Sana'a University Journal of Applied Sciences and Technology https://journals.su.edu.ye/index.php/jast
Vol. 2 | No. 1 | Page 103 – 106 | 2024 | ISSN : 2958-9568
Doi: 10.59628/jast.v2i1.768

Phytophthora infestans Culture Media
A Comparative Study of Cost-Effective V8 Agar and Rye Agar

Amira A. Al Harethi[1,*], Qais Y. Abdullah[1] , Hala J. Al-Jobory[1]
[1]Department of Biological Science, Faculty of Science, Sana'a University, Sana'a, Yemen.
***Corresponding author: a.alharethi@su.edu.ye**

ARTICLE INFO
Article history:
Received: Dec 1, 2023
Accepted: Jan 26, 2024
Published: Jan, 2024

KEYWORDS
Keywords:
1. *Phytophthora infestans*
2. Late Blight
3. V-8 Agar media
4. Ray Agar media

ABSTRACT
Phytophthora infestans is a pathogen that causes late blight in crops, such as potatoes and tomatoes, posing a serious threat to the Solanaceae family. Researchers have attempted to create an affordable and effective medium for isolating and cultivating this pathogen. The aim of this study was to evaluate the efficacy of low-cost V-8 agar for mycelial growth, sporangia production, and oospore production of *P. infestans* and compare it with Rye A medium. To achieve this, V-8 medium was modified by replacing V-8 juice with tomato. Mycelial growth and sporulation were measured on both media after 14 days of inoculation. It was observed as a white, fluffy growth in both media. The mean radial growth was larger on the low-cost V-8 medium (76 mm) than on Rye A medium (4.4 mm). The lemon-shaped sporangia with a semipapilated structure were recorded with an average length-to-width ratio of 28.6 µm to 30.6 µm, respectively. Oospore production was greater in the low-cost V-8 medium ($5x10^4$) than in Rye A medium ($1x10^4$). The present study demonstrated that low-cost, locally available materials such as tomato can be used as an alternative nutrient medium for the growth of *P. infestans*.

CONTENTS

1. Introduction

Phytophthora infestans is a destructive oomycete pathogen that causes significant damage to crops, including potatoes and tomatoes, particularly in the Solanaceae family. The pathogen can survive in infected tubers, seed pieces, and other plant debris, making it a persistent threat to crop health [1]. Culture media are an essential component of microbiology and play a crucial role in the growth and identification of microorganisms [2]. It is difficult to culture *P. infestans* on general media [3]. To support fungal growth, sporulation, and long-term storage, several semisynthetic and/or organic media have been developed, such as Rye seeds, sweet corn, pea seeds, soybean and carrot, field corn, bean meal, chickpea, oatmeal, cereal grains, V8 juice, and lima bean. These media have been used in agar-based systems to study the pathogen and develop strategies for controlling its spread and managing its impact on crops [4].

The cultivation of *P. infestans* requires the use of Rye Agar and V-8 juice Agar, which are expensive and not easily accessible in developing countries like Yemen. The high cost and scarcity of culture media pose a serious challenge to

microbiological research in such countries [5]. To overcome this challenge, researchers have attempted to formulate alternative culture media that are more affordable and accessible [6, 7, 8]. By using these alternative media, the cost of microbial research can be reduced, and the development of microbial research in developing countries can be facilitated.

The isolation of *P. infestans* from leaf samples is often hampered by the rapid overgrowth of bacteria, especially in cases with secondary bacterial infection [9]. To overcome this challenge, plant-pathogenic oomycetes can be effectively and rapidly isolated from infected plant tissues using selective media enriched with antibiotics, provided proper laboratory procedures are followed. Selective media used for oomycete pathogen isolation often contain antibacterial agents, such as penicillin, ampicillin, and rifampicin, which prevent the growth of both bacteria and fungi [10].
The aim of this paper is to compare the effectiveness of low-cost V8 Agar enriched with antibiotics and Rye Agar as culture media for *P. infestans*.

2. Materials And Methods

2.1. Material

The following materials were used on this study: ß-sitosterol, $CaCO_3$, agar, ampicillin, rifampicin, nystatin, flasks, measuring cylider, Pitri dich, and distilled water.

2.2 Culture media preparation

A low-cost culture medium like V-8 was prepared by blending 250 grams of fresh tomato with 150 ml of distilled water. Without filtering, add 1.5 g of ß-sitosterol and $CaCO_3$, mix well, and add 15 g of agar. The final volume was adjusted to 1 L using distilled water and autoclaved at 15 psi for 20 minutes. This medium was supplemented with antibacterial agents (100 mg/L of ampicillin, 20 mg/L of rifampicin) and a fungicide (50 mg/L of nystatin) [11].
The Rye agar medium was prepared using a slightly modified method for preparing Rye A medium, according to Caten and Jinks [12]. Sixty grams of Rye grain were soaked in distilled water under dark conditions at room temperature (25°C) for 24 hours. The liquid was retained, and the swollen or germinated grains were ground in distilled water and incubated at 68°C for 1 hour. The slurry was filtered through four layers of cheesecloth. The filtered liquid was combined with the retained liquid and used to prepare 1 liter of medium by adding 20 g and 15 g of glucose and agar, respectively. The mixture was then autoclaved at 121°C for 20 minutes at 15 psi.

