Vol. 20, No. 2 (2021) 569-580
Revista Mexicana de Ingeniería Química

Avocado seed hydrolysate as an alternative growth medium for fungi

Hidrolizado de semilla de aguacate como medio de cultivo alternativo para hongos

F. Martínez-Moreno[1], A. E. Jofre y Garfias[1], A. L. Hernandez-Orihuela[2], A. Martínez-Antonio[1]*

[1]*Laboratorio de Ingeniería Biológica. Departamento de Ingeniería Genética, Cinvestav Irapuato. Km. 8.6 Libramiento norte Irapuato-León. 36824. Irapuato, Guanajuato. México.*
[2]*Biofab México. 5 de Mayo 517. Irapuato. Centro. 36500. Guanajuato. México.*

Received: August 17, 2020; Accepted: January 2, 2020

Abstract

Fungi are diverse organisms that inhabit all niches on earth, with great metabolic diversity and various developmental structures. Cultivation of fungi is necessary for their genetic, biochemical, and morphological characterization. Due to their great metabolic capacity, fungi can grow on poorly processed raw materials. That quality has been taken advantage of to formulate inexpensive growth media such as those based on potato. Here, we test the suitability of a growth media consisting of hydrolysates of avocado seeds (ASM), which are by-products of avocado agroindustry, to cultivate six different fungal isolates coming from strawberry plants. We compared the performance of this medium versus PDA and V8, two well-known media among mycologists. We show that ASM is a competitive and suitable medium for fungal growth.

Keywords: Avocado seed hydrolysate, fungi alternative medium, strain isolated fungi, *Fusarium oxysporum*, *Alternaria alternata*, strawberry plant.

Resumen

Los hongos son organismos muy diversos que habitan todos los nichos de la tierra. Existen hongos benéficos y perniciosos, con gran diversidad metabólica y gran variedad de estructuras de desarrollo. El cultivo de hongos es necesario para su caracterización genética, bioquímica y morfológica. Debido a su gran capacidad metabólica, los hongos son muy adaptables para crecer en materias primas poco procesadas, y esta característica ha sido útil en la formulación de medios de cultivo económicos como el fabricado a base de papa. En este estudio, analizamos un medio de crecimiento que consiste de hidrolizado de semillas de aguacate, considerado un residuo en la agroindustria del aguacate, para cultivar seis cepas de hongos aislados de la corona de plantas de fresa. Comparamos el funcionamiento de este medio contra PDA y V8, dos medios bien conocidos entre micólogos. Los resultados indican que ASM es muy competitivo y adecuado para cultivar hongos y podría ser una alternativa sustentable para este propósito.

Palabras clave: Hidrolizado de semilla de aguacate, medio de cultivo alternativo, *Fusarium oxysporum*, *Alternaria alternata*, planta de fresa.

1 Introduction

To date, around 150.000 species of fungi have been described, although the total number is estimated to be closer to 1.5 million (Hawksworth, 2001). Fungi are eukaryotic organisms, uni- or multicellular, homo- or heterokaryotic, haploid, diploid, or dikaryotic. Fungi lack photosynthetic pigments; hence, they are chemoheterotrophs, which obtain their nutrients through extracellular digestion through enzyme secretion, followed by absorption of the solubilized nutrients. Their vegetative state of growth may be either visible on the surface or buried into the substrate. In any case, the structure is typically motionless, with large quantities of reproductive spores usually floating in the surroundings. Fungi reproduction can be sexual or asexual, with the subsequent production of spores (whose characteristics are important for the classification of fungi), while in yeasts, reproduction is carried out through a process of budding (Webster & Weber, 2007).

* *Corresponding author. E-mail*: agustino.martinez@cinvestav.mx
https://doi.org/10.24275/rmiq/Bio1951
ISSN:1665-2738, *issn-e*: 2395-8472

The fungi body is a system of elongated or filamentous hyphae, which are generally branched and have a thickness of 5 to 10 μm, linked together to form a mycelium. Filaments have a basic structural design of reticulated fibers made of various compounds, including polysaccharides such as glucans and chitin (N-acetylglucosamine), which confer rigidity. Fungi growth can be affected by stressful conditions, either nutritional or environmental, generating biphasic dimorphism and pleomorphism (Campbell & Reece, 2007; Tortora *et al*., 2007).

It is difficult to exaggerate fungi importance, going from their benefits for recycling biomass, the symbiotic association with plants, the relations they establish with ants for degrading cellulose and lignin, they also serve as human food (e.g., mushrooms), and some of them are used to produce foodstuffs and pharmacological substances. Besides, more than 8.000 known fungi species produce fungal diseases in pre- and post-harvest crops, which are of great importance from an economic point of view, with a cost of more than US $1 billion per year (Cleveland *et al*., 2003).

One important decision on the cultivation of fungi is the choice of the culture medium used to multiplicate, isolate, characterize, and preserve them. Media composition may vary, but a source of carbon, nitrogen, and microelements are always required (Steinberg, 1939). Potato dextrose agar (PDA) medium is frequently used for isolation and cultivation of fungi, as it supports an adequate growth and/or sporulation of most fungi (Booth, 1971; Beuchat, 1979; Rinaldi, 1982). Other commonly used media are papa-carrot-agar, dextrose-malt-agar, yeast-phosphate-agar extract, Czapek yeast lysate, malt-agar extract, cornmeal-agar, sucrose, jackfruit, and vegetable juice V8- agar (Burge *et al*., 1977; Adesemoye & Adedire, 2005; Marques *et al*., 2019). Additionally, some species require fine control of certain variables to achieve optimal growth, for instance, the pH of the medium, the incubation temperature, and the addition of certain supplements, which may be natural or artificial and are crucial for correct isolation, growth, and development (Beckman & Payne, 1983; Webb *et al*., 2015; Uppala *et al*., 2019).

Although most fungi can be cultivated in conventional media, various alternatives can be useful to expand the repertoire of media used in the mycology investigation. The use of alternatives over conventional media has a critical role in availability and economy for their production. At present, there is great interest in media made of agroindustry and biotechnological by-products (Tharmila *et al*., 2011; Ravimannan *et al*., 2014; Jayasiri *et al*., 2015; García-Amador *et al*., 2019; Gómez-Guerrero *et al*., 2019; Lobato-Calleros *et al*., 2020; Canino-Gómez *et al*., 2020; González-Leos *et al*., 2020; García-Sánchez *et al*., 2020).

