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FULL RESEARCH PAPER

Development of a semi-selective medium for isolation of *Xanthomonas campestris* pv. *musacearum* from banana plants

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Abstract Banana *Xanthomonas* wilt, caused by *Xanthomonas campestris* pv. *musacearum*, is a new threat to banana cultivation in eastern Africa. The causal bacterium grows slowly in culture and is easily overgrown by contaminants. A selective culture medium for isolation of *X. c.* pv. *musacearum* will facilitate disease study. A medium that suppressed saprophytic growth and possessed diagnostic characters for the pathogen was developed. Various carbon sources were tested with two isolates of *X. c.* pv. *musacearum*, and sucrose was selected as main carbon source. The susceptibility of *X. c.* pv. *musacearum* and other bacterial strains was tested with 29 different antibiotics. Cephalexin and cycloheximide had no effect on *X. c.* pv. *musacearum* but cephalexin inhibited most of the saprophytes and cycloheximide inhibited the fungal contaminants. Based on these studies, we have developed a semi-selective medium YTSA-CC containing yeast extract (1%), tryptone (1%), sucrose (1%), agar (1.5%), cephalexin (50 mg l^{-1}) and cycloheximide (150 mg l^{-1}), pH 7.0. The pathogen *X. c.* pv. *musacearum* was easily identified as yellowish, mucoid and circular colonies on YTSA-CC medium. This simple semi-selective medium was effective for isolation of *X. c.* pv. *musacearum* from infected banana tissues and soil, and it should be a valuable tool in ecological and epidemiological studies.

Keywords Antibiotic susceptibility · Banana *Xanthomonas* wilt · Carbon sources · Semi-selective medium · *Xanthomonas campestris* pv. *musacearum*

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Introduction

The banana *Xanthomonas* wilt (BXW) disease caused by the bacterium *Xanthomonas campestris* pv. *musacearum* (Tushemereirwe et al., 2004; *Xcm*) threatens the livelihood of millions of farmers in the Great Lakes region of eastern Africa. The disease was first identified in Uganda in 2001 and now has spread in epiphytotic proportions to almost all major banana-producing districts of the country. The disease has also been reported from the Democratic Republic of Congo (Ndungo, Eden-Green, Blomme, Crozier, & Smith, 2005), Rwanda (Svetlana Gantashova,

Institut des Sciences Agronomiques du Rwanda, ISAR, Rwanda, personal communication) and more recently has been identified in Tanzania (Mgenzi Byabachwezi, Agricultural Research and Development Institute Maruku, Tanzania, personal communication). The disease has a high potential of spreading into Kenya and Burundi because of the informal food-trade links that exist with affected countries. BXW was first reported more than 30 years ago in Ethiopia on *Ensete* species, which are closely related to banana (Yirgou & Bradbury, 1974). The disease attacks almost all varieties of banana, including a large group of agriculturally important bananas referred to as 'East African Highland Bananas', or EAHBs.

Economic impact of the disease is manifested as result of absolute yield loss or reduced bunch weights, and death of the mother plant and suckers that help in subsequent ratoon plant production cycles. Diseased fields cannot be replanted with banana due to soil-borne inoculum of the pathogen. Farmers are also unable to access clean planting material to re-establish new banana plantations. The symptoms of the disease are yellowing and wilting of leaves, and finally all leaves wither and the plant rots. Cross-sections of diseased pseudostems reveal yellowish droplets of bacterial ooze. The fruit ripens unevenly and prematurely with sections showing unique yellowish blotches in the flesh fingers and dark brown placental scars. The management of this disease by chemical control is impossible. Resistant varieties have been the best and most cost-effective method of managing bacterial diseases.

Ecology of *Xcm* and the epidemiology of BXW are not well understood. The pathogen probably survives in the soil and is spread by insects, cutting tools and infected planting material (Eden-Green, 2004). To carry out epidemiological and ecological studies, a simple selective medium is required for isolation of *Xcm* from the infected plants and soil. Semi-selective media have been developed for isolation of several *Xanthomonas* species and have proved useful for elucidating the ecology and epidemiology of related diseases (Davis, Rott, Baudin, & Dean, 1994; Di, Ye, Schaad, & Roth, 1991; Fessehaie, Wydra, & Rudolph, 1999; Mabagala & Saettler, 1992; Norman & Alvarez, 1994; Sijam, Chang, & Gitaitis, 1992; Wydra, Khatri-Chetri, Mavridis, Sikirou, & Rudolph, 2004). No specific medium for the isolation of *Xcm* has been reported. Considering that *Xcm* populations are often low and that this bacterium grows slower than bacterial saprophytes, it is important to develop a semi-selective medium for *Xcm.* The semi-selective medium should make *Xcm* easily recognizable and also limit the growth of saprophytic bacteria facilitating enumeration of the pathogen. Therefore, the objective of this study was to develop a simple semi-selective medium for isolation of *Xcm* from infected banana plants.