2.3 Isolation and inoculation of *P. infestans*

P. infestans was isolated from a single lesion of a naturally infected potato leaf obtained from the Seed Potato Production Center (SPPC) in Thamar, Yemen.
After 5–6 days, mycelium and spores appeared. The potato sections that had been inoculated were then placed on both the low-cost freshly prepared V-8 agar medium and Rye agar medium. They were incubated in darkness at 18–21 °C for two weeks [13]. Afterward, the process of subculturing and purification was carried out on separate low-cost fresh V-8 agar medium and Rye agar medium plates. There were three plates per treatment.

Table 1: Composition of low cost V8 Agar medium and Rye agar medium

Low cost V8 Agar Media		**Rye Agar Media**	
Ingredients g/L		Ingredients g/L	
Fresh tomato	250 g	Rye	60g
ß-sitosterol	1.5 g	Sucrose	20g
$CaCO_3$	1.5 g		
Agar	15 g	Agar	15g

2.4. Sporulation production:

Sporangial suspensions were obtained at the age of 14 days from cultures grown on both media using 10 ml of sterilized distal water to facilitate zoospore release. Mycelial fragments were eliminated by employing a dual layer of sterile cheesecloth. Subsequently, the number of sporangia was diluted in 20 ml of sterilized water

and quantified by placing a droplet of the suspension onto a hemocytometer slide, followed by microscopic examination [14].

3. Results and Discussion

3.1. Evaluation of the growth of *P. infestans* in both media

In this study, we evaluated the growth ability of *P. infestans* on two types of agar: low-cost V8 agar and Rye agar. The results showed that there was a notable difference in the growth rate of the isolates on these media. Specifically, we found that low-cost V8 agar was more effective than Rye agar in promoting the maximum mycelial growth of *P. infestans*. On V-8 agar, the mean radial growth reached 76 mm, whereas on Rye agar, it was only 4.4 mm (Figure. 1). These results are consistent with those of Sopee et al. [15], who demonstrated that media prepared from grains and fresh produce available in Thailand and other Asian countries were more supportive of the growth and sporulation of representative isolates compared to Rye agar, V-8 agar, and oatmeal media.

The low-cost V-8 medium is primarily composed of tomatoes, which are rich in flavonoids, folate, the phytosterol compound (β-sitosterol), sucrose, glucose, vitamin E, potassium, vitamin C, and various water-soluble vitamins [16]. The highest growth observed on the low-cost V-8 medium could be attributed to its nutrient content, vitamins, and sterols. As reported by Smith and Onions, a nutrient-rich medium promotes the growth of mycelia [17].

3.2. Colony morphology:

Colony characteristics and growth rates are essential for identifying *Phytophthora* species as a preliminary step. However, the biological and cultural features of the genus are also crucial for simpler identification. After 14 days following inoculation, both media were examined macroscopically, revealing the presence of a white, fluffy mycelium on their surfaces (Figure. 1).

Figure. 1: Mycelium growth of *P. infestans* on both A) Ray agar media and B) low-cost V-8 agar media

The mycelium was aseptate with sporangia that were lemon-shaped, tapering towards the base, and featured a notable semipapillate structure on their tops (Figure. 2). According to Shimelash and Dessie [18], the sporangia had an average length-to-width ratio of 28.6 µm to 30.6 µm, respectively.

Figure. **2**: Limoniform sporangia of P. *infestans* on both Ray agar media and V-8 agar media

Zoospore counting was conducted after 14 hours of incubation at 18°C. The oospore production was found to be higher in the low-cost V-8 medium ($5x10^4$) compared to Rye agar ($1x10^4$) (Figure. 3). This finding aligns with Medina and Platt's observation that sitosterol enhances oospore production. Moreover, the low-cost V-8 medium made with tomato juice effectively supported the growth and sporulation of *P. infestans*, making it a suitable alternative to the traditional V8-based media protocol [6].

Zoospore count	
Low cost V8 Agar	**Rye Agar**
$5x10^4$	$1x10^4$

Figure 3: Number of zoospores on both Ray agar media and V-8 agar media

4. Conclusion

Based on the findings, in comparison to Ray agar, the low-cost V-8 agar medium was the most effective medium for mycelium development and enhanced sporangia generation of *P. infestans* in vitro. Due to the unique properties of this medium, it should be possible to stimulate various fungal growth features of *P. infestans* and improve the possibilities for investigating both the organism and the disease it produces. Since materials for the low-cost V-8 agar medium are easily obtainable, unlike Rye seeds which are not easily available to everyone, accessible media are necessary in order to study the pathogen at the molecular level.