In this study, a potentially nutritious medium based on avocado seed hydrolysate (ASM) previously tested suitable to grow bacteria (Tzintzun-Camacho *et al*., 2016; Espinel-Rios *et al*., 2019; Palmerin-Carreño *et al*., 2019, Martínez-Antonio *et al*., 2019) was used to assess the growth of the following fungi: *Xylaria venosula* (Xve), *Fusarium oxysporum* (Fox), Fusarium proliferatum (Fpr), *Rhizoctonia solani* (Rso), *Clonostachys rosea* (Cro) and *Alternaria alternata* (Aal), all of them were isolated from strawberry plants. We assay two formulations of avocado seed hydrolysate media: one with 10X M9 salts (ASMS) and another without salts (ASM). Subsequently, we compare the efficiency of avocado seed hydrolysates media versus PDA and V8, two commonly used media for fungi's culture.

2 Materials and methods

2.1 *Fungal strains*

All the strains used here were isolated from the root and crown of strawberry plants with symptoms associated with "Strawberry's dry" in crop fields of Irapuato, Guanajuato, México. Six species were cultivated: *Xylaria venosula* (Xve), *Fusarium oxysporum* (Fox), *F. proliferatum* (Fpr), *Rhizoctonia solani* (Rso), *Clonostachys rosea* (Cro), and *Alternaria alternata* (Aal). *Fusarium oxysporum* (Fox) and *Alternaria alternata* (Aal) isolates were further used to study the morphological characteristics of reproduction and resistance structures using the microculture technique (Riddell, 1950).

2.2 *Identification of fungal strains*

Primers for internal transcribed spacer (ITS), flanking the rDNA 5.8S subunit, were used to amplify, sequence, and identify the previously isolated fungal strains; for this purpose, fungal mycelia were cultivated in PDA medium: ITS1-Fw 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4-Rv 5'-TCCTCCGCTTATTGATATGC-3'. We proceeded according to the indications given by Raja *et al*. (2017).

Table 1. Main contents of media used in this study.

Media	Content/L	pH	Commercial source	Agar/L
PDA	Potato starch (4g), dextrose (20g)	4.8	BD DIFCO, USA	
V8®	Carbohydrate (40g, sugars 15g), proteins (5g), dietetic fiber (5g), sodium (3.8g), data taken from the commercial label.	6.0	Campbells NJ, USA	18g
ASM®	Reduced sugars (20g), proteins (4g), others (2g)	6.1	Biofab México, Irapuato, MEX	
10X M9 salts	Na_2HPO_4-$2H_2O$ (75.2g), KH_2PO_4 (30g), NaCl (5g), NH_4Cl (5g)	7.0	Merck-Sigma Co, USA	

2.3 Culture media

Four different media were tested; the main components of these are reported in Table 1: papa-dextrose-agar was used at 24 g/L (PDA) (Becton, Dickinson and Company, NJ. USA); original vegetal juice V8 (800 mL distilled water, CaCO3 2g, V8 juice 200mL) (Campbells Co. USA), Avocado Seed Medium (ASM®) was prepared according to the methodology described by Tzintzun-Camacho *et al*. (2016) (Biofab México, Guanajuato. MEX), by diluting 1:10 the concentrated hydrolysate in distilled water, with a resulting pH of 6.1. ASM medium has a final concentration of 20 g/L of minimal soluble reduced sugars and a protein concentration of 2 g/L, in addition to several micronutrients present in avocado seeds. ASM was used alone, or a version supplemented with diluted M9 10X salts concentrate (see table 1); these media are referred onward as ASM and ASMS, respectively. When needed, 18 g/L microbiological agar (Becton, Dickinson and Company, NJ. USA) was supplied to get solid media and sterilized at 121 °C for 20 minutes without adjusting pH. The observation of developmental structures was performed by microculture (Riddell, 1950).

2.4 Growth conditions

2.4.1 Radial growth

Small pieces of agar from each isolate, previously grown in PDA, were set in 5 mm diameter filter paper discs and placed in the center of Petri dishes filled with different media. Plates were 60X15 mm containing a 10 mL medium. Plates were incubated in a growth chamber with fluorescent lamps at 54 μmol/m^2/s light intensity, 28 °C, and a 16/8 hr light/dark photoperiod. Mycelial growth was recorded as the inoculum radius at 24, 48, 72, and 96 hours.

2.4.2 Microculture

Micro culturing was assayed for *A. alternata* and *F. oxysporum* isolates and was based on the technique of Riddell (1950). In our case, 110X60 mm glass Petri dishes were used, glass rods were placed inside to hold the slide with its respective coverslips and sterilized with dry heat in an oven at 210 °C for 2 days. Then the slide was covered with media and inoculated with a small sample of mycelium. The microculture was incubated in a growth chamber with fluorescent lamps at 54 μmol/m^2/s light intensity, 28 °C, and a 16/8 hr light/dark photoperiod for five days. After incubation, 20 μL of lactophenol blue (Sigma-Merk Co. USA) were added and observed under a 40 and 100X objective light microscope for morphological description and photography (DM750 Leica Microsystems. DE).

2.5 Experimental design

Four repetitions of the experimental unit (Petri dish) were carried out for each isolate in each of the treatments (media), thus having a total of 96 plates. The mycelial growth was recorded at 24, 48, 72, and 96 hours after inoculation to determine the growth rate. The growth rates were obtained by linear regression, and the slope values were used to know the growth in mm per day (mm d^{-1}).

2.6 Data management and statistical analysis

Data records for each isolate were evaluated separately to get the growth equation and calculate the growth rate per day by linear regression and using the Statistical Analysis Software (SAS University Edition. USA). The four growth rate values were captured and assessed by analysis of variance (ANOVA) with a reliability of 95%. Multiple comparisons of means

were made using the Tukey test (HSD; $\alpha = 0.05$) to determine the medium with the best growth rate.

3 Results and discussion

3.1 The growth rate in different media

Radial growth rate (mm/day) was assessed at 24, 48, 72, and 96 hours after cultivation, as shown in Fig. 1. We observe that despite differences among media, fungal growth can be appreciated on all Petri dishes, as shown in mycelial growth photographs at 72 hours of culture in Fig. 2. The linear regression shows a high co-relationship between growth and incubation time with coefficients (R^2) ≥0.8489 (96% ≥0.9438). The ANOVA showed that the mean of the media-dependent growth rate for all strains is significant (P ≤0.0165).