Materials and methods

Bacterial strains and inoculum preparation

Sixteen isolates of *Xcm* (006/wks/05, KY44, 001/gyz/05, 002/wks/05, 007/wks/05, 036/lra/05, 038/lra/05, 031/kib/05, 033/kib/05, 045/kla/05, 019/iga/05, 003/muk/05, 041/nsg/05, 014/luw/05, 043/msd/05 and 034/kibo/05), for which Koch's postulate had been proved, were used as authentic pathogen cultures in this study. Strains of other *Xanthomonas* spp. used in this study are *X. campestris* pv. *campestris* (NCPPB528), *X. vasicola* pv. *vasculorum* (NCPPB206), *X. translucens* pv. *translucens* (NCPPB973), *X. campestris* pv. *hyacinthi* (NCPPB599) and *X. campestris* pv. *vesicatoria* (NCCPB881). A strain each of *Escherichia coli* and *Agrobacterium tumefaciens* from the senior author's laboratory collections were used as Gram-negative bacterial cultures. The cultures were maintained on YTSA (1% yeast extract, 1% tryptone, 1% sucrose, 1.5% agar). The same inoculum preparation method was used in all experiments, unless otherwise stated. The bacterial cultures were grown overnight in YTS (YTSA minus agar) medium at 28°C with shaking at 150 rpm and serially diluted 100-fold in YTS medium. The bacterial concentration of the overnight bacterial cultures was about 10^8 CFU (colony forming units) ml^{-1}. One hundred microlitres of the serially diluted cultures were plated per Petri plate (90 mm diam) using a bent glass rod.

Evaluation of basal medium

Four basal media were tested for the growth of *Xcm*. These include: LBA (1% tryptone, 0.5% yeast extract, 0.5% sodium chloride, 1.5% agar), YPGA (1% yeast extract, 1% peptone, 1% glucose, 1.5% agar), Minimal medium (3 g l^{-1} K_2HPO_4, 1 g l^{-1} NaH_2PO_4, 1 g l^{-1} NH_4Cl, 0.3 g l^{-1} $MgSO_4 \cdot 7H_2O$, 10 g l^{-1} sucrose, 1.5% agar), and YTSA. A single colony of *Xcm* was picked with a sterile loop and streaked on various media contained in 90 mm diameter Petri plates. The inoculated plates were incubated at 28°C, and observed daily for the appearance and growth of the colonies and their size. The optimal basal medium was selected based on time duration for appearance of colonies, growth (size) and number of isolated colonies formed on the plates of various media.

Evaluation of various carbon sources

The ability of the two *Xcm* isolates (006/wks/05 and KY44) to use different carbon sources was evaluated on basal YTA medium (1% yeast extract, 1% tryptone, and 1.5% agar) supplemented with various carbon sources at a concentration of 1% w/v (Table 1). The carbon sources tested were sucrose, glucose, glycerol, galactose, lactose, maltose, mannose, mannitol and D-cellobiose. Two plates were used for each treatment. CFU were counted following incubation at 28°C for 48 h. The plating efficiency was determined for each carbon source relative to the growth of the strains on the basal medium containing sucrose (YTSA) according to the following formula:

$$\text{Efficiency } \% = [(\text{CFU on basal medium containing candidate carbon source})/(\text{CFU on YTSA})] \times 100$$

Evaluation of antibiotics

Two *Xcm* isolates (006/wks/05 and KY44) along with two other Gram-negative bacterial strains (*E. coli* and *A. tumefaciens*) were tested for their susceptibility to 29 antibiotics using the plating method (Table 2). Antibiotic stock solutions were prepared in water, except for chloramphenicol, which was prepared in ethanol. All antibiotic solutions except chloramphenicol were filter-sterilized using 0.45 μm filters (Millex, Milipore USA) and added to lukewarm medium (approx 42–45°C). YTSA medium supplemented with various antibiotics at appropriate concentrations were poured in 90 mm Petri dishes. Concentrations of antibiotics ranged from 6 mgl^{-1} to 200 mgl^{-1} (Table 2). The serial dilutions of

Table 1 Growth of *Xanthomonas campestris* pv. *musacearum* on basal YTA medium supplemented with various carbon sources

