Available online at http://ajol.info/index.php/ijbcs

Int. J. Biol. Chem. Sci. 6(4): 1566-1573, August 2012

ISSN 1991-8631

Original Paper

http://indexmedicus.afro.who.int

Evaluation of culture media for growth and sporulation of *Phytophthora colocasiae* Racib., causal agent of taro leaf blight

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ABSTRACT

Phytophthora colocasiae was recently the most important pathogen of taro (*Colocasia esculenta*) in Cameroon and no information is yet available on its culture on common media. Six artificial media, V6 juice agar, V8 juice agar, V8m juice agar, potato dextrose agar, onion agar and taro leaf agar were assessed *in vitro* to determine the growth characteristics of the pathogen. After isolation of the pathogen on V6 juice agar, mycelial disks from pure culture were placed on each culture media and incubated at 24 ^{o}C for up to 21 days. After 7 days, colony diameter was highest on V6 juice agar (64 mm), followed by V8-m juice agar (57 mm), onion agar (55 mm), V8 juice agar (54 mm) and potato dextrose agar (54 mm). Sporulation was highest on V6 juice agar (18 x10^{6} sporangia/ml) and V8 juice agar (17 x 10^{6}sporangia/ml) after 21 days of incubation. *P. colocasiae* grows for 3-7 days in all the media tested and produces sporangia in some of the media after two to three weeks under laboratory conditions. V6 juice agar and V8 juice agar were the best media for isolation, growth and sporulation of the pathogen and can be recommended for the culture of the pathogen.

Keywords: *Phytophthora colocasiae*, culture media, *Colocasia esculenta*.

INTRODUCTION

Taro (*Colocasia esculenta* (L.) Schott) is a major staple food and remains an important crop to many cultural and agricultural traditions worldwide (Ooka and Brennan, 2000). It is an important staple food and source of income in the southern regions of Cameroon and some neighbouring countries (ISPP, 2010). Cultivated for its edible tubers and fresh leaves the crop is second to maize as the most widely eaten foodstuff in the production areas (Kughong, 2010). Mineral content and medicinal values of the cropare higher compared to other tuber crops (Onwuene, 1994; Misra and Sriram, 2002, Misra et al., 2007). Since 2010, taro was affected by leaf blight disease caused by *Phytophthora colocasiae* Racib in Cameroon. Epidemics of this disease occurred throughout the taro growing-areas during the rainy season causing up to 100% leaf and corm losses (Fontem and Mbong, 2011).The disease occurs on leaves as small, brown, water-soaked lesions that enlarge and coalesce into

DOI : http://dx.doi.org/10.4314/ijbcs.v6i4.16

large lesions with yellow exudate, ultimately leading to the defoliation and decay of the plants within few weeks (Omane, 2012).

Reports have revealed that *P. colocasiae* is relatively short-lived in infected leaf tissue, and the fungus seems to have a poor competitive saprobic ability (Narula and Meherotera, 1984). This contributes to the lack of success in isolating and growing the pathogen in artificial medium. This problem makes it difficult to have an accurate diagnostic and to conduct experiments which involve artificial inoculation using its reproductive structures. Sporangia and mycelia play important roles in inoculation and are essential in initiation and development of infection (Palomar et al., 1999). Several media have been used to isolate and culture *P. colocasiae* in other countries (Palomar et al., 1999; Lebot et al., 2003; Bernadovicova and Juhasova, 2005; Misra et al., 2008). Although, the pathogen has been identified in Cameroon (Ngoko, 2010; Fontem and Mbong, 2011), no information is available on its cultural characteristics. Furthermore, taro leaf blight being reported for the first time in Cameroon, comprehensive details of its culture media will help to design more effective diagnostic assays and control measures for the disease. This work was designed to assess cultural media for isolation and growth characteristics of the fungus.

MATERIALS AND METHODS

Isolation and purification of the pathogen

Field infected mature leaves showing typical symptoms of taro blight were collected from taro experimental farm of the Faculty of Agronomy and Agricultural Sciences, University of Dschang and carried to the Phytopathology Laboratory. Isolation of the fungus was done as described by Zhu et al. (2001). Leaf fragments showing young lesions were cut into approximately 1 cm^2 disinfected with 70% ethanol for 30 sec. They were rinsed twice with sterile distilled water, blotted dry on sterilized filter paper and plated on V-6 juice agar amended with 250 mg l^{-1} penicillin G, 250 mg l^{-1}ampicillin and 20 mg l^{-1} of nystatin (Djeugap, 2009). The plates were then incubated at room temperature for 2-3 days, then hyphal tips were transferred to freshly prepared V-6 juice Agar and this process was repeated several times to obtain pure culture.

