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Culture media used in the proliferation of edible mushrooms of the *PLEUROTUS* genus

Medios de cultivo utilizados en la proliferación de setas comestibles del género *PLEUROTUS*

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ABSTRACT

The present work is carried out with the objective of defining the culture media referred by the literature for the proliferation of the *Pleurotus* genus of edible mushrooms and assessing their application in the conditions of Cuba. The diversity of this genus includes at least 30 species, among them, *P. djamor*, *P. florida*, *P. pulmonaryius*, *P. sajor-cajou*, *P. citrinopileatus* and *P. ostreatus*, for which it is necessary to investigate the properties of the media used in the cultivation of the genus. Their classifications are exposed, according to the nature of their ingredients, the growth promotion of various microorganisms and their purpose, as well as their use. The constituents and conditioning of culture media for edible mushrooms, the most frequent preparation methods used in the preservation of *Pleurotus* strains, and possible defects in them, caused by incorrect handling, contaminants, pests and diseases, are disclosed, as well as the consumption and marketing of mushrooms.

Keywords: Microorganisms; Edible mushrooms; Culture media; Mycelium; *Pleurotus ostreatus*.

RESUMEN

El presente trabajo se realiza con el objetivo de definir los medios de cultivos referidos por la literatura para la proliferación del género *Pleurotus* de setas comestibles y valorar su aplicación en las condiciones de Cuba. La diversidad del género abarca al menos 30 especies, entre ellas, *P. djamor*, *P. florida*, *P. pulmonarius*, *P. sajor-cajou*, *P. citrinopileatus y P. ostreatus*, por lo que se hace necesario indagar en las propiedades de los medios utilizados en el cultivo del género. Se exponen sus clasificaciones, según la naturaleza de sus ingredientes, la promoción del crecimiento de diversos microorganismos y su finalidad, así como su utilización. Se dan a conocer los constituyentes y acondicionamiento de medios de cultivo para setas comestibles, los métodos de preparación de los más frecuentes utilizados en preservación de cepas de *Pleurotus y* posibles defectos en los mismos, provocados por una manipulación incorrecta, contaminantes, plagas y enfermedades, así como el consumo y comercialización de setas.

Palabras claves: Microorganismos; Setas comestibles; Medios de cultivo; Micelio; *Pleurotus ostreatus*.

1. INTRODUCTION

According to (Sánchez and Royse, 2001) edible mushrooms are macroscopic organisms that do not have chlorophyll and therefore are heterotrophs. They live on various organic materials and substrates which decompose in order to feed themselves. This is of vital importance for the reintegration of nutrients to nature and is part of the biogeochemical cycles. They absorb food molecules that can be nutrient-rich soil, manufactured food products, and the bodies of plants and animals, either dead or alive.

These authors affirm in their description that they are generally formed by intertwined hyphae that have the appearance of cottony white masses; these are called mycelium and are buried in the substrate. The mycelium is the most important part of the mushrooms which can live for many years. The growth of the mycelium only occurs at the tips, so it forms fructifications or radially plushy masses, which corresponds to the macroscopic structure of the mushrooms known as "fungus" or "mushroom", and constitutes the reproductive part by generating spores in the hymenium, which are also the basis for the identification of the fungus. However, according to (Sánchez, 2015) mushrooms are really the fruiting bodies or reproductive organs of some species of fungi such as basidiomycetes.

The cultivation of edible mushrooms is an ecological bioconversion system that human beings have used for thousands of years to obtain a food rich in vitamins and minerals, with beneficial protein values for the body and without the harmful effects of cholesterol and fats present in other foods, which is why they are highly desired for the human diet. According to (Holgado *et al.*, 2019) he refers that it is a crop that allows farmers to innovate in the capabilities of this biotechnology, and later replicate it in various communities and with different substrates.

The theoretical contributions of (Marín *et al*., 2018) are also very valuable, stating that edible mushrooms constitute a cultivable and renewable natural resource, for which their production and consumption must be adequately promoted among the population by both research and consulting institutions, in order to produce the adequate biological material or appropriate inoculum in terms of viability and efficiency, as well as to accede to the proper economic technology that guarantees its acceptable adoption and management by producers. They emphasize that the cultivation of mushrooms is an activity developed worldwide, being the greatest exponents of this activity the countries of Southeast Asia, Europe and the United States.

Likewise, (Quintana *et al*., 2018) ensure that in recent years the *Pleurotus* genus is the most studied and cultivated edible mushroom due to its ease of cultivation and nutritional quality. This fungus develops in nature preferably on residues of woody or fiber-rich material such as trunks, branches and bagasse. For its cultivation, materials that contain a similar composition to those used to grow in its natural environment can be used.