Carbon source	Mean (±SE) No. of colonies at 10^{-6} dilution	Mean (±SE) colony size at 10^{-6} dilution (mm)	Mean (±SE) plating efficiency (%)
None	61.3 ± 1.5	1.4 ± 0.3	55.8 ± 2.3
Sucrose	89.7 ± 5.5	2.2 ± 0.1	100.0 ± 0.0
Maltose	66.7 ± 3.7	1.5 ± 0.4	69.3 ± 1.0
Mannitol	60.7 ± 13.6	1.8 ± 0.1	59.1 ± 12.9
Glucose	77.3 ± 7.4	1.9 ± 0.2	84.2 ± 3.8
Galactose	72.0 ± 7.5	1.8 ± 0.2	73.9 ± 1.8
Glycerol	65.3 ± 5.6	1.6 ± 0.4	66.5 ± 4.3
Lactose	58.7 ± 9.6	1.8 ± 0.1	55.9 ± 9.6
D-Cellobiose	58.0 ± 5.5	1.5 ± 0.1	55.6 ± 3.3
Mannose	64.6 ± 3.8	1.7 ± 0.2	67.6 ± 6.9

Basal YTA medium is composed of 1% yeast extract, 1% tryptone, and 1.5% agar

YTSA medium is YTA containing 1% sucrose

Efficiency % = [(CFU on basal medium containing candidate carbon source)/(CFU on YTSA)] × 100

Table 2 Susceptibility of two isolates of *Xanthomonas campestris* pv. *musacearum*, *Agrobacterium tumefaciens* and *Escherichia coli* to various antibiotics

Antibiotic	Supplier/ Manufacturer	Concentration (mg l^{-1})	*E. coli*	*A. tumefaciens*	*Xcm* 006	*Xcm* KY44
Amikacin	Ciron	50	+	–	–	–
Amoxicillin	Sigma	50	–	+	+	–
Ampicillin	Sigma	50	–	+	+	–
Azithromycin	Cipla	50	+	–	–	–
Carbenicillin	Sigma	10–100	–	–	++	++
Cefazolin	Sigma	50	–	–	+++	+++
Cefotaxime	Duchefa	100	–	–	–	–
Cefuroxime	Sigma	50	–	–	–	–
Cephalexin	Sigma	10–200	–	–	+++	+++
Cephalosporin C	Sigma	50	–	–	–	–
Chloramphenicol	Sigma	50	–	–	–	–
Cycloheximide	BDH	150–200	+++	+++	+++	+++
Erythromycin	Fluka	50	+++	–	–	–
Gentamycin	Sigma	50	++	–	–	–
Kanamycin	Sigma	50	–	–	–	–
Ketoconazole	Cipla	50	+++	+++	+++	+++
Nalidixic acid	Sigma	50–100	–	–	–	–
Neomycin	Sigma	50	–	+++	+++	+++
Norfloxacin	Cipla	50	–	+	–	–
Pefloxacin	Wockhardt	50	–	–	–	–
Pentachloronitrobenzene	Sigma	100	+++	+++	+++	+++
Phenoxy methyl penicillin	Sigma	50	++	+	++	++
Polymixin B	Sigma	50	–	–	–	–
Oflaxacin	Cipla	50	–	–	–	–
Sulphamethoxazole + Trimethoprim (Septran)	Nicholas Piramal	100	–	–	–	–
Streptomycin	Sigma	50	–	–	–	–
Tetracycline	Sigma	50	–	–	–	–
Clavulanic acid (Timentin)	Sigma	100	–	–	–	–
Tobramycin	Sigma	6–25	+++	–	–	–
No antibiotic	–	–	+++	+++	+++	+++

+++, Resistant to antibiotic tested (60–100 colonies per plate); ++, partial resistant (30–60 colonies per plate); +, weak resistant (1–30 colonies per plate); –, susceptible (no colonies)

overnight-grown culture were plated on medium supplemented with various antibiotics. Two plates were used for each treatment. CFU were counted after an incubation period of 48 h at 28°C. All experiments were repeated three times.

Evaluation of semi-selective medium

The YTSA-CC medium (YTSA containing 50 mg l^{-1} cephalexin and 150 mg l^{-1} cycloheximide) was evaluated by growing two isolates of *Xcm* (006/wks/05 and KY44) and comparing the efficiency with YTSA basal medium. The serial dilutions (100-fold dilution) of overnight-grown culture were plated on YTSA-CC and YTSA medium. Two plates were used for each dilution. The growth of the two *Xcm* isolates was compared in terms of time of appearance and number of colonies recovered on both the media. Efficiency of the semi-selective medium was quantified according to the following formula:

$$\text{Efficiency } \% = [(\text{colonies on YTSA-CC})/(\text{colonies on YTSA})] \times 100$$

Sixteen isolates of *Xcm* and five strains of other *Xanthomonas* spp. were grown on YTSA-CC and checked for the recovery of colonies after an incubation period of 48 h at 28°C. All the experiments were repeated three times.

