

Replanting, Purification, and Preservation of *Cercospora*

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ABSTRACT

Cercospora is a group of Ascomycota fungi undergo meiosis after zygote formation of the short-lived and produce meiospores with the formation of free cells in a meiosporangium called ascus. Ascomycota indicates sexual compatibility bipolar and have cell walls composed of two layers (bi-layered). Phylogenetic tree based on 18S rDNA sequences showed that the Ascomycota and Basidiomycota have divergence from one another in the Paleozoic era, about 500 million years ago. *Cercospora* fungus is also a fungus that can cause disease is quite common in plants. In culturing the fungus *Cercospora* there are some processes that do the replanting, purification and preservation. Replanting isolate of the fungus *Cercospora* replanting which is the work of moving the fungus *Cercospora* isolates from the old medium into a new medium with a very high level of accuracy. In this case replanting process is done by using the method of puncture. Purification is a process of purifying the fungus *Cercospora* isolates from other microorganisms (fungi and bacteria) who participated grow during the isolation process, usually done by taking the hyphae at the very end of the isolates. Preservation is the storage and maintenance activities of the fungus *Cercospora* isolates using glycerol solution which will then be stored in the refrigerator in a certain period.

Keyword: fungi, cercospora, replanting, purification, and preservation

INTRODUCTION

Fungi Ascomycota zygotes undergo meiosis after the formation of the short-lived and produce meiospores with the formation of free cells in a meiosporangium called ascus. Ascomycota indicates sexual compatibility bipolar and have cell walls composed of two layers (bi-layered). Phylogenetic tree based on 18S rDNA sequences showed that the Ascomycota and Basidiomycota have divergence from one another in the Paleozoic era, about 500 million years ago. (Semangun, 2001).

Ascomycota is the defining characteristic of these fungi produce sexual spores in ASKI (single, ascus) sac-like, in contrast to the zygote fungi, sac fungi, in part contain the likelihood of their sexual stages in the macroscopic fruit bodies, or ascocarpus. Ascomycetes reproduce asexually by producing asexual spores in very large quantities, which are often dispersed by

wind. Asexual spores are produced on the tip of hyphae, often in long chains or in groups. Spores are not formed in the sporangia, as well as on Zygomycota. Spores called conidia open like that, from the Greek word which means "dust" (Hollyday, 1980)

As with other microorganisms *Cercospora* is a fungus that can be bred through pure breeding with growing media such as PDA. Microorganism growth medium is a material consisting of a mixture of nutrients (nutrients) required for the growth of microorganisms. Microorganisms utilize the nutrients in the form of media of small molecules that are assembled to construct components of cells. With the growth media can be done to isolate pure cultures of microorganisms and also manipulate the composition of growth media. (Indra., 2008).

Replanting of fungi or bacteria is the work of moving the fungus / bacteria from an old medium to a new medium with a very high level

of accuracy. For the first replanting that all the existing tools in conjunction with a medium to keep it sterile, it is to avoid any contamination (Dwijoseputro, 1998). Purification was purified from fungi and other microorganisms (fungi and bacteria) that participate to grow during the isolation process. The results of purification are pure fungal isolates. Preservation is the storage and maintenance of microbial activity within a specified period. The method chosen depends on the nature and purpose of preservation of microbes.

MATERIALS AND METHODS

Replanting

These methods include: preparation of the equipment to be used as LAF, needle stem loop, Bunsen burners, tissue, labels, masks, media PDA / MEA, alcohol, fungal isolates, parafilm. Replanting done inside, after the LAF is ready to use input devices and materials to be used in subsequent LAF wear a mask and spraying alcohol on hand to keep it sterile. Needle-shaped heating loop Bunsen burner until the rod with a really hot, then take the media PDA / MEA. After that take the isolates on the tube to the needle shaft use who do near burner Bunsen, who had been taken earlier isolates transferred to PDA media / new MEA, the transfer is done near the Bunsen burner, after the isolates were transferred to new media and then sealed and given parafilm the appropriate label.

Purification

Using a light microscope to isolate the fungus is a microscopic view so it looks hypha at the very outside, then take the hyphae of the fungus with a needle stem loop that is heated in the outer part because of the outer portion is the most good for the purification and has the smallest percentage for contamination , after taking the hyphae of the fungus isolates were then put on a PDA / MEA, after the isolates were transferred to the PDA / MEA is then sealed with parafilm and labeled accordingly.