The fungus was identified under light microscope based on cultural and morphological characteristics, followed by the identification guides and species descriptions by Ho et al. (1995) and Erwin and Ribeiro (1996). To confirm the identity of the pathogen, Koch's postulate was conducted; detached leaves from two to three-month old taro plants were inoculated with sporangial suspension from pure culture. These leaves were incubated at 24 ^oC for 5 days. All incubated leaves developed blight symptoms similar in appearance to those observed on diseased taro in the field. Noninoculated control detached leaves were disease free.

Composition of different media

The six different media tested were V6 juice Agar, V8 juice Agar, V8-m juice Agar, Potato dextrose Agar, Onion agar and Taro leaf Agar (Table 1). Media composition was adapted from Palomar et al. (1999), Zhu et al. (2001) and Djeugap et al. (2009). The six vegetables of V6 were obtained from Macedoine (Compagnie Générale de Conserve, Locmine, Cedex France) and the eight vegetables of V8 (French bean, celery, carrot, potato, lettuce, tomato, water leaf and sugar beet) were bought from local market. The vegetables were grounded with one litter of distilled water to make a mother solution from which 200 ml were taken for the medium.

Two hundred grammes of red bulb onion and 150 g of taro leaves were boiled in one liter of distilled water for 15 minutes and 30 minutes respectively for onion agar and

taro leaf agar and then strained. Each of the resulting juice was adjusted to one liter with distilled water. For Taro leaf agar, V-6 juice agar and V8 juice agar, 3 g of $CaCO_3$ was added to each medium.

Each prepared medium was transferred to a flask, supplemented with 1ml of vegetable oil (to encourage sporulation) and plugged with cotton and sterilized in an autoclave at 121 °C for 15 min. Prior to pouring the agar media into sterilized petri dishes, ampicillin (250 mg/l), penicillin (250 mg/l) and nystatin (20 mg/l) were added to each medium.

Evaluation of the different media

Mycelial disks of 4mm diameter were cut with a sterilized cock borer from 14 days-old pure culture and placed at the center of 9 cm diameter dishes containing each freshly prepared medium. The dishes were incubated at room temperature (22-24 °C) for 7 days. Data on mycelial growth diameter and cultural characteristics were recorded daily. Six plates were used per medium and the experiment was repeated thrice.

Sporangial suspensions were prepared from 14 and 21 day-old culture by adding 10ml of distilled water and a drop of Tween 80 to each plate and scraping the surface lightly with the edge of microscope slide to dislodge sporangia. After filtration through a double layer of cheesecloth to remove mycelial fragments, a drop of suspension was placed on a haematocymeter and mounted on a light microscope and the number of sporangia was counted at magnification 40 X. This experiment was repeated three times.

Statistical analysis

Analysis of variance was performed using SPSS statistical package to evaluate the values of mycelial growth and sporangia production; means were separated using Duncan Multiple Range Test at P=0.05.

RESULTS

Phytophthora colocasiae showed variable characteristics from creeping whitish mycelia with slight zone of striations in V6 juice agar, V8 juice agar, V8-m juice agar and taro leaf media to cottony and moderate cottony whitish mycelia in onion agar and potato dextrose agar (Table 2). The fungus grown in taro leaf agar, V6 juice, V8 juice agar and V8-m juice agar showed similar cultural characteristics. The mycelia were visible 2-3 days after introduction to the media; the surface was filled with creeping whitish mycelia with slight growth zone or striations. On potato dextrose agar and onion agar, the fungus produced cottony whitish aerial mycelia. The fungus grew in all the media, but colony diameter was significantly larger on V6 juice agar after one week. A similar growth pattern was observed on V8 juice agar, Onion agar, V8-m juice agar and potato dextrose agar, while on taro leaf agar mycelial growth was very low (Figure 1)

The spores observed were ovoid, hyaline, ellipsoid or fusiform, semipapillate, caducous and measured 27 to 52 x 16 to 29 µm (Figure 2). Microscopic examination of the culture revealed sporangial production of *P. colocasiae* in Onion agar, V6 juice agar, V8 juice agar and V8m juice agar after 14 days of incubation while no sporangia was found in potato dextrose agar and taro leaf agar. Production of sporangia after 21 days of culture was significantly higher in V6 juice agar and V8 juice agar than in Onion agar and V8-m juice agar. Few sporangia were recorded on potato dextrose agar after the same period. No sporangium was found in taro leaf agar medium throughout the experiment. Furthermore sporangial counts revealed 18 $x10^6$, 17 $x10^6$, 7 x 10^6, 7 x 10^6 and 1 x 10^6 sporangial/ml in V6 juice agar, V8 juice agar, onion agar, V8-m juice agar and potato dextrose agar respectively (Table 3).

