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Vol. 13(24), pp. 2425-2434, 11 June, 2014
DOI: 10.5897/AJB2014.13794
Article Number: E8470EA45342
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Physiological characteristics and pathogenicity of *Xanthomonas campestris* pv. *musacearum* strains collected from enset and banana in Southwest Ethiopia

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Received 14 March, 2014; Accepted 16 May, 2014

***Xanthomonas campestris* pv. *musacearum* (Xcm) is a deadly bacterial pathogen causing wilt of enset and banana plants since the first record in Keffa province in Southwest Ethiopia as early as the 1960s. The disease remains a dominant constraint to enset production although its impact on banana has declined over the past four decades. The disease is ravaging banana plantations and spreading at alarming rates since its recent outbreak in other east and central African countries, including Uganda, Democratic Republic of Congo, Rwanda, Tanzania and Kenya. Enset wilt management strategies such as sanitation have been recommended although it is tedious for farmers to apply them for various reasons. The efforts to develop enset clones tolerant/resistant to Xcm strains have not been efficient for inconsistent reactions/performance of the selected materials, mainly attributed to variations in the bacterial isolates used across the studies. Thus, it is important to determine ranges of variation within the pathogen and host populations for developing resistant varieties and further breeding work. The objectives of this study were to collect and characterize Xcm strains from enset and banana plants in three major enset growing zones of Southwest Ethiopia and determine host-pathogen interactions. Nineteen (19) Xcm strains were selected from a total of 72 isolates collected from leaf petioles of enset and banana plants infected with bacterial wilt in six districts of Sheka, Keffa and Bench-Maji zones. The bacterial strains were typically creamy to yellow mucoid, circular with dome-shaped colonies. The strains were Gram-negative, KOH and catalase positive, suppressed on asparagine medium and negative for nitrate reduction; most isolates (84.2%) were insensitive to 2% NaCl while few strains (15.8%) were retarded by 1% NaCl concentration. All the strains were positive to hypersensitivity test with reaction varying from chlorosis to necrosis on tobacco leaves. Six enset and two banana strains of Xcm were pathogenic to the susceptible enset '*Yeko*' and banana '*Butuza*' (AAA) clones. The banana strains induced typical bacterial wilting symptoms on both hosts that ultimately led to complete death (100%). The host-pathogen interaction evidenced differences mostly among the enset clones in their resistance/tolerance and variation in aggressiveness (virulence) between the bacterial strains. The enset clones '*Nobo*' and '*Gudiro*' were consistently resistant while '*Yeko'* was highly susceptible to the three Xcm strains, namely: Xcme-9, Xcme-10 and Xcme-19, whereas '*Chikaro*' was moderately tolerant to two strains (Xcme-10 and Xcme-19) but most susceptible to strain Xcm9. The strains were less or non-aggressive to the resistant clones '*Nobo*' and '*Gudiro*' but most aggressive on the susceptible plants while ranges of aggressiveness were demonstrated on '*Chikaro*'.**

Key words: Banana, *Ensete ventricosum*, enset bacterial wilt, *Xanthomonas campestris* pv. *musacearum*, Ethiopia.

INTRODUCTION

Xanthomonas campestris pv. *musacearum* (Xcm) has been the most devastating bacterial disease of enset (*Ensete ventricosum* (Welw.) Cheesman), threatening crop production in Ethiopia since it was first officially reported in 1968 (Yirgou and Bradbury, 1968, 1974; Ashagari, 1985; Weldemichael et al., 2006, 2008). The disease is widely distributed and has caused significant losses in almost all enset growing areas of the country over the past 45 years (Ashagari, 1985; Handoro and Weldemichael, 2007; Weldemichael et al., 2008), affecting farmers' livelihood. Ashagari (1985) reported that bacterial wilt was very severe in 23 out of 29 surveyed districts sampled in central, southern and southwestern Ethiopia. In general, unlike the report by Addis et al. (2004), the disease is more serious on enset than on banana where both crops are grown together in southwest Ethiopia.

Although *X. campestris* pv. *musacearum* was confined for many years as an endemic disease in Ethiopia, wide-spread outbreaks of bacterial wilt incited by the same pathogen have been reported on banana plantations, inflicting great losses, in other east and central African countries. In these regions, the bacterial wilt of enset is commonly known as banana Xanthomonas wilt and was first reported in Uganda in 2001, Democratic Republic of Congo in 2003, Rwanda in 2004, Tanzania in 2005 and Kenya in 2006 (Biruma et al., 2007; Smith et al., 2008; Tripathi et al., 2009). However, there has been no report on wild enset in these countries.

