Modified vegetables extract as substitution of v8-juice medium for cultivation of *Phytophthora* spp.

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**Abstract.** *Phytophthora* species is a pathogen that causes rot on plant parts both on roots, stems, leaves and fruit. One obstacle in investigating the mechanism of infection and virulence testing of *Phytophthora palmivora* is to obtain suitable media for its growth. The purpose of this research was to study the suitability of modified vegetables extract as substitution of V8 juice for growth medium of *Phytophthora* spp. This research consisted of several stages, which were, preparation of V8-Juice medium and vegetable medium, isolation of *P. palmivora* and *P. colocasia* from infected cacao pod and taro, and observation of *P. palmivora* and *P. colocasia* by their morphological characters. The results showed that this medium was effective for cultivation of *P. palmivora* and *P. colocasia*. There was no morphological difference was observed in the growth of *P. palmivora* both on V8-Juice medium and on vegetable extract medium. The appearance of hyphal swelling and characteristics of sporangium, oospore, chlamydospore and zoospore observed have the same characteristics in both types of media used. Modified vegetable media was also effective for cultivation of taro leaf blight pathogen *P. colocasiae*.

1. **Introduction**

*Phytophthora* species can infect all parts of plant tissue and all stages of plant development. This pathogen has 4 types of spores that may directly or indirectly cause infection so that they can survive in the soil or the body of a dead plant when the plant is not available to obtain an inoculum [1]. *Phytophthora* spp. is a pathogen that initially belongs to the Oomycetes class including algae group. Plant disease experts put it into a group of fungi because it can absorb nutrients and have mycelium, but it put back into algae groups [2]. Currently Oomycetes are classified in Kingdom Stramenopila or Chromista based on the nature of zoospores which have two types of flagella.

*Phytophthora* spp. can cause various kinds of rot disease in several types of plantation and horticultural commodities. High adaptability causes rapid development of infection, resulting in losses due to decreased plant productivity. One example of the most disputed disease in Indonesia is the cacao pod rot which is generally caused by *P. palmivora* showing the appearance of spot spots [3] on brown fruit which will increasingly spread on the surface of the fruit and are usually followed by growth of mycelium which forms sporangia in favorable conditions that can spread through the wind so that it can infect other plants very quickly [4].

Cocoa pod rot was first reported in 1833 in Sri Lanka. This disease was previously known to be caused by *P. omnivorous*, later known to be caused by *Pythium palmivorum* [5]. Furthermore, the pathogen was renamed by Butler to *P. palmivora* (Butler). *P. palmivora* has a very wide host range.
Lack of research on the development of infection and epidemiology of disease due to the difficulty of getting V8 juice as the main ingredient in making growth media used to identify the type of pathogen Phytophthora sp. become a limiting factor for the difficulty of finding the right control solution.

V8 juice is the main ingredient used in making growth media from the Oomycetes group, one of which is Phytophthora. The use of this media is able to facilitate the process of isolation from plant parts so it is very helpful in identifying both macroscopically and microscopically. However, since 2015, with the issuance of government regulations concerning the entry of imported food and beverage products, it will be difficult to get V8 media on the market because it is included as one of the list of beverages that are not allowed to be sold freely in Indonesia. Surely, it makes difficult for researchers in the field of microbiology in conducting research related to Phytophthora spp.

V8 juice as the main ingredient is composed of a mixture of tomatoes, carrots, celery, beets, parsley, slada, watercress, spinach, 2% salt, ascorbic acid, natural flavoring, and citric acid. V8 media is the media most often used in growing Phytophthora spp. to identify the morphological character of the pathogen, so that this media is the main media. Due to the difficulty of finding the main media, a study was carried out to obtain media that could replace the juice V8 as the main ingredient of the extract modified from the initial composition of V8 with several types of vegetables without having to use preservatives. The vegetables used are carrots, tomatoes, peppers, purple cabbage, red beets, celery, onions, garlic and kale which are mixed so that the formulation can function as a modification medium for the main V8 media replacement that can be used in growing Oomycota fungi. The purpose of this study was to determine the ability of modifying some vegetable extracts to replace V8 juice as the main ingredient in making growing media that is easily found or made by yourself so that it makes it easier during the isolation process.

2. Materials and methods

This research was conducted at the Disease Laboratory of the Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Hasanuddin Makassar. Testing several samples of fruit rot disease from several planting locations was conducted to evaluate the ability of Phytophthora spp. in growth media. Preparation of culture media from vegetable extracts was carried out in the same way as making media using V8 juice, 100 ml of vegetable extract mixed with 2 grams of CaCO$_3$ then centrifuged to separate the sediment so that clear liquid was obtained [6] then 15 grams of agar was added and 900 ml of H$_2$O were added.

Next the media was autoclaved and poured on a petri dish for the purpose of isolation. The process of isolating the symptomatic fruit tissue was done by cutting small and thin portions of the symptomatic cacao on the skin of the fruit (which was previously peeled off to reduce contamination of the external fungus) measuring 0.5 cm x 1 cm, each part consisting from half the healthy and half symptomatic fruit rot. Plant the part of the tissue was surface sterilized on the media, subsequently incubated until the fungus growth initiation at the optimal temperature of $P. ~palmivora$ growth which was 15-25°C. The fungus was identified to assure $P. ~palmivora$ species and subsequently purified to identify macroscopic and microscopic morphological characters. Morphological observations were then compared with observations in previous journals to determine the success of the media in cultivate Phytophthora.
3. Results and discussion

![Figure 1](image1.png)

**Figure 1.** Growth morphology of *P. palmivora* on V8 media, hyphae character when planting tissue (a) microscopic morphological form on the surface of the media (b) morphology after sub-culture (c)

- Mostly *Phytophthora* spp. of interest appeared to have special characteristics compared to high-level fungi. All of *Phytophthora*’s life was diploid, his cell wall consisted of cellulose and β 1,3-glucan. *Phytophthora* spp. does not produce sterols but requires β-hydroxy sterols for their sporulation [7]. Therefore, *Phytophthora* spp. requires to have special medium for culture.