Table 1: Composition of the six different media.

Medium	Ingredient	Amount/l
V6 Agar	V6 juice	150 ml
	Taro leaves	4 g
	$CaCO_3$	3 g
	Agar	15 g
	Distilled Water	850 ml
V8 Agar	V8 juice	200 ml
	$CaCO_3$	3 g
	Agar	15 g
	Distilled Water	800 ml
V8-m Agar	V8 juice	200 ml
	Taro leaves	4 g
	$CaCO_3$	3 g
	Agar	15 g
	Distilled Water	800 ml
Potato dextrose agar	Potato	200 g
	Dextrose	20 g
	Agar	15 g
	Distilled Water	1000 ml
Taro leaf agar	Taro leaves	150 g
	$CaCO_3$	3 g
	Agar	20 g
	Distilled Water	1000 ml
Onion Agar	Red bulb onion	200 g
	Agar	20 g
	Distilled water	1000 ml

Adapted from Palomar et al. (1999), Zhu et al. (2001) and Djeugap et al. (2009).

Table 2: Growth characteristics of *P. colocasiae* on six culture media 7 days after incubation.

Media	Cultural characteristics	Colony diameter** (mm)
V6juice-Agar	Mycelia of the fungus visible 2-3 days after introduction to the medium; surfaced filled with creeping whitish mycelia with slight growth zone or striations	64 a*
V8juice-Agar	Mycelia of the fungus visible 2-3 days after introduction to the medium; surfaced filled with creeping whitish mycelia with slight growth zone or striations	54 b
Oignon-Agar	Mycelia of the fungus visible 2-3 days after introduction to the medium; produced cottony whitish areal mycelia	55 b
V8 modifiedjuice-agar	Mycelia of the fungus visible 2-3 days after introduction to the medium; surfaced	57 b

	filled with creeping whitish mycelia with slight growth zone or striations	
Potatodextrose agar	Mycelia of the fungus visible 3-4 days after introduction to the medium; produced moderate cottony whitish areal mycelia	54 b
Taro leaf-Agar	Very poor growth, Mycelia of the fungus visible 3-5 days after surface filled with creeping reddish mycelia with slight growth zone or striations	24 c

*Means with the same letter are not significantly different according to Duncan multiple range test (P=0.05)
**Average of three trials with 10 plates for each medium.

Table 3: *In vitro* sporulation (x10^6 sporangia/ml) of *P. colocasiae* on common agar media for 14 and 21 days after incubation at 24 °C.

Milieux de culture	Day 14	Day 21
V-6 juice agar	7 a*	18 a
V-8juice agar	5 ab	17 a
Onion-agar	3 b	7 ab
V-8 m juice agar	5 ab	7 ab
PDA	0 c	1 b
Taro leaf agar	0 c	0c

*Values followed by the same letters for each column are not significantly different according to Duncan multiple range test (P=0.05).

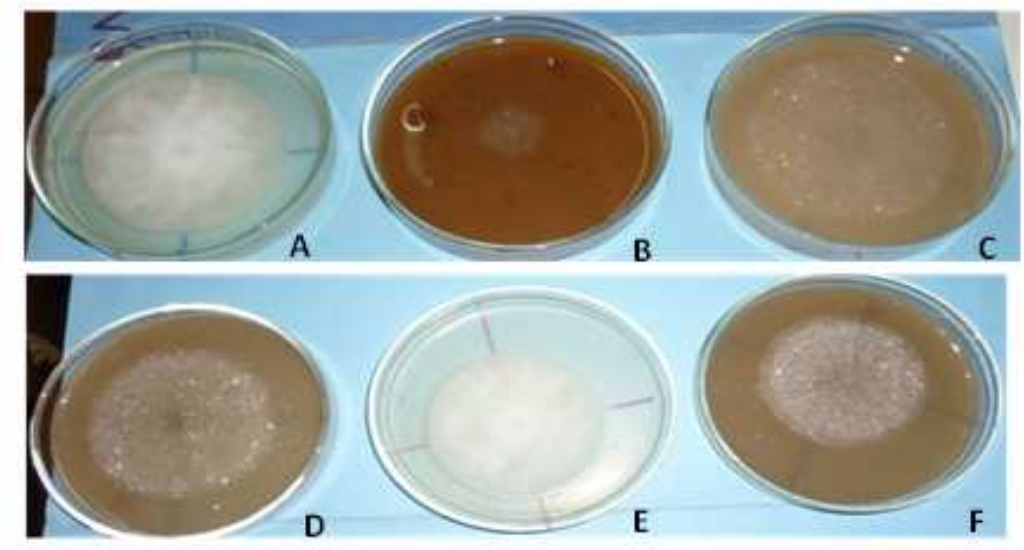

Figure 1: Colonies of *P. colocasiae*, grown on A) Onion Agar, B) Taro leaf Agar, C) V6 Juice Agar, D) V8 Juice Agar, E) Potato Dextrose Agar, F) V8-m Juice Agar.