Isolation of *X. campestris* pv. *musacearum* from infected banana plants

Different sample preparation methods were evaluated to isolate *Xcm* from leaf, fruit, peduncle, root, flower and pseudostem samples of infected banana plants. Five samples of each infected plant part were collected from five locations. Two methods were used to extract *Xcm* depending upon the type of plant part used. In the first method, the ooze was collected from the infected plant parts. The pseudostem of plants was sliced diagonally with sharp knife in the field and 100 µl of ooze that emanated from the cut surface was collected and suspended in 400 µl of sterile water. The fruits from the infected plants were brought to the laboratory, washed with sterile water and 70% ethanol. The fruit was cut with a sharp razor blade and 100 µl of ooze that came out of the cut surface was collected and suspended in 400 µl of sterile water. The male bud was brought to the laboratory, washed with sterile water, the flower bracts lifted, the male flower underneath the bract detached, and 100 µl ooze collected from the point of detachment (also called flower cushion) and suspended in 400 µl of sterile water. The ooze samples were serially diluted and 100 µl was spread on YTSA-CC medium plates.

In a second method, leaves, peduncles, roots and fruits were collected from the symptomatic plants, brought to the lab, and washed with sterile water. Small pieces of tissue were obtained by cutting the midrib of leaves or slicing peduncles, roots and fruits. The pieces were macerated in 500 µl of YTS medium in Eppendorf tubes (1.5 ml) with the help of small rod. The macerate was shaken at 100 rpm for 4 h at room temperature. The samples were serially diluted and 100 µl of the dilutions spread on YTSA-CC medium plates. Two plates were used for each treatment. Each experiment was repeated three times. The yellow and circular colonies resembling *Xcm* were identified after an incubation period of 48 h at 28°C.

Isolation of *X. campestris* pv. *musacearum* from soil

Soil samples were collected from five fields in which infected banana plants were either present or had died and were destroyed. Two samples of surface soil around infected plants were collected from each field. One gram of soil was suspended in 5 ml of sterile water and shaken at 100 rpm for 4 h at room temperature. The suspension was serially diluted and 100 µl was spread on YTS-CC medium plates. Two plates were used for each treatment. Yellow and circular colonies resembling *Xcm* were identified after an incubation period of 48 h at 28°C.

Identification of *X. campestris* pv. *musacearum*

The putative yellow colonies of *Xcm* isolated on YTSA-CC medium were transferred on YTSA medium for identification based on colony characteristics. The identity of the pathogen isolated from various experiments was confirmed by pathogenicity tests using in vitro plantlets of banana cv. Kayinja (Pisang awak) possessing 3–4 leaves and functional roots. The *Xcm* culture was grown by inoculating the single yellow colony from the plate in YTS broth and incubating at 28°C with shaking at 150 rpm till the OD at 600 nm reached 1.0. Ten millilitres of the broth culture was centrifuged at 5,000 rpm for 5 min, and the pellet suspended in 10 ml sterile double distilled water served as inoculum. One hundred microlitres of freshly prepared inoculum was injected with a syringe using 24 gauge needles into the pseudostem of each in vitro plantlet. The plants were incubated in normal laboratory conditions at room temperature and observed for symptoms.

Storage of *X. campestris* pv. *musacearum*

The purified bacterial cultures of *Xcm* were preserved on YTSA plates and slants at 4°C for short-term storage. For long-term storage the bacterial isolates were preserved as glycerol

stocks (50% in YTS). The glycerol stocks were preserved at –80°C and checked for pathogenicity every month.

Statistical analysis

The means and standard errors were calculated and analysed using SAS/STAT Software V8.

Results

Bacterial media

Colonies of *Xcm* appeared quickest after 48 h on YPGA and YTSA media, whereas the appearance was delayed (6–8 days) on LBA and minimal medium. There was no significant difference in number of bacterial colonies or their size on YPGA and YTSA media. The colonies were yellow mucoid on both media but very slimy on YPGA in comparison to YTSA medium. Therefore, YTSA medium was selected as the optimal basal medium for the development of the semi-selective medium.