Enset is cultivated only in Ethiopia (Zippel, 2005; Bizuayehu, 2008), being a multipurpose crop used for food, feed and fibre. The crop is said to ensure food security, especially in the face of recurrent drought and climate change (Brandt et al., 1997). Enset is supposed to have been domesticated and distributed in the higher areas of Keffa, in the southwestern part of the country (Westphal, 1975). Both contemporary techniques and classical classification systems indicate that there exists considerable diversity in the cultivated and wild enset populations of the country (Almaz et al., 2002; Birmeta et al., 2004; Bizuayehu, 2008).

To date, management of enset bacterial wilt has not been successful in Ethiopia for a number of technical and practical reasons. Although enormous extension efforts on sanitation (disinfection of farming and processing tools, roguing/eradication of infected enset plants) have been undertaken to curb the disease problem, the measures are not easy to implement by enset farmers. Enset is a giant, single-stemmed, herbaceous perennial plant (10-13 m high and >2 m in diameter) with a deep-rooted underground corm and a large pseudostem that make it difficult to uproot and bury or burn the infected mature trees. In addition, despite considerable diversity within cultivated and wild enset (Almaz et al., 2002; Birmeta et al., 2004; Bizuayehu, 2008), developing resistant/tolerant enset clones has not been effective. Among other problems, the reactions of most clones identified as tolerant/resistant were not consistent across locations and over time (Ashagari, 1985; Handoro and Michael, 2007, 2008). This inconsistent performance of the clones was partly attributed to variations in the bacterial wilt pathogen isolates used across the studies (Michael et al., 2008). Nevertheless, the interactions of host-pathogen-environment-related factors determine disease expression. Hence, the present studies addressed physiological characteristics, pathogenicity and host-pathogen interactions of *X. campestris* pv. *musacearum* strains collected from infected enset and banana plants in southwest Ethiopia.

MATERIALS AND METHODS

Collection of *X. campestris* pv. *musacearum*

During September - November 2011, a large number of tissue pieces (25 cm long) were randomly collected from leaf petioles of enset and banana plants with active bacterial wilt symptoms in farmers' fields, after checking for the presence of bacterial cells in the dissected sections. The sample areas were represented by 54 farmers' fields (2 plants per field) in six major enset-growing districts of three zones in southwest Ethiopia, namely Sheka, Keffa and Bench-Maji varying in altitude from 1450 to 2450 m.a.s.l. The bacterial cells were easily isolated and then purified on yeast peptone sucrose agar (YPSA) (5 g yeast extract, 10 g peptone, 20 g sucrose, 15 g agar per litre of distilled water) after incubating at 28°C for 48 to 72 h (Quimio, 1992; Schaad et al., 2001). The field-collected isolates were maintained as a stock culture on the same medium at 4°C and then used for further study. The cultural and physiological characterizations of the bacterial isolates were conducted in the plant pathology laboratory and the hypersensitivity and pathogenicity tests were undertaken in the greenhouse at College of Agriculture and Veterinary Medicine, Jimma University.

Characterization of *X. campestris* pv. *musacearum*

Gram reaction

The standard Gram-staining procedure (Schaad et al., 2001) and KOH solubility test (Fahy and Hayward, 1983) were conducted to determine Gram positive and negative bacterial isolates.

Growth on asparagine medium

The bacterial isolates that showed Gram negative reaction with mucoid thread were grown on asparagine medium

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(0.5 g asparagine, 0.1 g KH_2PO_4, 0.2 g $MgSO_4.7H_2O$, 0.5 g KNO_3, 0.1 g $CaCl_2$, 0.1 g NaCl and 15 g agar per litre of distilled water) at 28°C for 48 to 72 h (Dye et al., 1980). This diagnostic test is used to detect *Xanthomonas* because it is not able to grow on asparagine medium while other species, such as yellow *Enterobacteriaceae* and many Pseudomonas can. The growth of the bacteria on asparagine agar plates and broth were recorded and those isolates that were unable to grow on the medium were considered for further tests.

Growth on nutrient agar with 5% glucose

Each isolate was streaked on nutrient agar with 5% glucose (23 g nutrient agar, 5% glucose per litre of distilled water) and incubated at 28°C for 48 to 72 h. The colony growth characteristics such as form, elevation and color were scored on this medium to differentiate *X. campestris* from other *Xanthomonas* species (Bradbury, 1984).

Catalase and salt tolerance tests

A few drops of 3% hydrogen peroxide were added on the surface of the 48 h-old culture of each isolate on YPSA medium and bubble formation was recorded as positive for catalase activity (Dickey and Kelman, 1988). Salt tolerance of the strains was also tested by inoculating each isolate into nutrient broth in a test tube with varying NaCl concentrations (1 to 5%) (Hayward, 1964). The nutrient broth without salt (0%) was included as a negative control and the presence or absence of bacterial growth was examined after 12 to 14 days incubation at 25°C.