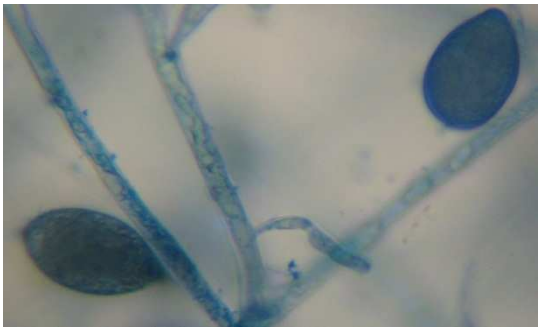

Figure 2: Sporangia and sporangiophores of *P. colocasiae* on V6 medium (x 400).

DISCUSSION

P. colocasiae showed variable characteristics in different media. According to Juhasova et al. (1987) and Juhasova (1992, 1999), the growth characteristics and distinguishing features of *Phytophthora* mycelium are very important for microscopic identification of *Phytophthora* isolate. Consequently, *P. colocasiae* was identified in various types of media. Generally slow growth in culture is characteristic for all *Phytophthora* and *P. colocasiae* in comparison with *P. infestans* show faster growth in V6 and V8 juice agar. These media have been found appropriate for the culture of *Phytophthora infestans* (Djeugap et al., 2009; Noumbo et al., 2002). Both V6 and V8 juice agar media were suitable for *P. colocasiae* cultivation in Cameroon. As reported by Papvizas et al. (1981), sporangium formation by a fungus is an important criterion for the selection of an appropriate medium for its isolation.

The poor development of the fungus on taro leaf agar medium may be due to its nutrient contents. According to Duncan (1988), nutrient content is very important in the expression of the fungus in culture media. The total absence of fructification in taro leaf agar throughout the experiment may be due to the high concentration of carbohydrates present in the medium or to the absence of some minerals which may be necessary or not for the development of the fungus. For instance, the production of sporangia by *Phytophthora cinnamoni* in axenic culture was reported to take place in the presence of certain ions and nutrient depletion (Trichilo and Aragaki, 1982). But it is not known whether the poor performance of *P. colocasiae* on taro leaf agar could be attributed to the high concentration of carbohydrates or to the lack of some minerals. However, the role played by nutrient in the growth of the fungus *in vivo* could be investigated.

Sporangia size and shape observed were within the ranges described by Palomar et al. (1999), Lebot et al. (2003), Bandyopadhyay (2011) and Oname et al. (2012). They were deciduous. In epidemiology, sporangia together with zoospores have been reported to be the main dispersal units and means of survival of *P. colocasiae* in corms and cormels (Erwin and Ribeiro, 1996; Misra and Chowdhury, 1997), as well as the principal means of dispersal of *Phytophthora* spp that ensure penetration of the host (Weste and Vithanager, 1979). Although they may not be well adapted for the survival of the pathogen, reports on sporangia of another Oomycete, *Peronospora tabicina*, causal agent of blue mould of tobacco, have been shown to travel several hundred kilometers to establish the epidemic of the disease (Aylor and Taylor, 1982). This might

explain the widespread of taro leaf blight in all the taro growing areas of Cameroon in June 2010 (Fontem and Mbong, 2011). But, further studies on the duration of *P. colocasiae* sporangia under atmospheric conditions will help to understand the sudden outbreak and the assessment of the risk of the long distance spread of the disease.

Conclusion

Base on the results, V6 juice agar and V8 juice agar were found to be appropriate for the growth and sporulation of *P. colocasiae*. This is the first report on the culture of taro leaf blight pathogen in Cameroon and will provide a basis for future research on pathogen characterization and management.

REFERENCES

Aylor DE, Taylor GS. 1982. Long range transport of Tobacco blue mould spores. *Agric. Met*, **27**: 217-232.