In recent years several authors have evaluated numerous mediums for cultivation of oyster mushroom (*Pleurotus ostreatus*), ranging from wheat straw, newspaper, pine needles and their mixtures (Jamil *et al*., 2019); wheat straw (Patel *et al*., 2019); wheat straw, water hyacinth and barley straw (Hussein *et al*., 2019); waste paper supplemented with corn stalk and wheat bran (Tesfay *et al*., 2020); rice husk (*Oriza sativa* L.), coffee husk (*Coffea arabica* L.) and sawdust (sawn wood particles) (Cruz *et al*., 2020); cellulose fibre rejects from industrial-scale recycling/pulping of waste paper (Grimm *et al*., 2021); sawdust and different organic manures (Orngu *et al*., 2021); banana leaf-midribs sticks (Chouhan *et al*., 2022); rice straw, wheat straw, corncobs, saw dust, rice husk, and sugarcane bagasse (Akter *et al*., 2022); and Potato Dextrose Agar, Sabouraud Dextrose Agar, Brain/Heart Infusion Agar and Yeast Extract Agar (Angulo *et al*., 2022).

Pleurotus are edible mushrooms, recognized for their high nutritional value. A wide range assumes the generic name of *Pleurotus*, and they have a mixture of water, carbohydrates and lipids. Its proteins are of high biological quality and contain nine of the essential amino acids necessary for mankind, including lysine and methionine. In addition, they are a source of vitamins, fibers and minerals, together with its medicinal properties (Fernández, 2021).

In accordance with all of the above, (Fernández, 2018) argues that, since olden times, ancient civilizations, such as the Chinese, Greek and Roman, used them for food and medicinal purposes; meanwhile the Aztecs used them in magical-religious rituals. For the most part they do not give a specific flavor to meals, and they can be enjoyed in a natural way, such as salads or accompanied by meat or eggs. It has been shown that they improve and strengthen the immune system, and increase the body's resistance against diseases and bacteria. They also have anti-inflammatory properties and help reduce the suffering of people with asthma, arthritis, kidney failure or brain hemorrhage.

From the bibliographic review accomplished on the subject, it was verified that in Cuba there have been attempts to cultivate them since 1989. However, the authors agree with (Fernández, 2018) when he states that for most Cubans, edible mushrooms are completely unknown and distant from the traditional food taste, an obstacle against which we will have to fight if we want to promote their consumption and include them in the Cuban diet in the future.

With regard to culture media, these constitute a very valuable tool for microbiologists, since they make it possible to recover the infectious agent, which at a certain moment may be affecting an individual or contaminating a food, an environment or a surface, and has made it possible to investigate various aspects, both of bacteria and mushrooms, which facilitate or promote the reproduction of certain microorganisms.
Therefore, the objective of the research is to know the most used culture media for the proliferation of the *Pleurotus* genus of edible mushrooms, which responds to a task of the research project titled "Development of technologies for the production of food from edible mushrooms in the agri-food chain" carried out by the University of Camagüey, Cuba.

2. MATERIALS AND METHODS

The information was extracted from the Google Scholar and Scielo database; in addition, several books and articles were consulted. The search strategy combined different keywords and logical operators: culture media and *Pleurotus* genus.

3. RESULTS AND DISCUSSION

(Barba and López, 2017) concluded that the diversity of the *Pleurotus* genus involves at least 30 species, including *P. djamor*, *P. florida*, *P. pulmonaryius*, *P. sajor-cajou*, *P. citrinopileatus* and *P. ostreatus*. Table 1 shows the taxonomic classification of the *Pleurotus ostreatus* specie.

Table 1. Taxonomic classification of *Pleurotus ostreatus*.

Kingdom	Fungi
Division	Basidiomycota
Subdivision	Basidiomycotina
Class	Basidiomycetes
Subclass	Holobasidiomycetidae
Order	Agaricales
Family	Tricholomataceae
Genus	*Pleurotus*

Specie	*ostreatus* (Jacq.: Fr) Kummer

Source: Own elaboration.

In the opinion of (Barba and López, 2017) the *Pleurotus* spp. is characterized by: being a genus of edible mushrooms, whose morphological characteristic in the tonality of the cap is variable and ranges from white, yellow, brown, gray to pale blue. It measures from 6 cm to 15 cm in diameter, since its size varies according to age and the conditions under which it has grown. The shape of the hat also depends on age, at first it is rounded and then it opens and widens, becoming more convex until it flattens, then the edge is raised and the whole ends up having a concavity similar to a dish.