Nitrate reduction

The ability of the isolates to reduce nitrate to nitrite was determined in a growth medium that contained 1 g KNO_3, 5 g peptone, 3 g yeast extract and 3 g agar per litre of distilled water in test tubes. 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 a positive result for nitrate reduction after three, five and seven days inoculation (Dickey and Kelman, 1988).

Hypersensitivity test

Forty-hour-old cultures of 19 selected bacterial strains (out of 72 collected isolates) were suspended in sterilized distilled water and adjusted to 0.3 O.D. at 460 nm (equivalent to about 10^8 CFU/ml bacterial cell concentration) using a spectrophotometer. An aliquot of 2 ml bacterial suspension was injected into the intercellular spaces of expanded leaves (2 leaves per plant) of one-month old tobacco plants (*Nicotiana tabacum* var. White Burley) with a sterile syringe. Similarly, treated plants with distilled sterile water were used as negative control. All the inoculated plants were then kept in a greenhouse with a daytime temperature of about 27 - 30°C for subsequent symptom development. The appearance of chlorotic to necrotic tissue around the injection point on inoculated leaves was considered as positive for the test (Quimio, 1992).

Pathogenicity test

Growing susceptible clones

One-year old suckers propagated from a susceptible enset clone, locally known as '*Yeko*', and a banana variety '*Butuza*' (AAA) were planted in a mixture of sterilized soil, sand and amended with composted manure (in a 2:1:1 ratio) in 25 L plastic pots (Quimio 1992). The suckers were grown for six months in the greenhouse at 25 - 30°C daytime temperature.

Inoculum preparation and inoculation

A total of eight Xcm strains (six from enset and two from banana), that showed positive reactions in the hypersensitivity test was selected and reproduced on nutrient agar in 90 mm Petri dishes incubated at 28°C for 48 h. The bacterial cell suspension of each strain was uniformly prepared as mentioned above and aliquots of 3 ml of the suspension (1 x 10^8 CFU/ml concentration) were inoculated using a sterile syringe to the second innermost leaf petiole of young (six month- old) enset and banana plants. In this case, the six enset strains were inoculated only into the susceptible enset plants while infectivity of the two banana isolates were tested both on the banana variety '*Butuza*' and the enset clone '*Yeko*'. Seedlings of both hosts treated in the same manner with distilled sterile water were included as negative controls. This experiment was laid out in a completely randomized design with three replications; each strain was tested on three plants per replication. The progressive wilting symptoms, including number of infected leaves, number of completely dead seedlings, and number of days to first symptom appearance and days to complete death were recorded at weekly intervals for three to four months. Finally, the presence and/or absence of bacterial mass was checked in the leaf petioles and pseudostems and re-isolated from symptomatic plants.

Enset clone-*X. campestris* pv. *musacearum* strain interaction in the field

This set of experiment was conducted under field conditions using four enset clones locally identified as '*Yeko*', '*Chikaro',* '*Nobo'* and '*Gudiro'* and three bacterial strains confirmed to be pathogenic in the previous trial. The enset clones were purposely selected based on their reactions to Xcm as susceptible, intermediate and tolerant/resistant in most surveyed farmers' fields in the six major enset producing sample districts. Suckers of each enset clone were transplanted at Gecha (1960 m a.s.l.) in Anderacha district of Sheka zone and carefully grown for about eight months. The three Xcm strains Xcme-9, Xcme-10 and Xcme-19, respectively represented Keffa, Sheka and Bench-Maji zones with their respective enset agroecology described as lowland (1450 m.a.s.l.), highland (2450 m.a.s.l.) and intermediate (1790 m.a.s.l.). Fresh bacterial inoculum of each selected Xcm strain was separately prepared as mentioned above and artificially inoculated (5 ml) with each enset clone following the procedures adopted by Michael et al. (2008). A row of five enset plants per clone was inoculated with sterile water and included as a negative control.

Experimental design, data collection and analysis

Each of the tests conducted in the laboratory was repeated at least three times with three replication per test including negative and positive control. The field experiment, consisting of 12 treatments (three bacterial strains by four enset clones) arranged in a factorial treatment combinations, was laid out in a randomized complete block design with three replications (five plants/treatment). The disease data, such as number of days to first symptom appearance, number of wilting and dead plants, and number of days to complete death, were recorded at fortnight intervals for five to six months. In addition, disease severity was assessed employing a 0 - 5 scale developed by Winstead and Kelman (1952), scoring 0 for a plant

Table 1. Physiological characteristics of *Xanthomonas campestris* pv. *musacearum* (Xcm) strains isolated from leaf petioles of enset and banana plants in southwest Ethiopia (2012).