Bandyopadhyay R, Sharma K. 2011. First Report of Taro (*Colocasia esculenta*) leaf blight caused by *Phytophthora colocasiae* in Nigeria. *Disease Notes*, **95** (5): 618

Berndovicova S, Juhasova G. 2005. Cultural characteristics of *Phytophthora* fungi, causal agent of ink disease on chestnut trees (*Castanea sativa* Mill.) in Slovakia. *Folia Oecologica*, **32**(2): 51-58.

Djeugap FJ, Fomtem DA, Tapondjou AL. 2009. Evaluation des milieux de cultures pour la croissance de *Phytophthora infestans*. Cameroon Bioscience Proceedings, 15: 85-92.

Erwin DC, Ribeiro OK. 1996. *Phytophthora Disases Worldwide*. American Phytopathological Society, APS Press: St Paul, MN, USA

Fontem DA, Mbong G. 2011. A novel epidemic of taro (*Colocasiae esculenta*) blight caused by *Phytophthora colocasiae* hits Cameroon (Abstract). Third Life Science Days held at the University of Dschang, Cameroon. 26-28 Mai 2011.

Ho HH, Ann PJ, Chang HS. 1995. The Genus *Phytophthora* in Taiwan, Monograph SeriesNo 15. Taipei, Taiwan, Institute of Botany. Academia Sinica.

ISPP (International Newsletter in Plant Pathology) 2010. Taro leaf blight in Cameroon and a solution in the Pacific. *ISPP Newsletter*, *40* (9).

Kughong FJ. 2011. Cameroon's Changing Weather linked to Crop Disease. http://www.wemmagazine.com/hot-topics/2011/nov/cameroon's-changing-weather-linked-to-crop-disease.aspx, Accessed on 23 Nov. 2011 10:02:41 GMT.

Lebot V, Herail C, Gunua T, Pardales J, Prana M, Thongjiem M, Viet N. 2003. Isozyme and RAPD variation among *Phytophthora colocasiae* isolates from South-east Asia and the Pacific. *Plant Pathology*, **52**: 303-313.

Misra AK, Sharma K, Misra MS. 2008. Effect of Benyl Amano purine on the pathogen growth and disesase development of taro blight caused by *Phytophthora colocasiae*. *Journal of Plant Pathology*, **90**(2): 191-196.

Misra RS, Sriram S. 2002. Medicinal value and export potential of tropical tuber crops. In *Recent Progress in Medicinal Plants; Crop improvement, Production Technology and Commerce* (vol. 5), Govil JN, Pandey J, Shivkumar BG, Singh VK (eds). SCI Tech Pub: USA; 376-386.

Misra RS, Maheswari SK, Sriram S, Misra AK, Sahu AK. 2007. Effect of borax for the control of taro leaf blight of Taro (*Colocasia esculenta* (L) Schott. *Journal of Root Crops*, **33**(1): 49-52.

Narula KL, Meherotera RS. 1984. The saprophytic survival of *Phytophthora colocasiae* in soils. *Indian Phytopathol*., **37**: 256-261.

Ngoko Z. 2010. Taro Leaf Blight in Cameroon? http://agro.biodiver.se/2010/

07/taro-leaf-light-in-cameroon/, Assessed on 15 July 2011 08:44:48 GMT.

Noumbo GRT, Fontem DA, Nyumia JB, Iloba C. 2002. Suitability of culture media on the growth and sporulation of *Phytophthora infestans* (Mont.) de Bary. *Journal of Tropical Agriculture, Food, Environment and Extension*, **3**(1): 9-12.

Omane E, Oduro K A, Cornelius E W, Opoku I Y, Akrofi A Y, Sharma K, Lava Kumar P, Bandyopadhyay R. 2012. First report of late blight of Taro (*Colocasia esculenta*) caused by *Phytophthora colocasiae* in Ghana. *Dis. Notes*, **96**(2): 292.

Onwuene IC. 1994. Tropical Root and Tuber crops. Production perspectives and future prospects. FAO plant production Paper 126; FAO, Rome, 228p.

Ooka J, Brennan BM. 2000. Crop profile for taro in Hawaii College of Tropical Agriculture and Human resources, University of Hawaii- Manoa, 2p.

Palomar MK, Mangaoang YC, Palermo VG, Edurise GM. 1999. Evaluation of culture media for sporangial production of *Phytophthora colocasiae* L. and control of taro blight using fungal antagonists. *Journal of Tropical Plant Pathology*, **35**: 7-16.

Papavizas GC, Browers JH, Johnson SA. 1981. Selective isolation of *Phytophthora capsici* from soil *Phytopathology*, **71**: 129-133.

Zhu J, Zhang Z, Yang Z. 2001. General research methods on pathogen of potato late blight (*Phytophthora infestans*). *Journal of Agricultural Sciences*, **24**: 112-114.