For *A. alternata*, the media with the best response were V8 (10.35 mm d^{-1}) and ASM (9.7 mm d^{-1}), and the medium with the lowest growth rate was ASMS (4.65 mm d^{-1}). A similar pattern was displayed by *F. oxysporum*, where ASM (11.47 mm d^{-1}) and V8 (11.92 mm d^{-1}) media supported the best growth rate, although with less difference compared to PDA (10.25 mm d^{-1}) and ASMS (10.12 mm d^{-1}). *C. rosae* shows the lowest growth rate in all media, although it displays a statistically better performance when grown on ASM (4.75 mm d^{-1}) medium. In the case of *F. prolyferatum*, the best media are V8 (11.32 mm d^{-1}), ASM (11.17 mm d^{-1}) and ASMS (10.7 mm d^{-1}), and, remarkably, this fungus tolerates the salts present in ASMS. Statistically, only PDA (8.32 mm d^{-1}) performs below. For *R. solani*, V8 (19 mm d^{-1}) and PDA (15.52 mm d^{-1}) where the best media, with V8 displaying all registries' major growth rate, the avocado media ASM (10.25 mm d^{-1}) and ASMS (6.52 mm d^{-1}) supported less growth for this fungus though comparable to the best growth displayed by other isolates. Finally, *X. venosula* had the best growth rates in PDA (7.25 mm d^{-1}), V8 (7 mm d^{-1}), and ASM media (6.12 mm d^{-1}), displaying the lowest growth of all the isolates in the ASMS medium (3.3 mm d^{-1}).

Considering the specific growth rate of each fungus and comparing the four media's performance for the growth of the six isolates' growth, we can observe that the medium with the best performance is V8 but statistically at the same level as the ASM medium, followed by PDA and finally ASMS. It seems like most fungal strains are affected by the salts present in this last medium, except for *F. proliferatum*.

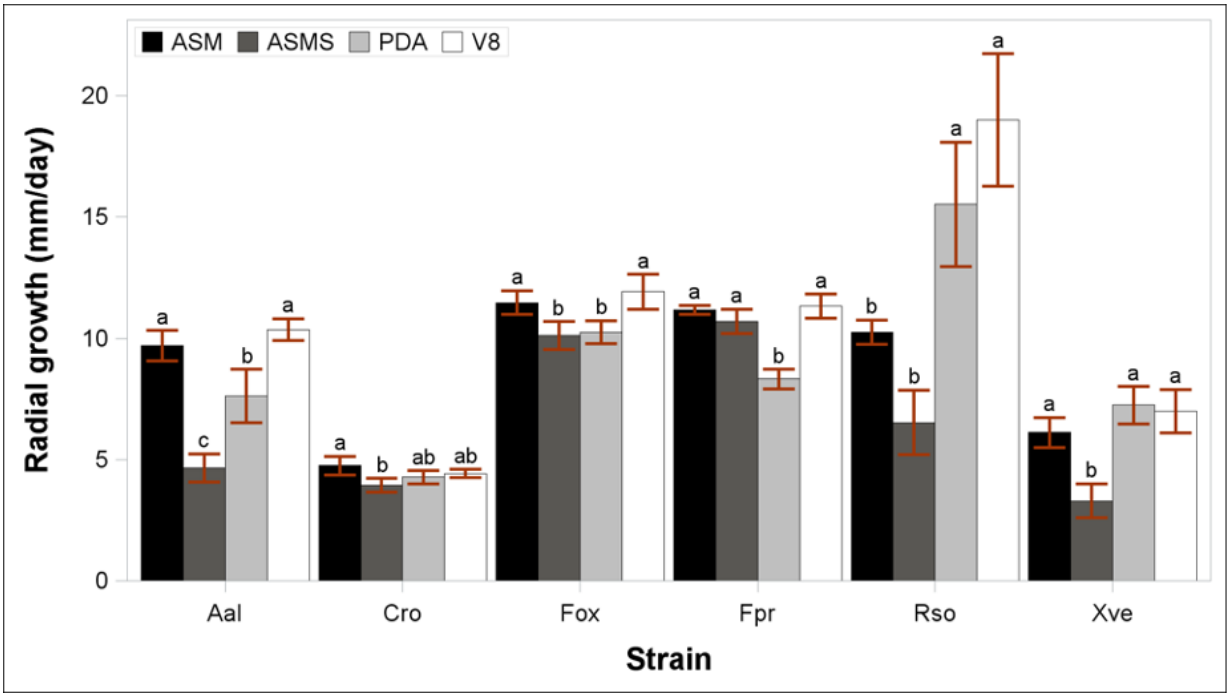

Fig. 1. Growth rate means for each isolate in different media. Error bars represent the standard deviation of radial growth compared to the mean. Means with the same letters in each isolate are statistically equivalent ($\alpha = 0.05$).