Xcm strains[1]	Gram staining	KOH test	Nutrient agar (5% glucose)	Catalase test	Nitrate reduction	NaCl concentrations (%)[2]					
						0	1	2	3	4	5
Xcme-1	-	+	+	+	-	+	+	+	-	-	-
Xcme-2	-	+	+	+	-	+	+	+	-	-	-
Xcme-3	-	+	+	+	-	+	+	+	+	+	-
Xcme-4	-	+	+	+	-	+	+	-	-	-	-
Xcme-5	-	+	+	+	-	+	+	+	-	-	-
Xcme-6	-	+	+	+	-	+	+	-	-	-	-
Xcme-7	-	+	+	+	-	+	+	+	-	-	-
Xcme-8	-	+	+	+	-	+	+	+	+	+	+
Xcme-9	-	+	+	+	-	+	+	+	-	-	-
Xcme-10	-	+	+	+	-	+	+	+	-	-	-
Xcme-11	-	+	+	+	-	+	+	+	+	+	-
Xcme-12	-	+	+	+	-	+	+	+	+	-	-
Xcme-13	-	+	+	+	-	+	+	+	+	-	-
Xcme-14	-	+	+	+	-	+	+	-	-	-	-
Xcme-15	-	+	+	+	-	+	+	+	+	+	+
Xcmb-16	-	+	+	+	-	+	+	+	+	-	-
Xcmb-17	-	+	+	+	-	+	+	+	+	+	-
Xcme-18	-	+	+	+	-	+	+	+	-	-	-
Xcme-19	-	+	+	+	-	+	+	+	+	+	-

[1]Xcm strains Xcme-1, Xcme-2 and Xcme-3 were collected from Gatimo, Yina and Kanga (Masha, Sheka zone)); Xcme-4, Xcme-5 and Xcme-6 were collected from Gebina, Chicha and Tugiri (Andiracha, Sheka zone); Xcme-7, Xcme-8 and Xcme-9 were collected from Ermichi, Kubito and Achany (Yeki, Sheka zone); Xcme-10, Xcme-11 and Xcme-12 were collected from Yerkichity, Dirbado and Damonechity (Giesha, Keffa zone); Xcme-13, Xcme-14, Xcme-15 and Xcmb-16 were collected from Sheda, Gawaty and Dachadifa (Bita, Keffa zone); Xcmeb-17, Xcme-18 and Xcme-19 were collected from Kuka, Maha and Ziagin (Shebench, Bench-Maji zone), respectively. [2]The salt tolerance test was replicated and three independent tests were conducted. The letters 'e' and 'b' stand for the host enset and banana, respectively. The sign '-' and '+' indicate negative and positive responses.

without visible symptom, 1 for one leaf wilted, 2 for two to three leaves wilted, 3 for four leaves wilted, 4 for all leaves wilted and 5 for completely dead plant. Finally, the incubation period, the number of days to complete death, disease severity index and incidence were computed for statistical analyses. The disease incidence (%) was calculated as the number of dead enset plants divided by the total number of inoculated plants per plot multiplied by 100, then transformed to arc sin square root values before analysis of variance, and treatment means were compared using Tukey's test with SAS software version 9.2 (SAS Institute Inc. 2008).

RESULTS

Physiological characteristics of *X. campestris* pv. *musacearum* strains

Colony characteristics

Nineteen bacterial strains including two isolates from banana were studied (Table 1), and the colonies were circular, dome-shaped and highly mucoid with a shiny appearance on the YPSA medium. All the strains showed Gram-negative reaction in Gram staining and did not dissolve in 3% KOH solution; instead they formed a thin strand of slime while mixing the bacterial cells and lifting with the inoculating loop.

The colonies of Xcm strains showed slight variations in colour and growth character on nutrient agar with 5% glucose medium. Most of the isolates from enset (68%) had slimy mucoid to mucoidal yellow colonies, whereas those identified from some enset clones and banana (32%) appeared creamy mucoidal to slightly yellow (Figure 1). The strains were catalase positive, producing gas bubbles when a 48-h-old colony of each strain was dissolved in a few drops of 3% hydrogen peroxide. The tested bacterial isolates failed to grow on asparagine medium and did not reduce nitrate to nitrite (Table 1).

In salt tolerance tests, 84.2% of the strains grew well on nutrient broth amended with 2% NaCl, while few strains (15.8%), including three from enset (Xcme-4, Xcme-6 and Xcme-14), were retarded by 1% NaCl, being highly sensitive to salt. On the other hand, 31.6% of the strains (Xcme-3, Xcme-8, Xcme-11, Xcme-15, Xcmb-17 and Xcme-19) demonstrated salt tolerance, being insensitive even up to 4 or 5% NaCl concentration (Table 1).

