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Research Article

Effect of Growth Media on the Pathogenicity of Enset Bacterial Wilt Pathogen *(Xanthomonas campestris pv. musacearum)*

Misgana Mitiku*, Alemar Said

Department of Plant Pathology, South Agricultural Research Institute, Areka Agricultural Research Center P.O.BOX 79, Areka, Ethiopia

ABSTRACT

The nutritional requirements of plant pathogenic bacteria vary with the bacterial species and even the bacterial isolates under consideration. Some media are considered general all-purpose media and support growth of a large variety of organisms. From previous work experience, disease/EBW (Enset Bacterial Wilt) development intensity of pure isolate is less compared to its fresh isolate. Chemical/nutrient composition of different media is relatively different. Some chemicals in the medium may affect the level of pathogenicity of *Xcm*. during its growth stage. Identification of appropriate media for *Xcm* pure culture isolation is important. Therefore the current research work done with the objective of identifying bacterial growth media that has effect on pathogenicity of *Xcm* (*X. campestris*). To do this the pathogenicity of the isolates has been evaluated by growing it on four different growth media (NA, NYA, YDC and one bulla based medium) and inoculated on to susceptible enset clone (Arkya) on pot experiment using Complete Randomized Design (CRD). Analysis of variance showed that among the tested growth media, there is no significant difference in pathogenicity of the bacterial wilt pathogen. So, we recommended that use of these growth media alternatively is possible at recommended rate of ingredients for pathogenicity and genotype screening experiment. But in terms of cost Nutrient Agar media (NA) is much cheaper than the rest of growth media so we recommended use it for isolation and identification work.

Keywords: Pathogenecity; Growth media; Enset bacterial wilt; Nutrient Agar

INTRODUCTION

Enset bacterial wilt caused by the bacterium *X. campestris pv. musacearum* (*Xcm*), XW symptoms were first observed on enset in Ethiopia in the 1930s [1]. The disease was, however, first identified in Ethiopia as *Xanthomonas* wilt in 1968 on enset and subsequently on banana in 1974 [2,3].

EXW invades the vascular system of enset, causing permanent wilting and eventual death of the plant. Primarily, EXW is transmitted *via* insects, contaminated tools and infected planting materials [4]. Symptomless enset and/or banana bunch and leaves used to wrap bunches for transport to markets are another important source of *Xcm* inoculum that may be responsible for its long distance spread [5].

Microbiological media can be prepared from known exact specifications of ingredients. They are made from highly purified and defined chemical compounds. Both the beef extract and peptone in nutrient broth are exact in composition. They are called synthetic media. Media containing imprecise composition of ingredients are called non-synthetic media. The knowledge on growth requirements of plant pathogenic bacteria is more essential for their mass production and pathogenicity.

The nutritional requirements of plant pathogenic bacteria vary with the bacterial species and even the bacterial isolates under consideration. The number of available media to grow bacteria is considerable. Some media are considered general all-purpose media and support growth of a large variety of organisms. From previous work experience, disease/EBW development intensity of pure isolate is less; compared to its fresh isolate. Chemical/nutrient composition of different media is relatively different. Some chemicals in the medium may affect the level of pathogenicity of *Xcm*. during its growth stage. Identification of appropriate media for *Xcm* pure culture isolation is important.

Objective

To identify bacterial growth media that has effect on pathogenicity of *Xcm*.

Correspondence to: Misgana Mitiku, Department of Plant Pathology, South Agricultural Research Institute, Areka Agricultural Research Center P.O.BOX 79, Areka, Ethiopia, Email: misganamitiku441@gmail.com

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MATERIALS AND METHODS

Preparation of the tested growth media

YDCA growth media: 1000 ml of YCDA (Yeast De glucose Calcium Carbonate Agar) media were prepared. For preparation of 100 ml of YCDA media 20 g of dextrose, 10 gram of yeast extract, 20 g of calcium carbonate, 10 ml of glycerol, 15 gram of agar-agar instead of bactero-agar and 990 ml of distilled water were used. For preparation of the media the above listed ingredients were mixed together gently and autoclaved at 121°C for 15 min then upon cooling the prepared media was poured on to Petri plate inside the hood ready for culturing of the pathogen.

Nutrient agar growth media: 28 g of nutrient agar powder in 1 litre of distilled water was suspended. Then the mixture allowed to heated and stirred to fully dissolve all components. Then the dissolved mixture allowed to autoclaved at 121°C for 15 minutes. Once the nutrient agar has been autoclaved, allowed it to cool but not solidify. Finally the nutrient agar was poured into each plate and leave plates on the sterile surface until the agar has solidified and ready for culturing of the pathogen.

Nutrient yeast agar growth media: 28 g of nutrient agar powder and 10 gram of yeast extract in 1 litre of distilled water was suspended. Then the mixture allowed to heated and stirred to fully dissolve all components. Then the dissolved mixture allowed to autoclaved at 121°C for 15 minutes.

Once the nutrient agar has been autoclaved, allowed it to cool but not solidify. Finally the nutrient agar was poured into each plate and leave plates on the sterile surface until the agar has solidified and ready for culturing of the pathogen.