Carbon utilization

There was a significant effect of carbon source on the recovery rate of *Xcm* (Table 1). The *Xcm* colonies were recovered on medium containing all the carbon sources tested. Colonies appeared fastest after 48 h and grew largest on YTA medium containing sucrose as the carbon source. Maximum number of colonies was recovered on medium containing sucrose followed by glucose and galactose. Fewer colonies were recovered on mannose, maltose, mannitol and glycerol compared to sucrose medium. Plain YTA medium also yielded growth of *Xcm* at a plating efficiency of about 55%. There was no significant difference in size and number of colonies recovered on D-cellobiose and lactose in comparison to YTA medium. The colonies recovered on sucrose medium were yellow mucoid and easily recognizable. Therefore, sucrose was selected as the main source of carbon for the semi-selective medium.

Susceptibility to antibiotics

The susceptibility of *Xcm* and other bacterial strains to various antibiotics is listed in Table 2. Both the isolates of *Xcm* were not inhibited by cephalexin, ketoconazole, cycloheximide, cefazolin, pentachloronitrobenzene and neomycin, and partially inhibited by phenoxy methyl penicillin and carbenicillin. The other two Gram-negative bacterial strains (*E. coli* and *A. tumefaciens*) were also not inhibited by ketoconazole, neomycin, cycloheximide and phenoxy methyl penicillin. The recovery of *Xcm* on medium supplemented with cephalexin was similar to that of cefazolin. Due to cost considerations, cephalexin and cycloheximide were selected as components of the semi-selective medium. Cephalexin was further evaluated to determine the optimal concentration for recovery of *Xcm* while suppressing other contaminants. YTSA supplemented with cycloheximide (150 mg l^{-1}) was used as the basal medium to which various concentrations (10, 25, 50 and 100 mg l^{-1}) of cephalexin were added. The number and size of *Xcm* colonies were similar at all the concentration tested, whereas other bacterial strains were weakly suppressed at concentration below 50 mg l^{-1}. Therefore, 50 mg l^{-1} was selected as the optimal concentration for cephalexin.

Evaluation of YTSA-CC medium

The final composition of the semi-selective medium YTSA-CC was yeast extract (1%), tryptone (1%), sucrose (1%), agar (1.5%), cephalexin (50 mg l^{-1}) and cycloheximide (150 mg l^{-1}), pH 7.0. The antibiotics were filter-sterilized and added to the medium before pouring into the plates. The growth of pure cultures of two *Xcm* isolates on YTSA-CC was compared to that on YTSA medium. Mucoid, yellowish and circular colonies appeared after 48 h and were approximately of the same size on both the media (Table 3). The number and size of colonies recovered were similar for both the isolates of *Xcm*. The efficiency of YTSA-CC medium was about 98%. The yellow, mucoid and circular colonies of all 16 isolates of *Xcm* and five other strains of *Xanthomonas* spp. were recovered on YTSA-CC medium after 48 h.

Table 3 Comparison of YTSA[a] and YTSA-CC[b] semi-selective medium in recovering colonies of *Xanthomonas campestris* pv. *musacearum* plated at different dilutions

Dilution of *Xcm* culture	Mean (±SE) No. of colonies		Mean (±SE) efficiency of YTSA-CC[c]	Mean (±SE) size of colonies (mm)	
	YTSA[a]	YTSA-CC[b]		YTSA	YTSA-CC
10^{-2}	Uncountable	Uncountable	–	–	–
10^{-4}	863.0 ± 26.6	843.7 ± 26.5	99.9 ± 5.1	1.3 ± 0.2	1.4 ± 0.3
10^{-6}	88.7 ± 5.8	87.0 ± 1.1	98.8 ± 4.9	2.7 ± 0.3	2.4 ± 0.3
10^{-8}	6.5 ± 2.2	6.1 ± 2.0	95.4 ± 2.4	3.3 ± 0.3	3.2 ± 0.2

[a] YTSA contains 1% yeast extract, 1% tryptone, 1% sucrose and 1.5% agar

[b] YTSA-CC medium is YTSA containing also 50 mg l^{-1} cephalexin and 150 mg l^{-1} cycloheximide

[c] Efficiency % = [(colonies on YTSA-CC)/(colonies on YTSA)] × 100

The number and size of colonies recovered were similar for all the strains of *Xanthomonas*.