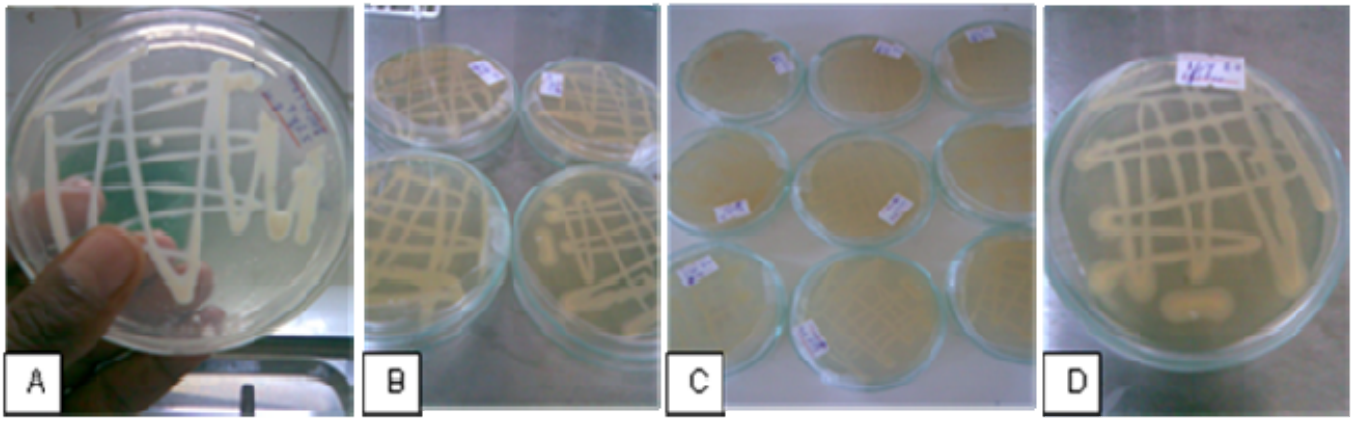

Figure 1. Colonies of *Xanthomonas campestris* pv. *musacearum* strains grown on nutrient agar with 5% glucose for 48 h at 28°C with creamy/slimy, less-mucoid (A and B) to mucoid and deep yellow (C and D) appearance.

Hypersensitive reaction of *X. campestris* pv. *musacearum* strains on tobacco leaves

All the 19 bacterial strains inoculated into the leaves of tobacco seedlings induced hypersensitive reactions ranging from small chlorotic spots to extensive necrotic areas around the injection point within 48 to 72 h. Some enset strains, such as Xcme-9, Xcme-10 and Xcme-19, showed more aggressiveness, inducing extensive necrosis, while untreated leaves appeared green.

Pathogenicity of Xcm strains on young enset and banana plants

Among Xcm strains that demonstrated an aggressively positive hypersensitive reaction, six isolated from enset and two from banana (Xcmb-16 and Xcmb-17) were found to be highly pathogenic on young seedlings of a susceptible enset clone '*Yeko*' and a banana variety '*Butuza*' (AAA). The number of days to first symptom appearance (incubation period) and days to complete death varied significantly ($P < 0.05$) among the bacterial strains (Table 2). The two banana strains Xcmb-16 and Xcmb-17 induced typical wilting symptoms on its host variety '*Butuza*' within 30 days, as compared to significantly ($P < 0.05$) longer incubation periods ranging from 45 to 60 days on the enset clone. In addition, some enset isolates Xcme-2, Xcme-6 and Xcme-13 took about two months (50 to 60 days) to infect and initiate first wilting symptoms. All eight strains eventually resulted in complete death of inoculated plants of both hosts within 45 to 90 days (Table 2).

The inoculated enset leaves first revealed gray to light yellow chlorosis around the inoculated areas that gradually turned to yellowish brown necrosis and finally the whole leaf dried and then the petiole collapsed. These external symptoms spread progressively to the remaining leaves, leading to complete death of the plant. Internally, groups of creamy to yellowish bacterial cells were evident in dissected petioles of symptomatic leaves and in cross-section of pseudostem of dying enset (Figure 2A - G) and banana plants (Figure 2H - J).

Interactions of enset clones with *X. campestris* pv. *musacearum* strains

There were highly significant ($P < 0.001$) differences among the selected strains, enset clones and their interactions in all disease parameters considered in the study (Table 3). The three strains Xcme-9, Xcme-10 and Xcme-19, respectively, collected from Sheka, Keffa and Bench-Maji zones of southwest Ethiopia, induced wilting symptoms on enset clone '*Yeko*' within short incubation periods of 52 to 53 days that subsequently resulted in higher wilt severity and complete death of the plants (Table 3). However, the same bacterial strains did not incite disease on all inoculated plants of clone '*Nobo*' 120 days after inoculation. Similarly, these strains showed infection symptoms only on the inoculated leaf of '*Gudiro*' plants after about 56 to 62 days incubation (Table 3). The infection did not progress and the plants entirely remained healthy even after the trial termination. Thus, the result indicated that enset clones '*Nobo*' and '*Gudiro*' showed high levels of resistance to the bacterial wilt strains, while '*Yeko*' was markedly susceptible.