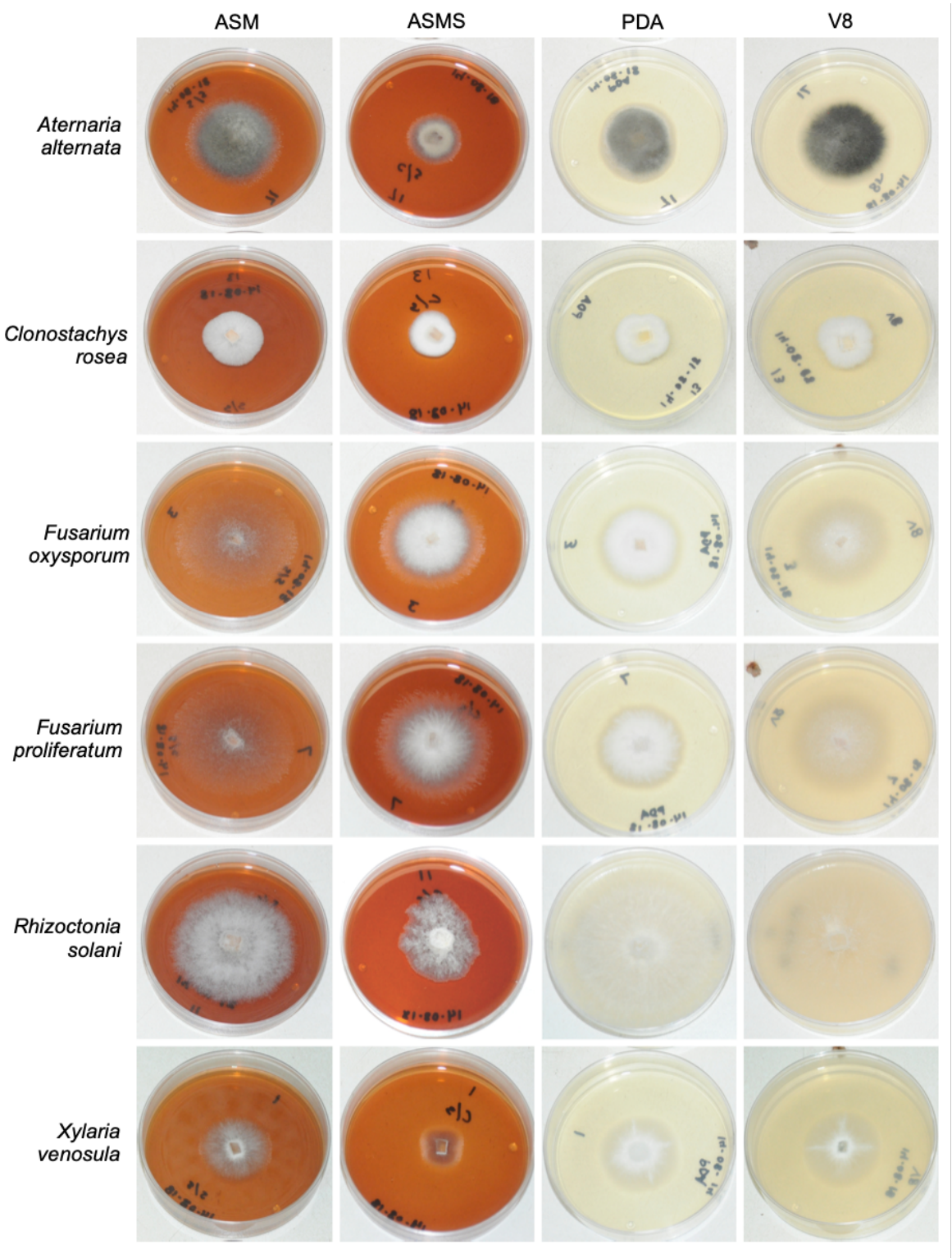

Fig. 2. Fungi mycelial growth in the tested media. Photographs show the grown of the different fungi strains after 72 hours of growth.

3.2 Morphological development on microculture

Direct observation of fungal microcultures is important to determine the development of specialized structures. The development of morphological structures unveils fungi' physiological status while gives information on their pertinence for some applications. For instance, high production of spores might be the choice for biological control. For other applications, the vegetative structure is preferred. Below, the main morphologies observed for *F. oxysporum* and *A. alternata* grew on different microcultures are described. We select these strains because they are rich in displaying diverse morphological structures and representatives of fungi pathogens.

3.2.1 Fusarium oxysporum

F. oxysporum reproduces asexually and usually produces chlamydospores, microconidia, and macroconidia with diversified shapes (Summerell *et al*., 2003; Hafizi *et al*., 2013; Murali Sankar *et al*., 2018). It has been reported that microconidia are uninucleate and display low germination efficiency ranging from 1 to 20%. On the other hand, macroconidia are multinucleate and germinate rapidly. Chlamydospores result from the structural modification of vegetative hyphae or a thick-walled conidial cell in both cases with accessory spores.

In our case, when observed under the microscope 5 days after incubation, *F. oxysporum* displays a higher proportion (approximately 6:1) of microconidia concerning macroconidia in the ASM medium. These microconidia are sessile, globose, smooth-walled and polymorphous (circular, ovoid and elliptic), with 2.5 to 15.9 μm length ($\bar{x}$ =7.2 μm) and 1.9 to 3.9 μm width ($\bar{x}$ =2.7 μm), most of them nonseptate (Fig. 3), these microconidia are produced from simple conidiophores with large phialides. It looks like large phialides originate a mass of microconidia, whereas short phialides produce solitary microconidia. Present macroconidia are slightly curved and fusiform with two and three septa, with a length of 16.5 to 25.1 μm ($\bar{x}$ = 21.6 μm) and width of 3.4 μm to 4.6 μm ($\bar{x}$ = 4.1 μm) composed of simple and branched conidiophores. Chlamydospores are not very abundant and with a regularly interspersed location.