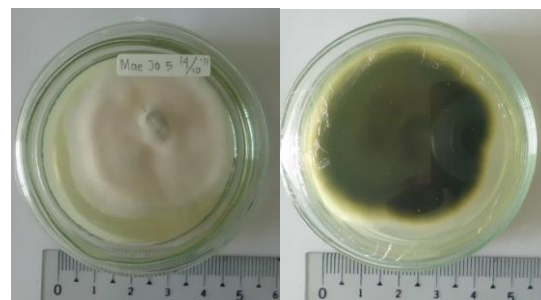
Preservation

Preservation of fungi using a solution of glycerol and performed in the first LAF isolates fungus that is taken to be preserved and the straw is cut into pieces less than 5cm and make sure the straw is already sterilized, then create a block on the isolates by using a straw block for a total of 10 isolates . Straw fuel is already in use and take a new straw is inserted with a toothpick in it to take the isolates that have been block earlier by inserting a straw into the block as much as 5 to 1 glycerol, then take a small bottle of liquid glycerol on the already available and include isolates contained in the straw into the liquid glycerol with the aid of a toothpick. Once inserted in a glycerol solution bottle is then labeled and put in refrigerator for further storage.

RESULTS AND DISCUSSION

Replanting

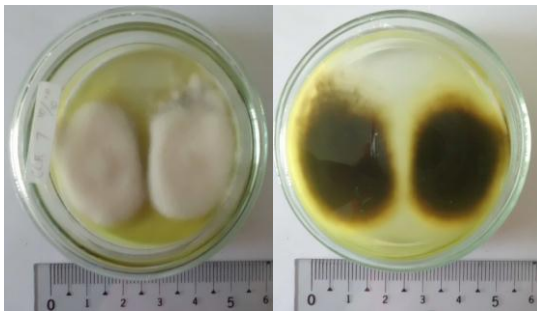
Based on the literature (Winarni, 1997) replanting microorganisms can be done by several methods such as scratch method, stocking, puncture, and castings. By looking at how replanting above are included in the puncture method is by way of dripping the needle loop in which there are inoculum, then put in the new media to cultivate a microorganism.



Picture 1. Isolates of the fungus
Cercospora replanting

Purification

Based on the literature (Anonymous, 2011e), which is purification / refining of these isolates were able to do some of the techniques of isolation technique and the technique calls monospora hyphae. Monospora isolation technique is one of the purification techniques are performed by isolating a single spore or take a single spore to be moved (inoculated) on the new medium. Monospora isolation technique requires high skills into practice, because it takes foresight in transferring a single spore in a new medium. While the technique is the technique of hyphae making purification is done by taking some collection of fungal hyphae to be moved to a new medium. Hyphae retrieval technique is relatively easier than the isolation monospora.



Picture 2. Isolates of the fungus *Cercospora* purification

Preservation

Storage of microorganisms in the refrigerator temperature of about -20°C to -85°C (cryopreservation / cryopreservation) is a good preservation method for most fungi, bacteria, and viruses. One of the storage system is the simplest and most popular for fungi and bacteria involves the use of porous ceramic beads (cryobeads) are suspended in a cryopreservation fluid, such as glycerol, in a small plastic bottle. Once inoculated with the culture, the excess solution should be taken using sterile pipette and the vial was kept in the refrigerator. Storage so that the method can survive long enough and well used to preserve isolates of the fungus *Cercospora*.



Picture 3. Isolates of the fungus *Cercospora* preservation

CONCLUSIONS

Cercospora fungus is a fungal group Ascomycota zygotes undergo meiosis after the formation of the short-lived and produce meiospores with the formation of free cells in a meiosporangium called askus. *Cercospora* fungus is also a fungus that can cause disease is quite common in plants. Replanting process is fungus / bacteria that is the work of moving the fungus / bacteria from an old medium to a new medium. Purification is a process of purifying the fungi / bacteria from other microorganisms (fungi and bacteria) that participate to grow during the isolation process, Preservation is a storage and maintenance of microbial activity within a specified period.

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