The enset clone '*Chikaro*' had differential reactions to the three bacterial strains. This clone was moderately attacked by Xcme-10 and Xcme-19 with respective disease incidences of 55 and 66% that were significantly ($P < 0.05$) different from strain Xcme-9, causing complete death (Table 3). The three Xcm strains caused the least disease severity (0 to 6.7%) and did not kill '*Nobo*' and '*Gudiro*' clones (0% incidence) although they showed high percentage of wilt severity index and incidences on

Table 2. Pathogencity of *Xanthomonas campestris* pv. *musacearum* (Xcm) strains inoculated with a susceptible enset clone '*Yeko*' and a banana variety '*Butuza*' in the greenhouse at College of Agriculture and Veterinary Medicine, Jimma University Ethiopia (2012).

Xcm strains[1]	Host	Incubation period (days)[2]	Mean number of days to complete death[3]	Wilt incidence[4]
Xcme-2	*'Yeko'*	50^{ab}	75^{a-d}	88.4 (100)
Xcme-6	*'Yeko'*	55^{a}	85^{ab}	88.4 (100)
Xcme-9	*'Yeko'*	35^{ab}	55^{de}	88.4 (100)
Xcme-10	*'Yeko'*	45^{ab}	65^{b-e}	88.4 (100)
Xcme-13	*'Yeko'*	50^{ab}	80^{a-c}	88.4 (100)
Xcmb-16	*'Yeko'*	45^{ab}	75^{a-d}	88.4 (100)
Xcmb-16	'*Butuza*'	30^{b}	45^{e}	88.4 (100)
Xcmb-17	'*Butuza*'	30^{b}	60^{c-e}	88.4 (100)
Xcmb-17	*'Yeko'*	60^{a}	90^{a}	88.4 (100)
Xcme-19	*'Yeko'*	45^{ab}	70^{a-d}	88.4 (100)
LSD (P < 0.05)		20.95	22.40	NS

[1]*Xanthomonas campestris* pv. *musacearum* strains Xcme-2, Xcme-6, Xcme-9 were collected from Masha, Anderacha and Yeki districts of Sheka; Xcme-10, Xcme-13, Xcmb-16 were collected from enset in Geisha and banana at Bita districts of Keffa; Xcmb-17 and Xcme-19 were collected from banana and enset at Shebench in Bench-Maji, respectively. [2]Mean number of days to first symptom appearance and [3]Mean number of days to complete death of the plants after inoculation (incubation period). [4]Disease incidence transformed to angular values before analysis and those figures in the brackets are actual mean values. Means followed with the same letter(s) in the column are not significantly different from each other according to Tukey's test. NS = non significant.

the plants of '*Yeko*' (up to 100%) (Table 3). This finding also showed *X. campestris* pv. *musacearum* populations varied from non- or least to high levels of aggressiveness or virulence.

DISCUSSION

In this study, 72 isolates of *X. campestris* PV. *musacearum* were identified from symptomatic enset and banana plants sampled in six districts of three major enset growing zones namely, Sheka, Keffa and Bench-Maji in southwest Ethiopia. The bacterial strains had circular, typical mucoid growth varying from light cream to very yellow colonies on nutrient agar with 5% glucose. In physiological tests, all the 19 bacterial isolates were Gram-negative, KOH and catalase positive, did not grow on asparagine medium and failed to reduce nitrate to nitrite. In addition, they demonstrated relatively varying growth sensitivity to NaCl concentrations and 84.2% of the isolates grew well on nutrient broth amended with 2% NaCl, while few strains (15.8%) were retarded by 1% NaCl, being highly sensitive to salt. The strains induced positive hypersensitive reactions, producing chlorotic to necrotic areas on tobacco leaves.

Eight of 19 strains were pathogenic to the susceptible enset clone '*Yeko*', including the two banana strains inoculated into enset and banana, exhibiting progressive bacterial wilting symptoms from yellowing of the inoculated leaves to complete death of the young plants of the respective hosts in the greenhouse. Besides, the characteristic external symptoms, creamy to yellowish pockets of bacterial cells were commonly encountered inside the leaf petioles and pseudostems of infected plants of enset and banana that were consistent with bacterial Xanthomonas wilt of both hosts infected in the fields. There were some discernible differences in cultural (creamy to yellow colony), physiological (salt tolerance) and hypersensitivity (chlorotic to necrotic) responses among Xcm strains. In the pathogenicity test, although all Xcm isolates eventually killed both enset and banana plants, the two banana strains Xcmb-16 and Xcmb-17 infected its host variety '*Butuza*' (AAA) within significantly ($P < 0.05$) shorter incubation time (30 days) and caused death with in 45 to 60 days than on enset plants. This may suggest that the banana strain is relatively more aggressive on banana than on enset. The banana cultivars such as 'Dwarf Cavendish', 'Giant Cavendish' and 'FHIA-17' with similar genome to '*Butuza*' were reported to be highly susceptible to Xcm strain in Uganda (Tripathi and Tripathi, 2009).

