Culture media influence on vegetative growth and mycelia weight of *Mycosphaerella fijiensis*: implication for inoculum production

Alfred Kumakech[1] & Tonny Opio[1]

[1] National Agricultural Research Organization (NARO), Ngetta Zonal Agricultural Research and Development Institute, P. O. Box 52, Lira, Uganda

Correspondence: Alfred Kumakech, National Agricultural Research Organization (NARO), Ngetta Zonal Agricultural Research and Development Institute, Lira, Uganda. E-mail: kumalfred@gmail.com

Received: March 05, 2023 Accepted: March 17, 2023 Published: July 01, 2023

DOI: 10.14295/bjs.v2i7.356 URL: https://doi.org/10.14295/bjs.v2i7.356

Abstract

Six (6) culture media (Potato Dextrose Agar, Malt Extract Agar, AFPA Base, Czapek Dox Agar, Nutrient Agar and Yeast Extract), were tested for their effect on colony growth and mycelia weight of *Mycosphaerella fijiensis*. The isolate of *M. fijiensis* (Kaw10) produced vegetative mycelial growth on all six-culture media. Culture media effect on the level of vegetative mycelial colonization and mycelia weight was significant. The highest and lowest vegetative mycelia colonization was recorded on malt extract (23.5 mm) and AFPA Base (4.2 mm), respectively. Similarly, culture media effect on mycelia weight was significant. Malt extract agar produced more mycelia weight (0.34g) than the control, PDA (0.11g). Collectively, our data identify malt extract agar as a good medium for improving growth of *Mycosphaerella fijiensis*.

Keywords: *Mycosphaerella* genus, mycelia, inoculum, culture media.

Influência do meio de cultura no crescimento vegetativo e no peso do micélio de *Mycosphaerella fijiensis*: implicações para a produção de inóculo

Resumo

Seis (6) meios de cultura (Ágar Batata Dextrose, Ágar Extrato de Malte, AFPA Base, Ágar Czapek Dox, Ágar Nutriente e Extrato de Leveduras) foram testados quanto ao seu efeito no crescimento da colônia e no peso do micélio de *Mycosphaerella fijiensis*. O isolado de *M. fijiensis* (Kaw10) produziu crescimento micelial vegetativo em todos os seis meios de cultura. O efeito do meio de cultura no nível de colonização micelial vegetativa e peso micelial foi significativo. A maior e menor colonização micelial vegetativa foi registrada no extrato de malte (23,5 mm) e AFPA Base (4,2 mm), respectivamente. Da mesma forma, o efeito do meio de cultura no peso dos micélios foi significativo. O Ágar Extrato de Malte produziu mais peso de micélio (0,34 g) do que o controle, BDA (0,11 g). Coletivamente, nossos dados identificam o ágar de extrato de malte como um bom meio para melhorar o crescimento de *Mycosphaerella fijiensis*.

Palavras-chave: gênero *Mycosphaerella*, micélio, inóculo, meio de cultura.

1. Introduction

Mycosphaerella fijiensis (anamorph *Pseudocercospora fijiensis*) is the causal agent of "Black Sigatoka disease of banana" (Churchill, 2011). *Musa* species are the primary hosts of *M. fijiensis* and disease symptoms depend on the levels of resistance of individual hosts. The ornamental plant Heliconia psittacorum is the only known alternative host of *M. fijiensis* (Gasparotto et al., 2005; Churchill, 2011).

Challenges of the *M. fijiensis*-banana pathosystem includes the slow growth rate of the fungus in vitro, and the difficulty of producing spore-based inoculum *in vitro* (Noar; Daub, 2016; Donzelli; Churchill, 2007). It is extremely difficult to produce enough inoculum of *M. fijiensis* for artificial inoculation. Although Twizeyimana et al. (2007) demonstrated the use of mycelia of *M. fijiensis* as inocula, he recommended the use of conidial suspensions instead of mycelia because of lack of information on the appropriate culture media for producing

large quantities of mycelia in a short time.