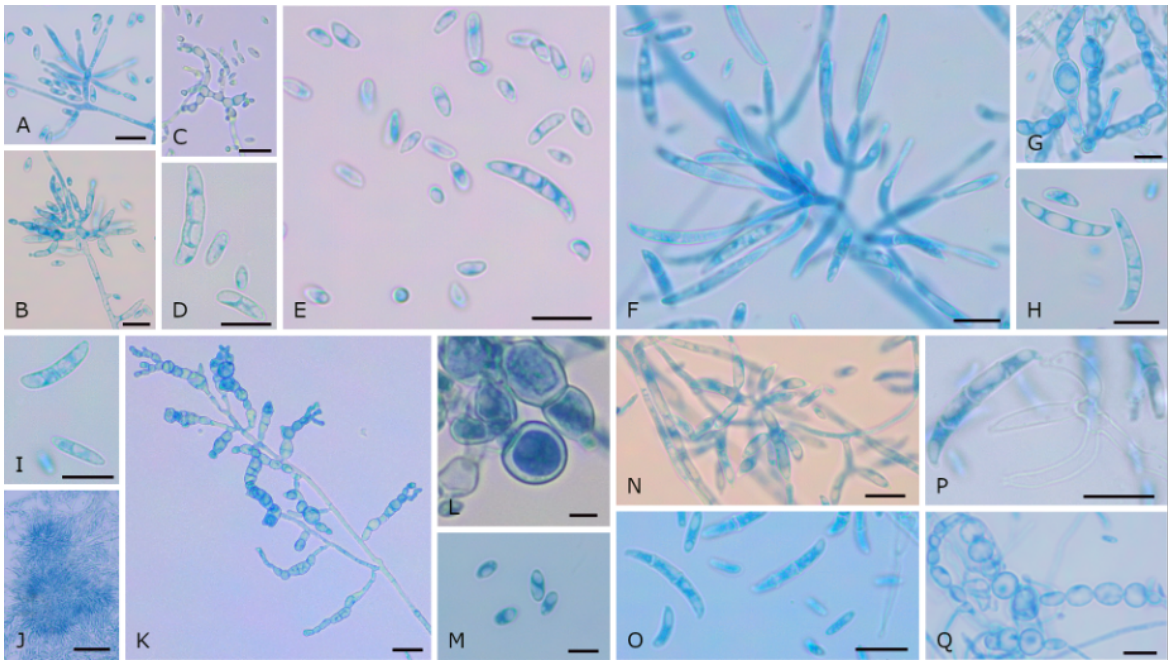

Fig. 3. Development structures of *Fusarium oxysporum*. A-E, ASM medium; F-J, ASMS medium; K-M, PDA medium; N-Q, V8 medium. A, B, F, N, P show of conidiophores' presence; D, E, H, I, M, O, P view of microconidia and fusiform macroconidia. C, G, K, L, Q presence of chlamydospores. Scale bars: A-I, N-Q= 10 μm; J= 50 μm; K= 20 μm; L, M= 5 μm.

In the ASMS medium, the formation of sporodochia and slightly higher macroconidia production were observed compared to the ASM medium. Microconidia were ovoid and elliptical, some of them slightly curved, with a length of 5.8 to 17.6 μm ($\bar{x}$ = 9.8 μm) and width of 2.1 to 3.9 μm ($\bar{x}$ = 2.9 μm), the majority without septa. Microconidia were formed from simple and branched conidiophores. Phialides are both large and short. Macroconidia are slightly curved and fusiform with up to four septa, with a length of 16.2 to 32.9 μm ($\bar{x}$ = 25.1 μm) and width of 3.2 to 5.1 μm ($\bar{x}$ = 4.1 μm) composed of simple conidiophores and long phialides. Chlamydospores are interspersed and terminal.

In PDA, macroconidia production was not observed but a low production of ovoid and elliptical microconidia, some of them with a septum, with lengths of 3.2 μm to 13.1 μm ($\bar{x}$ = 5.7 μm) and width of 1.8 to 3.7 μm ($\bar{x}$ = 2.7 μm), these are produced from single conidiophores. There were also observed long and short phialides producing solitary conidia. There was an abundant production of chlamydospores with interspersed and terminal locations. In V8, slightly higher production of microconidia was observed compared to PDA (ovoid and elliptical microconidia, some of them slightly curved) with 2.6 to 21.3 μm length ($\bar{x}$ = 9.7 μm) and 1.8 to 3.7 μm width ($\bar{x}$ = 2.5 μm). Most of them nonseptate and formed of simple conidiophores; short phialides producing solitary conidia were also observed. Macroconidia were slightly curved and fusiform with two and three septa, with lengths of 18.2 to 32.7 μm ($\bar{x}$ = 25.7 μm) and widths of 2.8 to 5.5 μm ($\bar{x}$ = 3.6 μm) produced from simple conidiophores and long phialides, it was observed the productions of chlamydospores also.

3.2.2 *Alternaria alternata*

For this fungus, there are reports of the formation of diverse spores, including didymospores, phragmospores, and dictyospores with similar conidiogenetic characteristics (Misaghi *et al*., 1978; Masangkay *et al*., 2000; Quayyum *et al*., 2005). There were observed melanized polymorphic conidia of dark brown color with a strongly marked rough surface in the ASM medium (Fig. 4), most of them containing only transverse septa, rarely muriforms, produced in chains of up to 3 conidia.

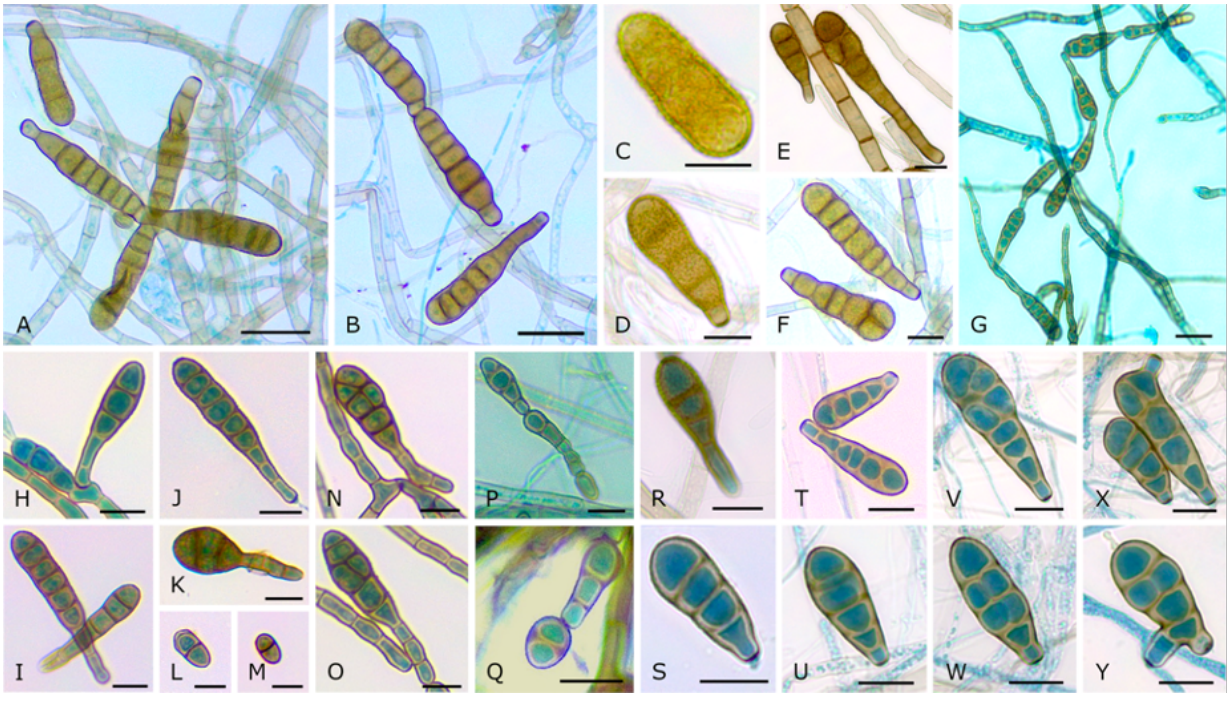

Fig. 4. Development structures of *Alternaria alternata*. A-F ASM medium. G-O ASMS medium. P, Q PDA medium. R-Y V8 medium. A, B, G, P view of conidia. A-D, H-M, Q-U conidia with transverse septa. E, F, N, O, V-Y dictyoconidia. H. conidiophore and conidia. X, Y: dictyoconidia producing secondary conidia. Scale bars: A, B, G= 20 μm; C-F, H-Y= 10 μm.