Isolation of *X. campestris* pv. *musacearum* from infected banana plants

The *Xcm* pathogen was isolated on YTSA-CC from banana leaf, fruit, peduncle, root, flower and pseudostem samples collected from various locations in Uganda. The pathogen was easily identified as yellowish mucoid and circular colonies from all the dilution levels on YTSA-CC, whereas mixed microbial populations of yellow mucoid colonies, creamish colonies and white fungal growth were observed on YTSA medium, except in fruit samples, where the number of saprophytes was low. But with the rotten fruit, many saprophytes were also observed on YTSA medium where as only typical yellow mucoid colonies were recovered on YTSA-CC medium. Colony size of the pathogen was about 2–2.3 mm on YTSA-CC medium. Maximum number of *Xcm* colonies was recovered from ooze collected from pseudostem samples followed by fruit and flower samples (Table 4). The *Xcm* colonies were recovered from ooze even at 10^{-6} dilution. The colonies recovered from macerate of leaf tissue, roots and peduncles were fewer in number as compared to ooze. It was observed that the number of colonies recovered from different plant parts varied with the advancement of disease. It was also noted that fresh ooze was better for pathogen isolation in comparison to stored samples.

Isolation of *X. campestris* pv. *musacearum* from soil

The pathogen was recovered from soil as yellowish mucoid colonies on YTSA-CC. Along with the yellow mucoid colonies, creamish colonies were also observed on YTSA-CC medium. Several types of saprophytes forming small reddish and creamish bacterial colonies and white fungal growth appeared on YTS medium, whereas only a few creamish colonies but no fungal growth appeared on YTSA-CC medium. By plating a highly diluted soil suspension (10^{-6} dilution) on YTSA-CC, creamish colonies of saprophytes were easily distinguishable from yellowish mucoid colonies of *Xcm*.

Identification and preservation of *X. campestris* pv. *musacearum*

The *Xcm* pathogen isolated from infected plants was purified, and identified by colony characteristics of mucoid, bright yellow, shiny, dome shaped and circular (Fig. 1). The pathogen identification was further confirmed by a pathogenicity test. The in vitro-grown banana plantlets artificially infected with *Xcm* culture showed symptoms of the disease within 10–11 days. The symptoms were yellowing and browning of leaves. After 4–5 weeks, the plants died. The bacterium was re-isolated from the infected plantlets confirming that symptoms were the result of infection with *Xcm*. The healthy control plants were

Table 4 Mean (±SE) number of *Xanthomonas campestris* pv. *musacearum* colonies isolated from diluted ooze or macerate of various plant parts on YTSA-CC semi-selective medium

Plant part	Dilution		
	10^{-2}	10^{-4}	10^{-6}
Pseudostem ooze	Uncountable	411.3 ± 21.0	38.3 ± 3.4
Leaf macerate	7.0 ± 1.7	0	0
Peduncle macerate	49.3 ± 10.9	2.7 ± 0.9	0
Fruit ooze	631.0 ± 43.7	56.3 ± 9.8	6.00 ± 2.1
Flower cushion ooze	209.7 ± 14.8	34.0 ± 5.5	3.00 ± 1.1
Root macerate	7.7 ± 1.2	0	0

YTSA-CC semi-selective medium contains 1% yeast extract, 1% tryptone, 1% sucrose, 50 mg l^{-1} cephalexin and 150 mg l^{-1} cycloheximide and 1.5% agar

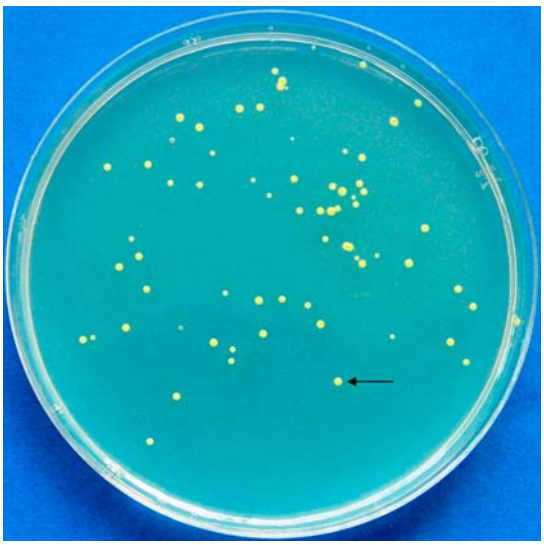

Fig. 1 Colonies of *Xanthomonas campestris* pv. *musacearum* on YTSA-CC medium. Arrow indicates mucoid, yellow, dome-shaped and circular colony of *Xanthomonas*

disease and pathogen-free. The *Xcm* cultures stored in glycerol at –80°C for a year retained infectivity in pathogenicity tests.