Current methods for evaluation of *Musa* species performance in response to *M. fijiensis* use conidia as inocula (Carlier et al., 2003). Production of conidia by *M. fijiensis* requires growth under relatively stringent conditions (Carlier et al., 2000). The fact that conidial production is highly variable, and in some cases significantly impaired by culture media and environmental requirements (Jacome; Schuh, 1993), further compounds the need for a potent source of inoculum.

The objective of the study was to investigate the effect of culture media on growth and weight of mycelia of *Mycosphaerella fijiensis*.

2. Materials and Methods

2.1 Culture media and reagents

Six culture media, namely, potato dextrose agar (PDA, Himedia, India), Malt extract agar (Oxoid, UK), AFPA Base (Oxoid, UK), Czapek Dox Agar (Oxoid,UK), Nutrient agar (Sigma Aldrich, USA) and Yeast Extract (Sigma Aldrich, USA) were procured and kept at room temperature (25 °C).

2.2 Mycosphaerella fijiensis isolate

Mycosphaerella fijiensis isolates (Mak Kaw 10) was isolated from a banana field in Uganda, Africa, using the procedure described by Stover (1976). Ascospore germination pattern was used to confirm the morphological feature of *M. fijiensis*. PCR - Polymerase Chain Reaction based molecular diagnostic assay was used to complement the morphological identification of *M. fijiensis*. Fungal cultures were maintained on potato dextrose agar PDA at room temperature (25 °C).

2.3 Culture media effect on vegetative growth and mycelia weight of M. fijiensis

The effect of culture media on vegetative growth and mycelia weight of *M. fijiensis* was tested in the Laboratory at Makerere University Agricultural Research Institute (MURIK), Kabanyolo according the procedure described by Partridge-Metz & Ambika (2011). The experiment was established following a Randomised Complete Block Design with 4 replications. Six culture media, namely, Potato Dextrose Agar (PDA), Malt Extract Agar, AFPA Base, Czapek Dox Agar, Nutrient Agar and Yeast Extract, were investigated.

Agar disks, 3 mm in diameter, from actively growing PDA culture of *M. fijiensis* isolate (Kaw 10) were aseptically placed at the centre of the different media, in a 9 cm diameter *Petri*-dish. The inoculated plates were sealed and incubated at 25 °C for 28 days. Colony growth was determined on the basis of linear dimensions using, a mathematical ruler. The mean diameter of the mycelia growth was recorded and mycelia from each plate was scraped off with a sterile scalpel and weighed on sterile filter paper to determine mycelium weight. The experiment was performed in duplicate.

2.4 Statistical analysis

Data on mycelial growth and weight of mycelial were analysed by analysis of variance (ANOVA) assuming normal distribution. Data analysis was performed using GENSTAT statistical package 16th edition. The data were subjected to ANOVA, and residual plots were used to check ANOVA assumptions. Hypotheses were rejected at $p < 0.05$ and means compared by *Tukey's* test.

3. Results

3.1 Culture media influence on vegetative growth and mycelial weight

The results of the effect of culture media on vegetative growth and mycelial weight of *M. fijiensis* is presented in (Tables 1 and 2). The isolate of *M. fijiensis* (Kaw10) produced vegetative mycelial growth on all six-culture media. Significant differences ($p < 0.05$) were observed in the level of vegetative mycelial colonization and mycelia weight (Table 1). Total vegetative mycelia colonization ranged from 23.5 mm for malt extract, to 4.2 mm for AFPA Base (Table 2). Significant difference in mycelia weight between Malt extract agar and PDA (control) was observed (Table 1). More mycelia weight was produced on malt extract agar-0.34g, than PDA-0.11g (Table 2). No significant difference was found between malt extract agar and PDA for mycelia

growth.

Table 1. ANOVA for effect of culture media on vegetative growth and mycelial weight of *Mycosphaerella fijiensis*.