Muriforms conidia were from 3 to 5 transverse septa, and a single longitudinal septum, lengths of conidia were 37 to 41 μm ($\bar{x}$ = 38.43 μm) and width from 13 to 14 μm ($\bar{x}$ = 13.56 μm). Conidia with only transverse septa (1 to 8) were also observed, most of them with 5 to 7 septa, lengths of conidia were from 11 to 51 μm ($\bar{x}$ = 35.99 μm) and widths from 5 to 12 μm ($\bar{x}$ = 9.7 μm). In the ASMS medium, dark brown polymorphic conidia were also formed, most of them with only transverse septa, in chains of up to 5 conidia. Muriform conidia were observed with 3 to 5 transverse septa and a single longitudinal septum, rarely 2. The lengths of the conidia range between 23 to 46 μm ($\bar{x}$ = 36.18 μm) and 8 to 12 μm width ($\bar{x}$ = 10.64 μm); conidia with only transverse septa (2 to 8) display lengths between 12 to 56 μm ($\bar{x}$ = 34.93 μm) and 5 to 12 μm width ($\bar{x}$ = 8.81 μm).

In PDA medium, low production of conidia was observed, most of them in an immature state with light brown color, produced in chains of up to 5 conidia with transverse septa (1 to 5), the lengths of conidia ranged from 7 to 33 μm ($\bar{x}$ = 16.78 μm) and 5 to 9 μm width ($\bar{x}$ = 6.82 μm).

In the V8 medium, dark brown polymorphic conidia were observed, with conical and oval forms, not produced in chains, and look muriforms with transverse septs. Muriforms conidia have 3 to 5 transverse septa, with a single, rarely two, longitudinal septum. Lengths of conidia go from 26 to 41 μm ($\bar{x}$ = 34.56 μm) and 9 to 12 μm width ($\bar{x}$ = 11.02 μm). On the other hand, conidia displaying only transverse septa (1 to 5), most of them with 4 septa, are 13 to 29 μm length ($\bar{x}$ = 22.89 μm), and 7 to 11 μm width ($\bar{x}$ = 9.49 μm).

The growth of mycelium depends on many factors that influence its development. The reported hyphal extension rates for filamentous fungi living in the rhizosphere vary from 0.4 to 17 mm /day, depending on temperature, humidity, colonized surface, species, and even the variability of isolates within the same species (Watt *et al*., 2006; Teixeira *et al*., 2017). In their study, Teixeira *et al*. (2017) show that the PDA medium was the least favorable for *Fusarium* isolates' mycelial growth. Their data show an average mycelial development between 3.8 and 5.4 mm/day in four different *Fusarium oxysporum* isolates grown on PDA medium. However, in corn flour-agar medium and malt-agar extract, they obtained the same or better (even twice as much) growth rates. The results of growth rates in PDA compared to other media are like those obtained here. We show that the PDA medium poorly supports mycelial growth compared to that on ASM or V8 media. The average growth rate in ASM and V8 media is about 45% higher than those reported in corn and malt-based media. In another study (Gordon *et al*., 2019) for *F. oxysporum* f.sp. *fragariae* they mention a mycelial growth without variation at pH = 5 and 7 at 20 °C. However, at 25 °C, some differences were found when modifying the pH. This temperature is close to our incubation conditions, so, at least in vitro and in a solid medium, it seems that the pH of the media can be a parameter that explains the difference in the growth rates with higher pH (ASM, ASMS, and V8). Ebihara & Uematsu (2014) reported a growth of 11.4 and 10.42 mm/day in two isolates of *Fusarium oxysporum* f.sp. *fragariae* in PDA medium, these data were like our results on other media. This information also shows that the isolate can behave differently within the same species, especially within a group as diverse as *Fusarium* (Aoki *et al*., 2014).

On the avocado seed hydrolysates media, the medium without salts (ASM) was more suitable for fungal growth than that including the salts (ASMS). ASM is statistically equal to V8 for growth rate in five of the six strains, except for *R. solani*, which displayed lower V8 and PDA performance. The addition of salts to the ASM medium did not favor the radial growth in these fungi; although *F. proliferatum* displayed an equal performance on ASM and V8, it was even better in both cases than that displayed on PDA. It could be expected that ASM behaves as V8 since both come from vegetal sources, with the difference that V8 originates from stems, leaves, and tubercles, whereas ASM originates from mature avocado fruit seeds. The descriptions given in this study could change in other media, as some authors (Olsson, 1995; Jennings & Lisek, 1999) observed that fungi could have different growth rate, morphologies, biomass, the diameter of the colony, etc., depending on their ability to explore, translocate and exploit the nutrients available in the culture media and, as mentioned, these capabilities might vary even between isolates of the same species.

Conclusions

Although it is impossible to test over all the fungi, with the fungi strains tested in this study, we conclude that ASM is as competitive as V8 vegetal juice and could be a practical, technical, and commercially suitable option as a fungal cultivation media, with the perk of being composed of agricultural by-products.

Acknowledgements

FINNOVATEG partially funded this study, grant MA-CFINN0944 (2019) from SICES, Guanajuato State Government, given to AM-A. The authors thank Alberto Camas Reyes and Diego Andrés Castro López for reviews on the manuscript and the anonymous referees for their constructive comments.

