

SUITABILITY OF CULTURE MEDIA ON THE GROWTH AND SPORULATION OF *Phytophthora infestans* (Mont.) de Bary

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ABSTRACT

Phytophthora infestans is the most important pathogen of solanaceous vegetables in Cameroon. However, research on this fungus is usually limited due to the difficulty of culturing the pathogen on common agar media, such as potato dextrose agar medium. Three culture media, rye A agar, V-8 agar and chickpea agar were assessed for *in vitro* mycelial growth and sporulation of *P. infestans*. The isolates were incubated at 18-21 °C in the dark. Mycelial growth and sporulation were rated on each medium after ten and twenty one days, respectively. Mycelial growth was larger on rye A followed by chickpea, while the highest number of sporangia was recorded on V-8 medium. Rye A and chickpea media supported mycelial growth and V-8 encouraged sporulation of *P. infestans*. V-8 agar medium was preferred over the other media for the culture of *P. infestans*.

Keywords: *Phytophthora infestans*, culture media, growth, sporulation.

INTRODUCTION

Phytophthora infestans (Mont.) de Bary causes severe late blight epidemics on potato and other solanaceous vegetables (Daggett *et al.*, 1993; Birch and Whisson, 2001). The pathogen infects the whole plant, although the foliage and potato tubers or tomato fruits are the most susceptible plant parts. The mycelium produces branched sporangiospores of unrestricted growth, which emerge through the stomata of the stems and leaves of the hosts. The sporangia, produced on sporangiospores become detached and drift off when ripe (Alexopoulos and Mins, 1979).

The fungus is very difficult to culture due to its strict localisation to certain plant parts and tissue only. The growth is slow especially on artificial nutrient culture media (Roger, 1951). *P. infestans* is very sensitive to tap water and as such distilled water is preferable in the media (Smith and Onions, 1983).

Culture media are useful in defining colony morphology which is very important in identification of the fungus (Abad *et al.*, 1994). Several media have been recommended as suitable for culturing of *P. infestans* (CAB, 1968; Caten and Jinks, 1968; Smith and Onions, 1983; Sozzi and Staub, 1987; Domsch *et al.*, 1990). However, the pathogen has been reported to grow poorly

on most culture media, except rye A medium (Hartman and Huang, 1995). In Cameroon, rye A seed is difficult to obtain unlike the components of other media. The objective of the study is to assess the suitability of three culture media on mycelial growth and sporulation of *P. infestans*.

MATERIAL AND METHOD

Isolation

Field infected huckleberry, potato and tomato leaves collected from four Divisions in the West and North-West provinces of Cameroon were carried to the Plant Pathology Laboratory, University of Dschang. They were sectioned into 4mm² pieces, immersed in 70% ethanol for 15 sec., surface sterilized in 10% sodium hypochlorite (NaOCl) for 2-5 minutes, rinsed briefly in sterilized distilled water and plated on V-8 agar. After 7 days of incubation at 18-21 °C in dark, hyphal tips were transferred to freshly prepared V-8 agar and this process was repeated several times to obtain pure culture.

Preparation of culture media

The media tested were rye A agar, V-8 agar and Chickpea Agar. They were prepared as described by Caten and Jinks (1968); Domsch *et al.*, (1990) and CAB (1968),

respectively. One millimetre of vegetable oil was added to one litre of each medium before sterilization to encourage sporulation (Mukalazi, 2000). After 30mn of sterilization at 0.5 atmosphere and 121°C in an autoclave, antibiotics (Rifamycin, 20µg/ml; Ampicillin, 100µg/ml) and fungicide (Rovral, 1.5µg/ml) were added to the media.

Assessment of mycelial growth and sporulation

A 4mm diameter of actively growing mycelial plug was cut with a sterilized cock borer from a pure culture and placed at the center of 9cm dishes containing freshly prepared rye A, V-8 or chickpea Agar media. After ten days of incubation at 18-21°C in dark, two perpendicular measurements of the radial growth of fungal colonies were taken. There were four plates per treatment and this was replicated twice.

Sporangial suspensions were prepared from 21 days old culture by adding 10ml of distilled water and a drop of Tween 20 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 the suspension was placed on a haematocymeter and mounted on a microscope and the number of sporangia was scored at 40X. This experiment was repeated twice.

Data analysis

Analysis of variance was performed using MSTATC statistical package to evaluate the values of mycelial growth and sporangia production; means were separated by Fisher's Least Significant Difference (LSD) at $P = 0.05$ (Obi, 2002).