5. References

[1] Forbes, G., Morales, J., Restrepo, S., Pérez, W.,Gamboa, S., Ruiz, R., Cedeño, L., Fermin, G., Andreu, A., Acuña, I. and Oliva, R.,. *Phytophthora infestans* and *Phytophthora andina* on Solanaceous Hosts in South America.In: K. LAMOUR, ed. *Phytophthora*: a global perspective. Oxfordshire: CAB International. CABI *Plant Protection Series*, **2013**. 2, cap. 6, pp. 48-58. https://www.cabi.org/ForestScience/ebook/20133113100

[2] Basu S, Pal A, Desai PK . Quality control of culture media in a microbiology laboratory. Indian J Med *Microbiol*. **2005**, 23(3):159-163. doi:10.4103/0255-0857.16586

[3] Noumbo GR, Fontem DA, Nyumia JB and Iloba C. Suitability of culture media on the growth and sporulation of *Phytophthora infestans* (mont.) de Bary. Agro-Science **2002**. Vol. 3(1): 9-12

[4] David Smith, Jacqueline Kolkowski. Chapter 6 - Fungi, Maintaining Cultures for Biotechnology and Industry, *Academic Press*, **1996**. Pages 101-132, ISBN 9780123619464.

[5] Ombelet S, Ronat J-B, Walsh T, Yansouni CP, Cox J, Vlieghe E, et al. Clinical bacteriology in low-resource settings: today's solutions. *Lancet Infect Dis*. **2018**.18. doi:10.1016/B978-012361946-4/50007-7

[6] Medina, M.V. Comparison of different culture media on the mycelial growth, sporangia and oospore production of *Phytophthora infestans*. *American Journal of Potato Research*. **1999** 76: 121-125. doi.org/10.1007/BF02853576

[7] Peters, R.D., H.W. Platt and R. Hall. Long-term survival of *Phytophthora infestans* in liquid media prepared from autoclaved seeds. Cana*dian Journal of Plant Pathology*.**1998**. 20: 165-170 doi.org/10.1080/07060669809500422.

[8] Raza, W., M.U. Ghazanfar, M. Asif, I. Ul-Haq, M. Zakria and L.K.T. Al-Ani. Morphological characterization of *Phytophthora infestans* and its growth on different growth media. Sarhad *Journal of Agriculture*, **2022**. 38(4): 1189-1202. doi: 10.17582/journal.sja/2022/38.4.1189.1202

[9] Axel, C., Zannini, E., Coffey, A., Guo, J. H., Waters, D. M., and Arendt, E. K. Ecofriendly control of potato late blight causative agent and the potential role of lactic acid bacteria: a review. *Appl. Microbiol. Biotechnol*. **2012** 96, 37–48. doi: 10.1007/s00253-012-4282-y

[10] Sarker, S.R.; McComb, J.A.; Burgess, T.I.; Hardy, G.E.S. Antimicrobials in *Phytophthora* isolation media and the growth of *Phytophthora* species. *Plant Pathol.* **2020** ,69, 1426–1436. doi:10.1111/ppa.13224

[11] Gamboa, S., Perez, W., Andrade-Piedra, J.L. and Forbes, G. Laboratory manual for *Phytophthora infestans* work at CIP. Lima, Peru: *International Potato Center*. **2019**: 78pp doi:10.4160/9789290605331 .https://hdl.handle.net/10568/105663

[12] Caten, C. E. and J. L. Jinks.. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. *Can. J. Bot*. **1968** 46: 329-348. doi:10.1139/B68-055

[13] Flier, W.G., L.J. Turkensteen, G.B.M. Van Den Bosch, P.F.G. Vereijken, A. Mulder. Differential interaction of *Phytophthora infestans* on tubers of potato cultivars with different levels of blight resistance. *Plant Pathology*. **2001**. 50: 292-301. https://doi.org/10.1046/j.1365 3059.2001.00574.x.

[14] Torres-Londono, G.A. Morphological Characterization,Virulence, and Fungicide Sensitivity Evaluation of *Phytophthora Palmivora*. PhD thesis, *Michigan State University*. **2016**. p.129. https://doi.org/10.1139/b68-055

[15] Sopee, J., Sangchote, S. & Stevenson, W.R. Modified agar-based media for culturing *Phytophthora infestans* . *Phytoparasitica* **2012**. 40, 269–278 .https://doi.org/10.1007/s12600-012-0218-4. doi.org/10.1007/s12600-012-0218-4

[16] Beecher GR. Nutrient content of tomatoes and tomato products. Proc Soc *Exp Biol Med*. **1998** ;218(2):98-100. doi:10.3181/00379727-218-44282a. doi: 10.3181/00379727-218-44282a

[17] Smith, D., and Onions, A. H. S. The Preservation and Maintenance of Living Fungi. *Commonwealth Mycological Institute*, **1983**. Kew, UK. https://doi.org/10.1111/j.1365-3164.1994.tb00033.xs

[18] Shimelash, D., and B. Dessie. Novel characteristics *of Phytophthora infestans* causing late blight on potato in Ethiopia. *Current Plant Biology* **2020**. 24:100172. https://doi.org/10.1016/j.cpb.2020.100172