Contamination Factors in Tissue Culture Media

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Abstract

Plant tissue culture is a plant propagation technology from plant cells, tissues and organs in solid or liquid media under aseptic conditions. The main advantage of tissue culture technology is that it can produce planting material of high and uniform quality. Tissue culture planting media is the media needed so that isolated plant cells or tissues can grow and develop into complete plants. However, this culture medium is also suitable for the rapid growth of bacteria and fungi. culture media, equipment used to transfer explants to the media, planting materials used, as well as space for planting and growing explants. Proper sterilization of tools can prevent contamination.

Keywords: Media, Sterilization, Tissue culture

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Introduction

Plant tissue culture is a technology for propagating plants from plant cells, tissues, and organs on solid or liquid media in aseptic conditions. Whole plants can be regenerated from small tissues or plant cells on suitable culture media under controlled environmental conditions (Gaikwad, A.V., S.K. Singh, and R. Gilhotra, 2017). In addition to plant propagation, tissue culture can also be used to produce double haploids, cryopreservation, propagation of new varieties of crops, preservation of rare and endangered crops and plants that are difficult to propagate, as well as to produce secondary metabolites and transgenic plants. The main advantage of tissue culture technology is that it can produce high-quality and uniform planting material. This activity can also be carried out throughout the year in disease-free conditions anywhere regardless of the season or weather (Jain, 2016).

Plant tissue and cell culture media generally consist of some or all of the following components: macronutrients, micronutrients, vitamins, amino acids or nitrogen supplements, sugars, other unspecified organic supplements, ingredients, compaction or support systems, and growth. Regulator.

Plant tissues or cell cultures require various combinations of nutrients, minerals, plant growth substances, vitamins and sugars as a source of carbon. However, this culture medium is also suitable for the rapid growth of bacteria and fungi. Microorganisms that attack plant tissues or cell cultures generally grow rapidly, so they will deplete nutrients and produce toxins that can affect the growth and kill plant tissues (Misra, A.N. and M. Misra, 2016).

Contamination by bacteria and fungi is a problem that often attacks plant tissue cultures. Sources of contamination can come from equipment made of glass or plastic, culture media, equipment used to move explants to the media, planting materials used, as well as planting and growing rooms for explants (Bhojwani, S.S. and P.K. Dantu, 2013). The preparation and maintenance of tissue culture systems requires sterilization of culture media, culture containers, and sterilization of the surface of cultivated seeds or plant tissues, as well as the sterilization of all equipment used for tissue culture activities. Fungal spores or bacterial cells that come into contact with the growth medium will contaminate the explant quickly. To prevent contamination, it is necessary to sterilize the equipment. Sterilization is useful for killing and cleaning all forms of live microbes in the equipment and planting materials used (Misra, A.N. and M. Misra, 2012).

Sterilization can be defined as an effort to kill microorganisms including in the form of spores, sterilization is a technique to clean and free an object from all microorganism life (protozoa, fungi, bacteria, and viruses). Sterilization of culture media can be done using hot steam at a temperature of 121^{o} C, with a pressure of 15-17 psi, for 20-30 minutes. The tool used for sterilization is an autoclave. Sterilization of the media should not be too long because it can cause the breakdown of sugars, degradation, vitamins, and amino acids, inactivation of cytokinin and zeatin riboside and changes in the pH of the media. The sterilized media can be stored first in the storage room after at least 3 days of storage and the media is safe from contaminants, the culture media is ready to be used for planting.

Method

Time and Place

This research was carried out at the G10 Agrotech Laboratory, Jl. Sei Bahorok, Babura, Medan City. The research was conducted from June to August 2024.

Sterilization Tools

This method is used mainly for the sterilization of media, liquids and laboratory equipment. Laboratory equipment that can be sterilized using this method is as follows: Equipment made of good quality plastics such as polypropylene, polymethylpentene, polyallomer, Tefzel, polytetraluoroethylene (PTFE), and Telon FEP, Equipment made of glass such as culture bottles, beakers, and pipettes (Sri Wulandari, 2021).