RESULTS

Fungal isolates were characterized by downy and white mycelium on the different media. All the isolates grew on all media, but colony diameter was significantly ($P=0.05$) larger on rye A. the five isolates tested showed a similar amount of growth on rye A, while on chickpea agar medium, mycelial growth of 2H₃, 3P₁ and 2T₃ was better than that of isolates 1H₂₁ and 4P₆. Radial growth on V-8 varied among isolates, colony growth of isolate 2H₃ and 3P₁ was greater than that of isolates 1H₂₁ and 4P₆. In general the greatest radial growth of all isolates occurred on rye A and chickpea media (Table 1.)

The spores observed were ovoid, ellipsoid to limoniform, tapering to the base, caduceous, semi papilate and average 16.00µm and 30.17µm respectively in length and width. The highest number of spores was recorded on V-8 medium, where all the isolates had sta-

tistically the same number of spores. More sporangia were produced in isolates 2H₃ and 3P₁ than the others on rye A. Two isolates 2H₃ and 4P₆ did not sporulate on chickpea medium (Table 2).

DISCUSSION

The isolates tested grew in all the media, but the growth was very slow on V-8 agar medium. This poor development of the fungus on the medium had been reported by Harman and Yuang (1995). Sporulation was low on rye A and chickpea media. These media are rich in carbohydrates which are conducive to the growth of most fungi. The highest number of sporangia recorded on V-8 medium could be due to the high proportion of vitamins on the medium. As reported by Smith and Onions (1983), rich media encourage the growth of mycelial and reduce sporulation.

Classification of fungi is based on morphological characteristics. For instance, sporangium and colony morphology was used by Feichenberger *et al.* (1984) to separate *Phytophthora palmivora* from *P. citrophora*. Sporangium formation by a fungus is one criterion used to select an appropriate medium for selective isolation of the species (Papvizas *et al.*, 1981). According to Trognitz (1998), production of sporangia is the most important phenotypic characteristic for host susceptibility.

The cost of a medium only is probably not viable criterion for choosing a culture medium. However, if rye A and V-8 agar media were found as good media for the growth and sporulation of *P. infestans*, the availability of the media recipes could be a deciding factor, especially in Cameroon, where rye seed is not grown.

Spore size and shape were similar to those described by Hooker (1990). Most of the sporangia were dislodged from sporangiosphere. Sporangia caducity is a highly reliable character in identifying *Phytophthora* species (Feichenberger *et al.*, 1984; Hedaithy and Tsao, 1979). Epidemiologically, sporangia could be a source of primary inoculum and might explain the rapid spread of late blight disease in the field. As reported by Sunseri and Johnson (2001), sporangia propagate the life cycle of *P. infestans*. Sporulation of the fungus also contributes to epidemics severity. For instance, the most prominent feature contributing to the destructiveness of *Peronocleospora sorghi* on maize was reported to be the production of sporangia in large number allowing a single infected plant to pose a threat to an entire maize-growing district; the greater capacity a pathogen has for sporulation, the greater the threat of epidemic increases (Duck *et al.*, 1987).

Table 1: Radial growth of *P. infestans* colonies on common agar media for 10 days at 18°C.

Original host	Isolate	Rye A	V-8	Chickpea
Huckleberry	2H ₃	43 ^a	18 ^b	43 ^a
	1H ₂₁	36 ^a	10 ^c	30 ^b
Potato	3P ₁	44 ^a	25 ^b	43 ^a
	4P ₆	40 ^a	15 ^c	35 ^b
Tomato	2T ₃	44 ^a	14 ^c	44 ^a

* Values followed by the same letter for each row are not significantly different according to LSD (P=0.05).

Table 2: In vitro sporulation (x 10⁵ sporangia /ml) of *P. infestans* on common agar for 21 days at 18°C

Original host	Isolate	Rye A	V-8	Chickpea
Huckleberry	2H ₃	4 ^b	8 ^a	0 ^c
	1H ₂₁	1 ^c	5 ^a	3 ^b
Potato	3P ₁	3 ^b	5 ^a	1 ^c
	4P ₆	1 ^c	5 ^a	0 ^c
Tomato	2T ₃	1 ^c	5 ^a	3 ^b

* Values followed by the same letter for each row are not significantly different according to LSD (P=0.05).

Although V-8 agar medium supported the least colony growth and the best sporulation of the fungus, the ingredients are available in Cameroon. The medium was therefore preferable to rye A and chickpea and choice medium for recovering *P. infestans* from planting materials.

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