Bulla based growth media: 28 g of nutrient agar powder and 30 g of bulla in 1 litre of distilled water was suspended. Then the mixture allowed to heated and stirred to fully dissolve all components. Then the dissolved mixture allowed to autoclaved at 121°C for 15 minutes. Once the nutrient agar has been autoclaved, allowed it to cool but not solidify. Finally the nutrient agar was poured into each plate and leave plates on the sterile surface until the agar has solidified and ready for culturing of the pathogen.

Test plant and pathogen inoculums preparation

Suckers of 7 month old of the clone Arkya were planted in pots of 3 liter capacity with sterile soil mix of 1:1 loam soil and sand respectively and maintained until they reach up to one year. These plants were planted in randomized complete design with 3 replications. Each plot consisted of 1 row of 3 plants. There was a spacing of 0.5 m between rows and 1.5 m between replications. There was one control plant in each replication.

The inoculum was obtained from Hawassa research site by collecting bacterial ooze on plants that were infected by EBW. Bacterial suspension was prepared from these ooze using sterile distilled water and cultured on these tested media (NA, NYA, YDC and one bulla based medium). Then after 48 hours of incubation under 28°C set incubator, grown bacterial colonies were harvested and bacterial suspension for inoculation at a cell concentration 108 cfu/ml (adjusted to 0.3 OD at 460 nm using spectrophotometer) was prepared for inoculation (Figure 1).

Figure 1: During planting and inoculum preparation.

Inoculation of enset clones/Arkya/and disease assessment

The plants were inoculated at the base of the newly expanding central leaf petiole using a 10 ml capacity sterile hypodermic syringe with metal needle. Numbers of live and, hence, injected leaves per plant were recorded at the time of inoculation. The control plants were inoculated with the same volume of sterile distilled water. Disease assessment was done at 15, 21, 30 and 45 days after inoculation. The number of infected plants per clone at each disease assessment period was recorded.

Disease severity assessment was made using 0-5 disease scoring scale; where 0=no visible disease symptom, 1=yellow necrotic and 1 leaf wilted, 2=2-3 leaves wilted, 3=4 leaves wilted, 4=all leaves wilted, and 5=the whole plant dead.

Disease severity scales were transformed into Percentage Severity Index (PSI) for analysis following the formula suggested by Wheeler as below.

$$PSI = \frac{Sumofnumericalratings}{No.ofplantsscored \times \max imumscorescale} \times 100$$

Enset bacterial wilt incidence was made, as mean percentage of enset plants showing typical wilt disease symptoms (yellowing and necrosis of leaves, complete wilting of leaves and the whole plants) of inoculated enset clones using the following formula

$$PSI = \frac{Sumofnumericalratings}{No.ofplantsscored \times \max imumscorescale} \times 100$$

The area under disease progress curve was computed from disease severity data recorded at different DAI for each plot following the formula advised by Campbell and Madden.

$$AUDPC = \sum_{i=1}^{n-1} 0.5\left(X_i + X_{i+1}\right)\left(t_{i-1} - t_i\right)$$

where n is the total number of disease assessments, ti is the time of the ith assessment in days from the first assessment date and xi is the disease severity of *Xcm* at the ith assessment. AUDPC value was expressed in % days because severity (x) is expressed in percent and time (t) in days (Figure 2).

Figure 2: During inoculation and disease assessment.

Data analysis

The collected data were analyzed using SAS software version 9.1 for windows.

RESULTS AND DISCUSSION

During the experiment all the tested growth media showed initial yellowing symptoms on the inoculated leaves after 30 days after artificial inoculation. Analysis of variance showed that among the tested growth media, there is no significant difference in pathogenicity of the bacterial wilt pathogen. But in terms of cost Nutrient Agar media (NA) is much cheaper than the rest of growth media (Tables 1-3) [6-8].

Table 1: Significance of mean square value for disease severity and total area of disease progress curve for four growth media.

Source of variation	DF	DS	PSIf (%)	TAUDPC (%-days)
Treat	3	0.075	29.659	45708.97
Error	8	0.092	37.051	84478.44
CV (%)		7.84	7.82	12.55

Table 2: Mean values of disease severity, final percent severity index and total area under disease progress curve for the tested media.

Media type	DS (1-5 scale)	PSIf (%)	TAUDPC (%-days)
NA	3.88	77.77	2133.2
NYA	4.00	80.00	2350.0
YDCA	3.66	73.33	2379.9
Bulla based	4.00	80.00	2400.0
CV	7.84	7.82	12.55
LSD	0.83	16.67	796.29

Table 3: Cost of each growth media types.

SN.	Types of growth media	Cost (ETB)
1	Nutrient agar (NA)	3600
2	Nutrient yeast agar (NYA)	7200
3	Yeast De glucose Calcium Carbonate Agar (YDCA)	15,050
4	Bulla based	3900

CONCLUSION AND RECOMMENDATION

The number of available media to grow bacteria is considerable. Some media are considered general all-purpose media and support growth of a large variety of organisms. The nutritional requirements of plant pathogenic bacteria vary with the bacterial species and even the bacterial isolates under consideration. Therefore, the current study was conducted to evaluating the effect of different bacterial growth media on pathogenicity of *Xcm*.

The result of the study depicted that, all tested growth media didn't have effect on the pathogenicity of enset bacterial wilt pathogen so; we recommended that use of these growth media alternatively is possible at recommended rate of ingredients for pathogenicity and genotype screening experiment. But in terms of cost Nutrient Agar Media (NA) is much cheaper than the rest of growth media so we recommended use it for isolation and identification work.

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