The standard temperature and pressure required in the sterilization process using autoclaves performed at high temperatures for short periods of time is preferred compared to lower temperatures for longer periods of time. Some of the standard temperatures or pressures used are 115 °C/10 psi, 121 °C/ 15 psi, and 132 °C/27 psi. (psi = pounds per square inch). However, in general, the temperature and pressure used are 121°C/ 15 psi (Gupta, N.V. and Shukshith K.S., 2016). The usual time setting with this wet sterilization method is 10 – 15 minutes. This condition is very effective at killing bacterial and fungal spores (Ikenganyia, E.E., M.A.N. Anikwe, T.E. Omeje, and J. O. Adinde, 2017). It is important to note that the sterilization time is calculated after the autoclave reaches its normal state of 121°C and 15 psi pressure, rather than starting at the press of the "on" button (Gupta, N.V. and Shukshith K.S., 2016). In addition, do not leave the culture medium for too long in the autoclave because it can result in chemical changes in the medium so that it can cause poor plant growth (Ikenganyia, E.E., M.A.N. Anikwe, T.E. Omeje, and J. O. Adinde, 2017).

Sterilization is a useful activity to prevent contamination of tissue culture equipment, culture media, and planting materials used. Equipment sterilization can be done by wet sterilization using autoclaves, dry sterilization using ovens, flame sterilization, and sterilization using glass bead sterilizers. Sterilization of tissue culture media can be done using an illustrating membrane under positive pressure and autoclave with a sterilization time adjusted to the volume of the media liquid (Sri Wulandari, 2021).

Sterilization Media

Media sterilization starts from mixing all the ingredients needed to make the media, cooking until boiling, and pouring into culture bottles. The medium is poured into a culture bottle of 25 ml, and covered with a bottle cap. Then the media is sterilized using an autoclave with a temperature of 121^0C for 30 minutes. The sterilized media is stored in a culture chamber for 6 days before use.

Results and Discussion

Bacteria

Contamination caused by cysts is characterized by the presence of mucus on the surface of the media. Bacteria grow on the incoming tissue culture media due to lack of sterility in the media, lack of cooking in the media manufacturing process and occur due to the residual water left behind in the sterilization process "evaporation during autoclave"

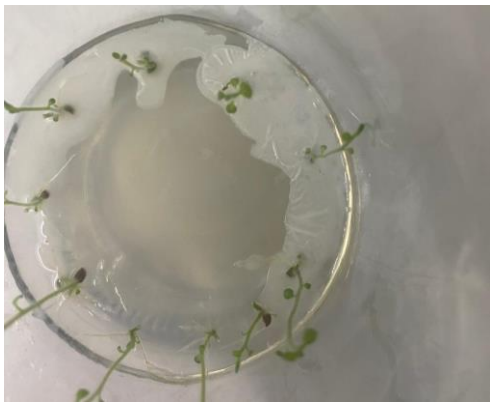

Figure 1. Bacteria

Mushroom

Contamination is marked by white to gray hyphae that cover the surface of the culture medium. This is proven by the difference in the color of the mushroom in the culture bottle such as mushrooms that are white and also black. The group of fungi that have unblocked hyphae belong to the kingdom chromista, protozoa and the class of zygomycetes dapus. This group of fungi produces a large number of spores in the sporangium. The group of chromista and protozoa fungi will form spores with flagellates called zoospores, in this study no fungi of contaminants that produce zoospores were found. The class zygomycetes produce spores within the sporangium with sporangiums with sporangiums with a simple spore stalk shape. The microscopic character of Groups III and IV leads to the fungal characteristics of the class zygomycetes due to the direction of spreading growth and rough surface, having unblocked hyphae and producing spores in the sporangium. According to oratmangun, species of the zygomycetes class found in tissue culture contamination are Rhizopus sp and Mucor sp. In this study, the Mucor sp type was found because the white or grayish-white colonies had spores and unblocked hyphae (Desta Andriani, et.al.2021).

Figure 2. Mushroom

Conclusion

Failure is caused by contamination in the form of fungi in the media and planting material. Contamination is caused by many things, one of which is the imperfection of the sterilization process. In addition, the incubation place, which is considered clean, is actually not sterile. Coupled with the discontinuity of the air conditioner, the temperature of the laboratory rises under certain conditions so that at that time microbes enter the culture bottle, if the bottle cap is not strong.

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