Discussion

The YTSA-CC medium is the first semi-selective medium developed for the isolation of *Xcm* from infected banana plants. This medium inhibited growth of the most saprophytes and allowed contamination-free isolation of the pathogen from various plant parts such as fruits, peduncles, flowers, leaves, roots and pseudostems. The pathogen could be isolated on YTSA medium from ooze of infected fruit samples during the early stages of infection when saprophytic contamination was very low. However, *Xcm* could not be efficiently isolated on YTSA from rotten fruits at advanced stages of disease development. The pathogen was also isolated from soil using YTSA-CC medium at higher dilutions. A few saprophytes from soil sample also grew on this semi-selective medium but could be easily distinguished from *Xcm* based on colour and morphology of colonies.

The YTSA was selected as the basal medium based on the recovery and plating efficiency of *Xcm* isolates. Previously, it was reported that YPGA basal medium with glucose as the carbon source had excellent plating efficiencies for many xanthomonads (Roumagnac, Gagnevin, & Pruvost, 2000). We have noted that there was no significant difference in the number of bacterial colonies on YPGA containing yeast extract, peptone and glucose, and YTSA containing yeast extract, tryptone and sucrose, but the colonies were very slimy on YPGA in comparison to YTSA medium. Most of the xanthomonads are known to produce extracellular polysaccharides on media containing glucose (Schaad & Stall, 1988).

We have tested various carbon sources supplemented with YTA basal medium for recovery of *Xcm* and selected sucrose as the main source of carbon for the semi-selective medium as the maximum number of colonies were recovered and also colonies were yellow mucoid and easy to

recognize. The plating efficiency of *Xcm* on YTSA-CC was almost similar to that on YTSA medium. There was no significant difference in size of colonies recovered on YTSA-CC medium in comparison to YTSA medium. YTSA-CC medium allowed the recovery of *Xcm* from bacterial suspension, plant parts and soil samples. The recovery rate was almost similar on YTSA-CC medium in comparison to non-selective YTSA medium. Like many other common bacterial media, *Xcm* produces a polysaccharide slime and yellow pigment on YTSA-CC medium resulting in the production of distinctive yellow, translucent, shiny and dome-shaped colonies, which are easily distinguished from saprophytes. It has been reported that the colonies of *Xanthomonas* spp. appeared to be yellowish on some media like YPGA, NGA, XTS and MSP due to xanthomonadin production (Mohan & Schaad, 1987; Roumagnac et al., 2000; Schaad & Forster, 1985; Wydra et al., 2004) compared to whitish or creamish colonies on other media like CCM, MXP, BSCAA (Claflin, Vidaver, & Sasser, 1987; Randhawa & Schaad, 1984; Wydra et al., 2004).

Among the antibiotics, cephalexin is a narrow-spectrum cephalosporin (beta-lactam antibiotic), which inhibits some Gram-negative bacteria (Bryan & Godfrey, 1991). Several xanthomonads are resistant to narrow-spectrum cephalosporins (Weng, Chen, Lee, Lin, & Tseng, 1999). We have observed that cephalexin suppressed growth of most of the saprophytes with no effect on *Xcm* isolates. The colonies were recovered with all the 16 isolates of *Xcm* tested on YTSA-CC medium. Similarly, colonies of several other *Xanthomonas* spp. like *X. campestris* pv. *campestris*, *X. vasicola* pv. *vasculorum*, *X. translucens*, *X. gardneri* and *X. vesicatoria* were also recovered on this medium. This medium can also be used for the isolation of the *Xanthomanas* spp. pathogens from other crops. In *Xanthomonas* spp., the resistance for cephalexin is associated with the presence of a gene coding for a beta-lactamase (Weng et al., 1999). Also, the commercial synthesis of cephalexin is currently done using bacteria belonging to the genus *Xanthomonas* (Rhee, Lee, Rhee, Ryu, & Hospodka, 1980). Cycloheximide is fungicidal and inhibited all the fungal saprophytes in our samples.

The YTSA-CC, simple semi-selective, medium proved to be useful for isolating the *Xcm* from various parts of infected plants from different locations and from soil. The *Xcm* isolated from infected banana plants on YTSA-CC medium can be tentatively identified based on the colony characteristics being yellow, mucoid, dome shaped and circular. This semi-selective medium was used to recover several *Xcm* isolates from different banana-growing regions of Uganda. The newly developed YTSA-CC semi-selective medium is useful for isolation of *Xcm* from banana plants and soil, and can be further used for studies on ecology and epidemiology of BXW.

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References

Bryan, L. E., & Godfrey, A. J. (1991). β-lactam antibiotics: mode of action and bacterial resistance. In V. Lorian (Ed.), *Antibiotics in laboratory medicine* (pp. 599–664). Baltimore, USA: Williams & Wilkins.