References

Adesemoye, A. O., & Adedire, C. O. (2005). Use of cereals as basal medium for the formulation of alternative culture media for fungi. *World Journal of Microbiology and Biotechnology 21*, 329-336. https://doi.org/10.1007/s11274-004-3907-4

Aoki, T., O'Donnell, K., & Geiser, D. M. (2014). Systematics of key phytopathogenic Fusarium species: current status and future challenges. *Journal of General Plant Pathology 80*, 189-201. https://doi.org/10.1007/s10327-014-0509-3

Beckman, P. M., & Payne, G. A. (1983). Cultural techniques and conditions influencing growth and sporulation of *Cercospora zeae-maydis* and lesion development in corn. *Phytopathology 73*, 286-289. https://doi.org/10.1094/phyto-73-286

Beuchat, L. R. (1979). Comparison of acidified and antibiotic-supplemented potato dextrose agar from three manufacturers for its capacity to recover fungi from foods. *Journal of food protection 42*, 427-428. https://doi.org/10.4315/0362-028X-42.5.427

Booth, C. (1971). Chapter II Fungal culture media. In *Methods in Microbiology* (Vol. 4, pp. 49-94). Academic Press. https://doi.org/10.1016/S0580-9517(09)70007-8

Burge, H. P., Solomon, W. R., & Boise, J. R. (1977). Comparative merits of eight popular media in aerometric studies of fungi. *Journal of Allergy and Clinical Immunology 60*, 199-203. https://doi.org/10.1016/0091-6749(77)90124-5

Campbell, N. A., & Reece, J. B. (2007). *Biología*. Pp. 160-177. Ed. Médica Panamericana.

Canino-Gómez, J., Limón-Rodríguez, B., Rivas-García, P., Escamilla-Alvarado, C., Morones-Ramírez, J., & Alcalá-Rodríguez, M. (2020). Omega fatty acids production in an isolated native *Chlorella* sp. from northeast Mexico with improved growth using urine as nutritive medium. *Revista Mexicana de Ingeniería Química 19*, 161-176. https://doi.org/10.24275/rmiq/Bio1606

Cleveland, T. E., Dowd, P. F., Desjardins, A. E., Bhatnagar, D., & Cotty, P. J. (2003). United States Department of Agriculture-Agricultural Research Service research on pre-harvest prevention of mycotoxins and mycotoxigenic fungi in US crops. *Pest Management Science: formerly Pesticide Science 59*, 629-642. https://doi.org/10.1002/ps.724

Ebihara, Y., & Uematsu, S. (2014). Survival of strawberry-pathogenic fungi *Fusarium oxysporum* f. sp. fragariae, *Phytophthora cactorum* and *Verticillium dahliae* under anaerobic conditions. *Journal of general plant pathology 80*, 50-58. https://doi.org/10.1007/s10327-013-0476-0

Espinel-Rios, S., Palmerin-Carreño, D. M., Hernandez-Orihuela, A. L., & Martinez-Antonio, A. (2019). A Plackett-Burman design for substituting MRS medium components with avocado seed hidrolysate for growth and lactic acid production by *Lactobacillus* sp. *Revista Mexicana de Ingeniería Química 18*, 131-141. https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n1/Espinel

García-Amador, R., Hernández, S., Ortiz, I., & Cercado, B. (2019). Use of hydrolysate from agave bagasse for bio-hydrogen production in microbial electrolysis cells. *Revista Mexicana de Ingeniería Química 18*, 865-874. https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n3/Garcia

García-Sánchez, M. E., Robledo-Ortiz, J. R., Jiménez-Palomar, I., González-Reynoso, O., & González-García, Y. (2020). Production of bacterial cellulose by *Komagataeibacter xylinus* using mango waste as alternative culture medium. *Revista Mexicana de Ingeniería*

Química 19, 851-865. https://doi.org/10.24275/rmiq/Bio743

Gómez-Guerrero, A. V., Valdez-Vazquez, I., Caballero-Caballero, M., Chiñas-Castillo, F., Alavéz-Ramírez, R., & Montes-Bernabé, J. L. (2019). Co-digestion of Agave angustifolia haw bagasse and vinasses for biogas production from mezcal industry. *Revista Mexicana de Ingeniería Química 18*, 1073-1083. https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n3/Gomez

González-Leos, A., Bustos, M. G., Rodríguez-Castillejos, G. C., Durán, L. R., & Del Ángel, J. D. Á. (2020). Kinetics of lactic acid fermentation from sugarcane bagasse by *Lactobacillus pentosus*. *Revista Mexicana de Ingeniería Química 19*, 377-386. https://doi.org/10.24275/rmiq/Alim618

Gordon, T. R., Stueven, M., Pastrana, A. M., Henry, P. M., Dennehy, C. M., Kirkpatrick, S. C., & Daugovish, O. (2019). The effect of pH on spore germination, growth, and infection of strawberry roots by *Fusarium oxysporum* f. sp. fragariae, cause of fusarium wilt of strawberry. *Plant disease 103*, 697-704. https://doi.org/10.1094/PDIS-08-18-1296-RE

Hafizi, R., Salleh, B., & Latiffah, Z. (2013). Morphological and molecular characterization of *Fusarium solani* and *F. oxysporum* associated with crown disease of oil palm. *Brazilian Journal of Microbiology 44*, 959-968. https://doi.org/10.1590/S1517-83822013000300047

Hawksworth, D. L. (2001). The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological research 105*, 1422-1432. DOI: https://doi.org/10.1017/S0953756201004725

Jayasiri, S. C., Hyde, K. D., Ariyawansa, H. A., Bhat, J., Buyck, B., Cai, L., Dai, Y-C., Abd-Elsalam, K. A., Ertz, D., Hidayat, I., Jeewon, R., Jones, E. B. G., Bahkali, A. H., Karunarathna, S. C., Liu, J-K., Luangsa-ard, J. J., Lumbsch, H. T., Maharachchikumbura, S. S. N., McKenzie, E. H. C., Moncalvo, J-M., Ghobad-Nejhad, M., Nilsson, H., Pang, K-L., Pereira, O. L., Phillips, A. J. L., Raspé, O., Rollins, A. W., Romero, A. I., Etayo, J., Selçuk, F., Stephenson, S. L., Suetrong, S., Taylor, J. E., Tsui, C. K. M., Vizzini, A., Abdel-Wahab, M. A., Wen, T-C., Boonmee, S., Dai, D. Q., Daranagama, D. A., Dissanayake, A. J., Ekanayaka, A. H., Fryar, S. C., Hongsanan, S., Jayawardena, R. S., Li, W-J., Perera, R. H., Phookamsak, R., de Silva, N. I., Thambugala, K. M., Tian, Q., Wijayawardene, N. N., Zhao, R-L., Zhao, Q., Kang J-Ch., & Promputtha I. (2015). The Faces of Fungi database: fungal names linked with morphology, phylogeny and human impacts. F*ungal diversity 74*, 3-18. https://doi.org/10.1007/s13225-015-0351-8

Jennings D.H and Lisek G. (1999). *Fungal Biology: Understanding the Fungal Lifestyle*. Pp. 85-109. 2nd Ed. Springer. Berlin, GE.