Source of variation	d.f.	Mycelia growth				Mycelia weight			
		s.s.	m.s.	v.r.	F pr.	s.s.	m.s.	v.r.	F pr.
Replication	3	1.708	0.5693	2.36		0.00316	0.001053	1.26	
Culture media	5	878.608	219.652	911.42	<.001	0.30448	0.07612	90.98	<.001
Residual	12	2.892	0.241			0.01004	0.000837		
Total	20	883.208				0.31768			

Source: Authors, 2023.

Table 2. Effect of culture media on vegetative growth and mycelial weight of *Mycosphaerella fijiensis*.

Culture media	Mean values	
	Mycelia growth (mm)	Mycelia weight (g)
Malt Extract Agar	23.5[a]	0.34[a]
AFPA Base	4.2[c]	0.00[c]
Czapek Dox Agar	17.6[b]	0.03[c]
Nutrient agar	4.7[c]	0.00[c]
Yeast Extract	17.3[b]	0.04[c]
PDA (control)	20.7[a]	0.11[b]

Note: [abc] Means followed by the same letter are not significantly different ($p > 0.05$). Source: Authors, 2023.

4. Discussion

Fungal nutritional requirements are important for successful cultivation (Zahra et al., 2011). The type and concentration of carbon and nitrogen sources as well as Carbon (C) and Nitrogen (N) ratio play important roles on fungal growth (Zahra et al., 2011; Gao et al., 2007). Selection of the basal medium is the first step in the optimization of medium. In this study, six culture media (Malt Extract Agar, AFPA Base, Czapek Dox Agar, Nutrient Agar, Yeast Extract and PDA) were tested in order to identify a good medium for the improvement of growth of *M. fijiensis*. Results from this study have demonstrated that colony growth and weight of *M. fijiensis* are greatly influenced by solid media type.

Highest and lowest mycelia growth was observed on Malt Extract Agar (23.5 mm) and AFPA base (4.2 mm), respectively. Vegetative mycelia weight also developed to a lesser extent on PDA (0.11g) than Malt extract agar (0.34g) yet PDA and Malt extract agar were statistically grouped together (Table 2) when measuring vegetative mycelia colonization, indicating that mycelia colonization may not necessarily translate into higher mycelia weight with *M. fijiensis* grown on agar based media. Compared to the other media, malt extract agar contains proper formulation of C, N and nutrient sources essential for *M. fijiensis* growth.

It contains dextrose which provides a carbon and energy source. Additionally, malt extract agar contains digest of animal tissues (peptones) which provides nutritious source of amino acids and nitrogenous compounds for growth. The acidic pH (5.5) of the malt extract agar provides optimum environment for the growth of *M. fijiensis*. The poor vegetative colonisation of *M. fijiensis* on AFPA Base and Nutrient Agar could be due to the less acidic pH of the two media (AFPA-6.5 and Nutrient Agar-7.0).

The possibility to produce sufficient quantity of mycelia as inocula, presents the opportunity to improve growth of *M.fijiensis* as well as early screening procedure in Musa breeding programs. Artificial inoculation methods are needed for early screening of *Musa* genotypes. Production of mycelia of *M. fijiensis* is highly variable and significantly impaired by culture media. The choice of culture media is therefore critical. The use of mycelia as inocula has several advantages including reliability and reduced time needed to generate and prepare mycelia

inoculum, and the ability to assess the virulence of isolates that sporulate poorly *in vitro*.

5. Conclusion

Weight of mycelia of *Mycosphaerella fijiensis* varied with the type of culture media. Malt Extract Agar was identified as the best medium. The choice of culture media for *M.fijiensis* growth is important. For this purpose, the present study recommends the use of Malt Extract Agar for *Mycosphaerella fijiensis* mycelia production.

6. Acknowledgments

We thank Makerere University Agricultural Research Institute, Kabanyolo (MUARIK) for offering facilities required to conduct this study. This research was funded by the National Agricultural Research Organisation (NARO).

7. Auhors' Contributions

Kumakech Alfred: Conceptualization, investigation, methodology, data analysis, writing and, supervision. *Opio Tonny*: data collection.

8. Conflicts of Interest

No conflicts of interest.

9. Ethics Approval

Not applicable.