The 19 bacterial strains detected in infected and symptomatic enset and banana trees thus conform to the phytopathogenic bacterial species *X. campestris* pv. *musacearum*. These findings are in agreement with the pioneer work of Yirgou and Bradbury (1968, 1974) who first detected and identified enset bacterial wilt on enset and banana plants in Keffa province of southwest Ethiopia. Based on physiological, biochemical and pathological characteristics of the bacterial strains, the

Figure 2. Typical characteristic symptoms of bacterial wilt caused by *Xanthomonas campestris* pv. *musacearum* on infected enset and banana plants under field conditions in southwest Ethiopia. External progressive wilting symptoms (A - C) and dead enset plant (D), pocket of bacterial cells in dissected leaf petiole (E) and yellowish bacterial cells oozing out of cross-section of leaf petioles (F) and pseudostems of enset (G). External early wilting symptom (H) and finally dead (I) and yellowish bacterial exudates in the stem cross-section (J) of banana plant.

authors proposed the epithet *X. musacaerum* sp.n. as causal agent of the disease on both hosts (Yirgou and Bradbury, 1968, 1974). Later, the species was grouped as *X. campestris* pathovar and named as *X. campestris* pv. *musacearum* providing detailed classical taxonomic descriptions (Bradbury, 1986; Young et al., 1991). The results of molecular genetic analyses (Rep-PCR and RAPD) indicate that the present populations of *X. campestris* pv. *musacearum* in central and east Africa (Ethiopia, DR Congo, Rwanda, Tanzania and Uganda) are homogenous regardless of time of isolation, geographic location and hosts (Aritua et al., 2007, 2008; Odipio et al., 2009; Lewis Ivey et al., 2010). Nevertheless, based on fatty acid methyl esters and *gyrase* B gene analyses, Aritua et al. (2008) proposed reclassification of this species as a pathovar within *X. vasicola* (*X. vasicola* pv. *musacearum*). As commented by Ivey et al. (2010), we also suggested that several research data should be generated on many collections of *X. campestris* pathovars (including the *Musacearum* strains from enset and banana) through host-pathogen interaction (cross-inoculation) and molecular analyses.

Table 3. Interaction of enset clones inoculated with *Xanthomonas campestris* pv. *musacearum* isolates in incubation period (days), disease severity index and incidence (%) and days to complete death under field conditions at Gecha (Sheka) in southwest Ethiopia (2011/2012).

Enset clones[1]	Bacterial isolates[2]	Incubation period (days)[3]	Disease severity index (%)[4]	Days to complete death[5]	Incidence[6]
Gudiro	Xcme-9	61.7^{ab}	6.7^{c}	0.0^{c}	1.6^{c}(0.0)
	Xcme-10	55.8^{bc}	6.7^{c}	0.0^{c}	1.6^{c}(0.0)
	Xcme-19	62.5^{ab}	6.7	0.0^{c}	1.6^{c}(0.0)
Nobo	Xcme-9	0.0^{e}	0.0^{d}	0.0^{c}	1.6^{c}(0.0)
	Xcme-10	0.0^{e}	0.0^{d}	0.0^{c}	1.6^{c}(0.0)
	Xcme-19	0.0^{e2}	0.0^{d}	0.0^{c}	1.6^{c}(0.0)
Chikaro	Xcme-9	46.7^{d}	100^{a}	86.7^{b}	88.4^{a}(100)
	Xcme-10	67.5^{a}	68.9^{b}	110.0^{a}	66.0^{b}(83.4)
	Xcme-19	63.3^{a}	71.2^{b}	100.0^{ab}	54.8^{c}(66.7)
Yeko	Xcme-9	51.7^{cd}	100^{a}	86.7^{b}	88.4^{a}(100)
	Xcme-10	51.7^{cd}	100^{a}	88.3^{b}	88.4^{a}(100)
	Xcme-19	53.3^{cd}	100^{a}	90.0^{b}	88.4^{a}(100)
Coefficient of variation (cv)		9.25	2.21	12.46	13.89

[1]Enset clones Gudiro, Nobo, Chikaro and Yeko; and [2]*Xanthomonas campestris* pv. *musacearum* isolates Xcme-9, Xcme-10 and Xcme-19 were obtained from Sheka, Keffa and Bench-Maji zones of southwest Ethiopia. [3]Incubation period indicates mean number of days between inoculation and first symptom appearance and 0.0 means no diseased plant was recorded until end of the trial (120 days after inoculation). [4]Disease severity index (%) calculated from wilt score based on 0 - 5 scale of Winstead and Kelman (1952). [5]Mean number of days to complete death of the plants after inoculation date. [6]Figures in the brackets are the original/untransformed mean values of disease incidence. Means (of three replications) with in each column followed with the same letter(s) are not significantly (P<0.05) different from each other according to Tukey's test.

