wilted and dead *enset* plant due to artificial inoculation of the pathogen (D).

Discussion

Cultural, physiological, biochemical and pathogenicitical characterization of bacterial isolates are important tool for their identification. For Xcm identification, the growth and color of isolates can be determined on the basis of colony traits by using YPSA medium. The mucoid growth; yellow colony color; dome-shaped, circular and shiny colony are usually the characteristics of Xcm. These results are in harmony with the findings of Gizachew

(2000), Kidist (2003) and Befekadu *et al.* (2014). Gram staining reaction is a necessary initial step for the identification and classification of bacteria. The present study found Gram negative nature of Xcm bacterium that has fragile cell walls, which are bound by an outer membrane. This membrane is readily disrupted on exposure to 3% KOH releasing the viscous DNA (Fahy and Hayward, 1983).

The Xcm bacterium produced gas bubbles when these were mixed with a drop of H_2O_2 on glass slide; this finding gives a clue for presence of aerobic bacteria (Sands, 1990). In Kovacs oxidase test positive isolates produced purple color when mass of bacterial growth is rubbed on filter paper impregnated with oxidase reagent. Similar results were found by Dickey and Kelman (1988). According to Aneja (1996) and Dickey and Kelman (1988), hydrolyses of casein and gelatin are signs of proteolytic activities, and the present Xcm pathogen showed positive reaction for both. A color change from green to blue is an indication of utilization of both citrate and malate as a source of energy (Aneja, 1996). And, in the present findings, Xcm isolates were found positive for utilization of both citrate and malate compounds.

Detection of pathogen variability through characterization was the objectives of this research work, and hence, variability of isolates was observed in their morphological features on YPSA and nutrient broth with 5% glucose. The color and growth conditions were variable among most isolates. Most of them showed light yellow to deep yellow colony and there were isolates that grew fast and isolates with slow growth. In line with this result, Kidist (2003) reported the presence of variability among Xcm isolates right from field at which the difference in bacterial ooze color from one clone to another. In contrast to these findings, Gizachew (2000) did not find any difference between Xcm isolates, collected from enset, that were collected from Gurage and North-Omo zones in southern Ethiopia. *Xanthomonas campestris* pv. *musacearum* isolates also showed variability in physiological requirements, as many isolates were capable of growing on medium containing 3–5% NaCl and the others' was not. And, there was variation among isolate growth that was also observed in slight change from the normal recommended temperature for the growth of Xcm (28–30 °C) to 26 and 32 °C that resulted in failure of 36 and 30% isolates growth, respectively. This variability among isolates might be due to their genetic diversity. Phenotypic characterization and PCR-based studies conducted on both *enset* and banana Xcm isolates from Ethiopia also revealed possibility of variation among the isolates (Tsehaye, 2009). However, Aritua *et al.* (2008) reported the absence of genetic diversity amongst Xcm population based on the similarity of the rep-PCR profiles of the isolates. Many authors also found out that strain of Xcm causing bacterial wilt of *enset* and banana exhibit a high level of genotypic similarity (Aritua *et al.*, 2007; Tripathi *et al.*, 2008; Odipio *et al.*, 2009).

Conclusions

Based on EBW causal organism identification, through cultural, morphological, physiological and pathogenicity test results, the causative agent of the disease was confirmed to be Xcm. In cultural, morphological and physiological characterization assays, most isolates that were collected from the southwestern *enset* growing areas of Ethiopia had different appearances in colony color, growth types and tolerance abilities to different NaCl concentrations and temperature extremes, but similar in many biochemical characteristics. In spite of the fact that we used a limited number of

Xcm isolates, the result obtained suggests the presence of population diversity among Xcm isolates. Therefore, there seems a need for further study on its genetic diversity before drawing a meaningful inference.

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