10. References

Carlier, J., Foure, E., Gauhl, F., Jones, D. R., Lepoivre, P., Mourichon, X., Pasberg-Gauhl, C., & Romero, R. A. (2000). Black Leaf Streak. *In*: 'Diseases of Banana, Abaca and Enset, (Ed. DR Jones), (CABI Publishing: Wallingford), pp. 37-79.

Carlier, J., Waele, D. D., & Escalant, J. V. (2003). Global evaluation of *Musa* germplasm for resistance to Fusarium wilt, Mycosphaerella leaf spot diseases and nematodes: Performance evaluation. *In*: INIBAP Technical Guidelines 7. Vézina, A. and Picq, C. (Eds.). The International Network for the Improvement of Banana and Plantain, Montpellier, France.

Churchill, A. C. L. (2011). *Mycosphaerella fijiensis*, the black leaf streak pathogen of banana: progress towards understanding pathogen biology and detection, disease development, and the challenges of control. *Molecular Plant Pathology*, 12(4), 307-328. https://doi.org/10.1111/j.1364-3703.2010.00672.x

Donzelli, B. G. G., & Churchill, A. C. L. (2007). A quantitative assay using mycelial fragments to assess virulence of *Mycosphaerella fijiensis*. *Phytopathology*, 97(8), 916-929. https://doi.org/10.1094/PHYTO-97-8-0916

Gasparotto, L., Pereira, J. C. R., Urben, A. F., Hanada, R. E., & Pereira, M. C. N. (2005). *Heliconia psittacorum*: hospedeira de *Mycosphaerella fijiensis*, agente causal da sigatoka-negra da bananeira. *Fitopatologia Brasileira*, 30(4), 423-425. https://doi.org/10.1590/S0100-41582005000400016

Gao, L., Sun, M. H., Liu, X. Z., & Che, Y. S. (2007). Effects of carbon concentration and carbon to nitrogen ratio on the growth and sporulation of several biocontrol fungi. *Mycological Research*, 111(1),87-92. https://doi.org/10.1016/j.mycres.2006.07.019

Leiva-Mora, M., Alvarado-Capó, Y., Acosta-Suárez, M., Cruz-Martín, M., Sánchez, C., & Roque-Morales, B. (2008). Enhanced sporulation, morphological and pathogenic characterization of *Mycosphaerella fijiensis*, causal agent of *Musa* Black leaf streak. *Centro Agrícola*, 35(2), 33-39. http://cagricola.uclv.edu.cu/descargas/pdf/V35-Numero_2/cag062081603.pdf

Noar, R. D., & Daub, M. E. (2016). Bioinformatics prediction of polyketide synthase gene clusters from *Mycosphaerella fijiensis*. *PLoS One*, 11(7), 1-31. https://doi.org/10.1371/journal.pone.0158471

Partridge-Metz, S., & Ambika, C. (2011). Culture Media Influence on vegetative growth and In vitro conidia production of Magnaporthe oryzae. *Cell and Plant Science*, 2, 9-11.

Twizeyimana, M., Ojiambo, P. S., Tenkouano, A., Ikotun, T., & Bandyopadhyay, R. (2007). Rapid screening of *Musa* species for resistance to black leaf streak using in vitro plantlets in tubes and detached leaves. *Plant Disease*, 91(3), 308-314. https://doi.org/10.1094/PDIS-91-3-0308

Jacome, L. H., & Schuh, W. (1993). Spore production and artificial inoculation techniques for *Mycosphaerella fijiensis var.* difformis. *Tropical Agriculture* (Trinidad), 70(1), 33-38. https://journals.sta.uwi.edu/ojs/index.php/ta/article/view/7063

Zahra, A., Afshin, E., Musaalbakri, A. M., Muhajir, H., Rosfarizan, M., & Arbakariya, B. A. (2011). Nutritional requirements for the improvement of growth and sporulation of several strains of *Monascus purpureus* on solid state cultivation. *Journal of Biomedicine and Biotechnology*, 46, 1-9. https://doi.org/10.1155/2011/487329