The enset clone by Xcm isolate interaction study showed significant differences in incubation period, severity index, date to complete death and disease incidence under field conditions (Table 3). This result demonstrates that there are remarkably contrasting responses in tolerance/resistance to the bacterial wilt strains among enset clones grown in southwest Ethiopia. The two clones '*Nobo*' and '*Gudiro*' are highly resistant, with little or no infection symptoms as opposed to the partial to complete death of inoculated plants of '*Chikaro*' and '*Yeko*' clones. There were no wilting symptoms, except on the inoculated leaves of '*Gudiro*' trees treated with three isolates Xcme-9, Xcme-10 and Xcme-19, four months after inoculation. Similarly, Handoro and Michael (2007) observed very low bacterial wilt infection on '*Meziya*' enset clone artificially inoculated with three Xcm isolates originating from Sidama, Dawro and Kembata with 8.3, 5.7 and 2.6%, respectively, as compared to 75 - 100% death on '*Arkia*' clone by the same isolates under field conditions. The '*Meziya'* trees that showed slight yellowing symptoms recovered from infection and became healthy after four to six months (Handoro and Michael, 2007). Michael et al. (2008) also reported that some clones, such as '*Buacho'* and '*Wonigoro*' (Sidama collection) and '*Bazeriet*' and '*Dere*' (Gurage collection) recovered from initial infections. The *in vitro* plantlets and potted plants of *Musa balbisiana* and *cv.* 'Nakitembe', inoculated with a *Xanthomonas* wilt isolate, have recovered and appeared healthy after showing initial symptom of infections under screen house conditions (Tripathi and Tripathi, 2009).

Although many authors speculated hypersensitive type of resistance that are also common in nonhost crops like tobacco and maize, some kinds of physical and/or biochemical defense reactions are perhaps operating in the resistant hosts of enset and banana clones. Thus, further investigation on the resistance mechanisms and/or pathogenicity factors with histopathological and biochemical techniques through to genomics are worthwhile.

The present study also indicates that there is variation in pathogenicity within the bacterial populations. Among the three Xcm isolates; on '*Chikaro*' clone, Xcme-10 and Xcme-19 caused significantly lower disease severity index, incidence and longer time to complete death as compared to isolate Xcme-9. Xcme-9 was found to be highly aggressive to '*Chikaro*' and '*Yeko*' plants, while all strains were slightly or non-aggressive to '*Gudiro*' and '*Nobo*'. Handoro and Michael (2007) tested the pathogenicity of five Xcm isolates artificially inoculated into '*Meziya*' (resistant) and '*Arkia*' (susceptible) enset clones and observed variations in disease incidence. The Sidama and Dawro isolates were very aggressive (100%) followed by Kembata, Gurage and Hadiya strains with wilt infection of 75.0, 66.7, and 58.3%, respectively, on the susceptible clone '*Arkia*'. Tripathi et al. (2008) found significant variation (P<0.0001) in susceptibility of eight banana cultivars but reported no variation in pathogenicity

(wilt incidence) between 16 Xcm isolates tested on the eight cultivars. However, these isolates were significantly (P<0.0001) different from each other in two important parameters, incubation period for appearance of symptoms and number of days to complete wilting of plants (Tripathi et al., 2008).

In conclusion, Xanthomonas bacterial wilt is widespread in different enset growing regions of Ethiopia including Sheka, Keffa and Bench-Maji zones. It is found to be an important disease of enset, but with minor intensity on banana plants. The bacterial strains identified from enset and banana in these areas showed similar cultural, morphological and physiological characteristics to the species *X. campestris* pv. *musacearum* with some differences in salt tolerance and aggressiveness. The host-pathogen interaction evidenced diversity among the enset clones in their resistance/tolerance and variation in virulence between the bacterial strains. The enset clones '*Nobo*' and '*Gudiro*' are consistently resistant while '*Yeko'* is highly susceptible to the three Xcm strains. '*Chikaro*' is moderately tolerant but most susceptible to third one. The three strains are least or non-aggressive towards the resistant clones '*Nobo*' and '*Gudiro*' but most aggressive on the susceptible '*Yeko*' with varying pathogenicity levels to '*Chikaro*' ranging from moderate to high aggressiveness. Therefore, this study implies that the response of '*Nobo*' and '*Gudiro*' enset clones should be verified in major enset producing areas including yield and quality assessment. In addition, aggressive (virulent) Xcm strain should be used in screening and testing enset landrace collections in endeavor to identify resistant/-tolerant clones for sustainable management of enset bacterial wilt.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors acknowledge College of Agriculture and Veterinary Medicine, Jimma University for financial support and sincere appreciation goes to the enset farmers for their collaboration during sample collection and field experiment.

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