![Figure 2](image2.png)

**Figure 2.** Microscopic morphology of *P. palmivora* on V8 media

- Isolation of plant parts on vegetable extract media showed very characteristic characters in the media, *P. palmivora* growth looked front (a) *P. palmivora* growth looked back (b) pathogenic morphological form when mycelium grew on media (c) morphological form microscopically on the surface of the media (d)
The results of plant tissue isolation showed the growth of pathogenic mycelium from the typical Oomycetes class on vegetable extract media. Mycelium in media exhibited a characteristic when initial isolation from plant tissue. The morphology of plant tissue growth on the media was fungus mycelium growing on the top of the surface of the isolated fruit skin, but on the surface of the media mycelium growth appeared to be in the middle of the media and there was something similar to bacterial growth. Morphology of isolates was found in the use of vegetable extract media (figure 3) and was similar with cultivate in V8 media (figure 1). The traits that distinguish the Oomycetes class from the fungus are that all genera from the Oomycetes class have a sexual phase that produces oospores, whereas the fungus does not have oospores but zigospore, basidioles and ascospores. Oomycetes mycelium is diploid while the fungus is haploid or dikaryotic [8].

![Image](image_url)

**Figure 4.** Morphological isolates isolated from three districts macroscopically on vegetable extraction media front and back, Gowa Regency (a), Luwu Regency (b), Pinrang Regency (c)

The results showed a macroscopic appearance of morphology on vegetable extract media appear the same growth when cultivate in V8 media (figure 1). Morphologically in figure 4, it can be seen that there are several forms of *P. palmivora* mycelium that are often found when observing on media, namely the stellate and rossaceous types, and there are also other types. Mostly *Phytophthora* mycelium on media has a star-like or stellate and rosaceous form. Some isolates have growth types whose mycelium appears to be in the middle of the media and there are similar bacterial growths that grow on the surface of the media almost similar to when isolated early from plant tissue.

Morphological differences in mycelium that appear on the media due to the use of media from vegetable extracts, so that growth is not included in the type of stellate and rossaceous colonies. The use of different media for the isolation of *Phytophthora* species shows diverse growth morphologies [9]. Although macroscopically different on the media, but microscopically the same that is *P. palmivora* this is shown by the spore character on the microscope.
Figure 5. Microscopic morphology of *P. palmivora* on vegetable extract media, a typical form of hyphal swelling (a) sporangium (b) chlamydospora (c) zoospore (d) oospore that is formed from oogonium and antheridium with two types of antheridium namely amphigynous and paragynous (e) abundant and branched sporangium as well as the formation of chlamydospores with the characteristics seen on a microscope (f).

Microscopic identification shows that there were 4 types of spores and the characteristic of hyphae such as hyphal swelling containing a lot of nuclei and septation less. Formation of hyphal swelling varies from several observation locations in the form of terulose, coralloid [10], and loops. In hyphal swellings the nucleus usually forms a branch point which is then followed by globular swelling (rounded) having thick walls called chlamydospores that appear to grow abundantly and are located at the tip or in the middle of hyphae. Chlamydospores form globose down to the subglobose, located internally in the mycelium with a diameter measuring 32-42 μm [11]. The morphology of chlamydospores is less different between species and is rarely used for identification purposes [10]. The morphology of observed sporangium was 2-3 different formation of sporangium but was commonly found in *P. palmivora* like ovoid with prominent papillae and produces zoospores (swimming spores) with flagella [10].

Microscopically observations of the three forms of asexual spores namely sporangium, chlamydospora and zoospores can be clearly identified because they can develop well on the media. Oospores (sexual spores) was identified to have spherical oogonia with antheridium amphigynous. Oogonium grows through antheridium and the antheridium surrounds the oogonium stems and oospores are oval and produce spherical oogonia with antheridium paragynous. The observed oospores were a form of sexual spores resulting from the fertilization of the two types of mating, oogonium and antheridium [12]. 20 sporangia were produced in one sporangium stalk separated from the stalk (caducity) and after sporangium separates a spore stalk or pedicle was a very short measuring 5 μm. Sporangium length ranges from 40-60 μm and width 25-40 μm with length-width ratio is 1.4-2. Figure 5 shows that the microscopic morphology of vegetable extract media was very effective in replacing V8 because all types of spores was identified clearly almost the same as when using V8 (figure 2).
4. Conclusion
Using of vegetable extracts was very effective medium in replacing V8 juice as the main ingredient in making *P. palmivora* growth media. The isolation of pathogens from plant tissue purification resulted in the characteristic pathogen growing on the media and morphologically macroscopic on petri dishes showed the same appearance when grown with V8 media. In addition, the use of vegetable extract media was very effective to form asexual spores namely sporangium, chlamydospores, and zoospores. They developed well in medium with sexual spores at low temperatures (20°C) in darkness and appropriate nutrition. Not only that, in several other studies that use media from vegetable extracts were able to grow the type of *Phytophthora* on the tops of cacao plants and on *P. colocasiae* causing blight of taro plant leaves (not yet published). This shows that this medium was useful to grow several types of *Phytophthora* spp. in the Oomycetes group.

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