Claflin, L. E., Vidaver, A. K., & Sasser, M. (1987). MXP, a semiselective medium for *Xanthomonas campestris* pv. *phaseoli*. *Phytopathology, 77*, 730–734.

Davis, M. J., Rott P., Baudin, P., & Dean, J. L. (1994). Evaluation of selective media and immunoassays for detection of *Xanthomonas albilineans*, causal agent of sugarcane leaf scald disease. *Plant Disease, 78*, 78–82.

Di, M., Ye, H., Schaad, N. W., & Roth, D. A. (1991). Selective recovery of *Xanthomonas* spp. from rice seed. *Phytopathology, 81*, 1358–1363.

Eden-Green, S. (2004). How can the advance of banana *Xanthomonas* wilt be halted? *Infomusa, 13*, 38–41.

Fessehaie, A., Wydra, K., & Rudolph, K. (1999). Development of a new semiselective medium for isolating *Xanthomonas campestris* pv. *manihotis* from plant material and soil. *Phytopathology, 89*, 591–597.

Mabagala, R. B., & Saettler, A. W. (1992). An improved semiselective medium for recovery of *Xanthomonas campestris* pv. *phaseoli*. *Plant Disease, 76*, 443–446.

Mohan, S. K., & Schaad, N. W. (1987). An improved agar plating assay for detecting *Pseudomonas syringae* pv. *syringae* and *P. syringae* pv. *phaseolicola* in contaminated bean seed. *Phytopathology, 77*, 1390–1395.

Ndungo, V., Eden-Green, S., Blomme, G., Crozier, J., & Smith, J. (2005). Presence of banana xanthomonas wilt (*Xanthomonas campestris* pv. *musacearum*) in

the Democratic Republic of Congo (DRC). New Disease Reports, 11, http://www.bspp.org.uk/ndr/july2005/2005-29.asp.

Norman, D. J., & Alvarez, A. M. (1994). Rapid detection of *Xanthomonas campestris* pv. *dieffenbachiae* in anthurium plants with a miniplate enrichment/ELISA system. *Plant Disease, 78*, 954–958.

Randhawa, P., & Schaad, N. W. (1984). Selective isolation of *Xanthomonas campestris* pv. *campestris* from crucifer seeds. *Phytopathology, 74*, 268–272.

Rhee, D. K., Lee, S. B., Rhee, J. S., Ryu, D. D. Y., & Hospodka, J. (1980). Enzymatic biosynthesis of cephalexin. *Biotechnology and Bioengineering, 22*, 1237–1247.

Roumagnac, P., Gagnevin, L., & Pruvost, O. (2000). Detection of *Xanthomonas* sp., the causal agent of onion bacterial blight, in onion seeds using a newly developed semi-selective isolation medium. *European Journal of Plant Pathology, 106*, 867–877.

Schaad, N. W., & Forster, R. L. (1985). A semi-selective agar medium for isolating *Xanthomonas campestris* pv. *translucens* from wheat seeds. *Phytopathology, 75*, 260–263.

Schaad, N. W., & Stall, R. E. (1988). *Xanthomonas*. In N. W. Schaad (Ed.), *Laboratory guide for identification of plant pathogenic bacteria* (2nd ed., pp. 81–94). St Paul, MN, USA: APS.

Sijam, K., Chang, C. J., & Gitaitis, R. D. (1992). A medium for differentiating tomato and pepper strains of *Xanthomonas campestris* pv. *vesicatoria*. *Canadian Journal of Plant Pathology, 14*, 182–184.

Tushemereirwe, W., Kangire, A., Ssekiwoko, F., Offord, L. C., Crozier, J., Boa, E., Rutherford, M., & Smith, J. J. (2004). First report of *Xanthomonas campestris* pv. *musacearum* on banana in Uganda. *Plant Pathology, 53*, 802.

Weng, S. F., Chen, C. Y., Lee, Y. S., Lin, J. W., & Tseng, Y. H. (1999). Identification of a novel beta-lactamase produced by *Xanthomonas campestris*, a phytopathogenic bacterium. *Antimicrobial Agents and Chemotherapy, 43*, 1792–1797.

Wydra, K., Khatri-Chetri, G., Mavridis, A., Sikirou, R., & Rudolph, K. (2004). A diagnostic medium for the semi-selective isolation and enumeration of *Xanthomonas axonopodis* pv. *vignicola*. *European Journal of Plant Pathology, 110*, 991–1001.

Yirgou, D., & Bradbury, J. F. (1974). A note on wilt of banana caused by the enset wilt organism *Xanthomonas musacearum*. *East African Agricultural and Forestry Journal, 40*, 111–114.