Lobato-Calleros, C., Alvarado-Ambriz, S., Hernández-Rodríguez, L., & Vernon-Carter, E. J. (2020). Wet processing coffee waste as an alternative to produce extracts with antifungal activity: *In vitro* and *in vivo* valorization. *Revista Mexicana de Ingeniería Química 19*, 135-149. https://doi.org/10.24275/rmiq/Bio1612

Marques, G. L., Silva, T. P., Lessa, O. A., de Brito, A. R., Reis, N. S., Fernandes, A. G. de A., Oliveira, J. R., & Franco, M. (2019). Production of xylanase and endoglucanase by solid-state fermentation of jackfruit residue. *Revista Mexicana de Ingeniería Química 18*, 673-680. https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n2/Marques

Martínez-Antonio A., Minchaca-Acosta AZ., Hernández-Orihuela AL., Rosales-Colunga LM., Estévez-Palmas JM., Espinel-Ríos S. (2019). Medio de cultivo de semilla de aguacate. México. Patente No. 366669B. CDMX. *Gaceta de la Propiedad Industrial del Instituto Méxicano de la Propiedad Industrial*, 4884(Julio), 116.

Masangkay, R. F., Paulitz, T. C., Hallett, S. G., & Watson, A. K. (2000). Characterization of sporulation of *Alternaria alternata* f. sp. sphenocleae. *Biocontrol Science and Technology 10*, 385-397. https://doi.org/10.1080/09583150050114981

Misaghi, I. J., Grogan, R. G., Duniway, J. M., & Kimble, K. A. (1978). Influence of environment

and culture media on spore morphology of *Alternaria alternata*. *Phytopathology 68*, 29-34. https://doi.org10.1094/Phyto-68-29

Murali Sankar, P., S. Vanitha, A. Kamalakannan, P. Anantha Raju and Jeyakumar, P. (2018). Prevalence of *Fusarium oxysporum* f. sp. ciceris causing wilt in whickpea and its pathogenic, cultural and morphological characterization. *International Journal of Current Microbiology and Applied Sciences 7*, 1301-1313. https://doi.org/10.20546/ijcmas.2018.702.159

Olsson S. (1995). Mycelial density profiles of fungi on heterogeneous media and their interpretation in terms of nutrient reallocation patterns. *Mycological Research 99*, 143-153. https://doi.org/10.1016/S0953-7562(09)80878-2

Palmerín-Carreño, D. M., Hernández-Orihuela, A. L., & Martínez-Antonio, A. (2019). Production of D-lactate from avocado seed hydrolysates by metabolically engineered *Escherichia coli* JU15. *Fermentation 5*, 26. https://doi.org/10.3390/fermentation5010026

Quayyum, H. A., Dobinson, K. F., & Traquair, J. A. (2005). Conidial morphology, virulence, molecular characterization, and host-parasite interactions of selected Alternaria panax isolates on American ginseng. *Botany 83,* 1133-1143. https://doi.org/10.1139/b05-086

Raja, H. A., Miller, A. N., Pearce, C. J., & Oberlies, N. H. (2017). Fungal identification using molecular tools: a primer for the natural products research community. *Journal of Natural Products 80*, 756-770. https://doi.org/10.1021/acs.jnatprod.6b01085

Ravimannan, N., Arulanantham, R., Pathmanathan, S., & Niranjan, K. (2014). Alternative culture media for fungal growth using different formulation of protein sources. *Annals of Biological Research 5*, 36-39.

Riddell, R. W. (1950). Permanent stained mycological preparations obtained by slide culture. *Mycologia 42*, 265-270. https://doi.org/10.1080/00275514.1950.12017830

Rinaldi, M. G. (1982). Use of potato flakes agar in clinical mycology. *Journal of Clinical Microbiology 15*, 1159-1160. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC272272/pdf/jcm00155-0213.pdf

Steinberg, R. A. (1939). Growth of fungi in synthetic nutrient solutions. *The Botanical Review 5*, 327. https://doi.org/10.1007/BF02878505

Summerell, B. A., Salleh, B., & Leslie, J. F. (2003). A utilitarian approach to Fusarium identification. *Plant disease 87*, 117-128. https://doi.org/10.1094/PDIS.2003.87.2.117

Texeira, L., Coelho, L., & Tebaldi, N. D. (2017). Characterization of *Fusarium oxysporum* isolates and resistance of passion fruit genotypes to fusariosis. Revista Brasileira de Fruticultura, 39(3). https://doi.org/10.1590/0100-29452017415

Tharmila, S., Jeyaseelan, E. C., & Thavaranjit, A. C. (2011). Preliminary screening of alternative culture media for the growth of some selected fungi. *Archives of applied science Research 3*, 389-393.

Tortora, G. J., Funke, B. R., & Case, C. L. (2007). *Introducción a la Microbiología*. Pp. 187-198. Ed. Médica Panamericana.

Tzintzun-Camacho, O., Sánchez-Segura, L., Minchaca-Acosta, A. Z., Rosales-Colunga, L. M., Hernández-Orihuela, A., & Martínez-Antonio, A. (2016). Development of bacterial culture medium from avocado seed waste. *Revista Mexicana de Ingeniería Química 15*, 831-842. http://rmiq.org/ojs311/index.php/rmiq/article/view/1046

Uppala, S. S., Zhou, X. G., Liu, B., & Wu, M. (2019). Plant-based culture media for improved growth and sporulation of *Cercospora janseana. Plant disease 103*, 504-508. https://doi.org/10.1094/PDIS-05-18-0814-RE

Watt, M., Silk, W. K., & Passioura, J. B. (2006). Rates of root and organism growth, soil conditions, and temporal and spatial development of the rhizosphere. *Annals of botany 97*, 839-855. https://doi.org/10.1093/aob/mcl028

Webb, K. M., Brenner, T., & Jacobsen, B. J. (2015). Temperature effects on the interactions of sugar beet with Fusarium yellows caused by *Fusarium*

oxysporum f. sp. betae. *Canadian Journal of Plant Pathology 37*, 353-362. https://doi.org/10.1080/07060661.2015.1071283

Webster, J., & Weber, R. (2007). *Introduction to Fungi*. Pp.1-32. Cambridge University Press.