In the lower portion of the hat there is a set of structures called sheets, which are white and form the hymenium, and where the basidiums are found. These last ones contain the spores that are sexual considering the fertile part. The spores seen under the microscope are elongated, almost cylindrical and measure from 7 µm to 11.5 µm X 3 to 5.6 µm in diameter. The cap and the foot also have a superficial cover that protects them and is called the cuticle. Regularly, the fungus that has a somewhat short foot or stipe has a pleasant flavor.

Its cultivation has had a rapid development and acceptance in the market, due to its nutritional properties, flavor and consistency, and in small and medium-sized industries due to the variety of residues and organic materials at which it is capable of growing, and in particular with wide temperature intervals. Regarding nutritional content, *P. ostreatus* contains most of the essential amino acids and minerals; includes vitamins such as thiamin (B1), riboflavin (B2), ascorbic acid, nicotinic acid, and pantothenic acid; folic acid, tocopherol, pyridoxine, cobalamin and provitamins such as ergosterine and carotenoids.

They also contain essential minerals such as calcium, phosphorus, potassium and iron, in addition to their low fat, carbohydrate and sodium content, which make them valuable against cardiovascular ailments and hypertension, in addition to being characterized by its organoleptic properties, reflected in their appearance, pleasant aroma, and use for the preparation of numerous dishes.

3.1. Culture media

Based on the generalization of the composition of existing media and their use in different fields, culture media have been defined by various authors, including: (Rodríguez and Zhurbenko, 2018; Barba and López, 2017).

(Rodríguez and Zhurbenko, 2018) underline that culture media are a set of elements or substances that guarantee microorganisms or other cells the necessary nutrients for their conservation and/or development. Hence, these elements or substances can be of organic or inorganic, natural or artificial origin. Its purpose is to guarantee the growth of the cell organism, its identification or differentiation within a group of them and even inhibit the development of others.

For (Barba and López, 2017) the culture medium is a laboratory technique that consists of a gel or a solution that contains the necessary nutrients to allow, in favorable conditions of pH and temperature, the growth of viruses, microorganisms, cells, plant tissues or even small plants. Depending on what is desired to grow, the medium will require some conditions. They point out that they are generally dried in the form of a fine or granular powder before being prepared; because already prepared they can be in a solid, semi-solid or liquid state. They suggest that the ultimate goal of the culture medium is varied: antibiogram, identification or multiplication.

For the authors of this work, the arguments proposed by (Rodríguez and Zhurbenko, 2018) and (Barba and López, 2017) are of interest because they show that a culture medium is the set of nutrients, growth factors

and other components that provide the necessary substances for the development of microorganisms such as bacteria, molds, microscopic and macroscopic fungi. They assume that the metabolic diversity of these microorganisms is considerable enough that the variety of culture media is enormous, and there is no universal culture medium suitable for all of them.

In addition, it is indispensable to emphasize that the work accomplished by (Rodríguez and Zhurbenko, 2018) covers all the elements necessary for the development of various microorganisms. In addition, multiple conditions of the media are reflected which are essential for the growth of bacteria and plants, thus this is taken into account in this work.

Classification of culture media: As pointed out by (Rodríguez and Zhurbenko, 2018), culture media can be classified taking into account very diverse criteria. So far, there is no single classification; only some authors or commercial firms agree on the simplest classifications, based, for example, on physical condition, or on the scale on which they are used.

- ✓ Depending on the nature of its ingredients.

Proteinic: Contain peptones, hydrolysates or extracts, that is, products obtained from the lysis or partial hydrolysis of proteins, whose composition is not exactly known.

Synthetic: Do not contain protein products on their formulation.

Intermediates: Contain nitrogenous compounds of protein origin, but of known composition or structure, such as amino acids, together with other known organic and inorganic substances.

- ✓ According to the growth promotion of certain microorganisms.

Enrichment: Generally liquid media (may be semi-solid in some cases) containing substances that stimulate the growth of microorganisms and allow the development of the microbial population from a reduced number of cells of certain germs that can be found in presence of others that, in favorable conditions, stop the development of the former.

Selective: Mostly solid, contain substances that inhibit the growth of certain microorganisms, that is, prevent the growth of undesirable species.

Electives: Tolerate the growth of several species of microorganisms, facilitating at the same time the identification of colonies, ensuring the minimum nutrients necessary for their development.

- ✓ According to its purpose.

Common: Favor the development of most microorganisms without specifically satisfying any nutritional requirement.

Special: Its composition meets the vital nutritional requirements of a specific microorganism that only finds optimal conditions for its development in this environment.

Differentials: Allow the differentiation between microorganisms.

Selective: Include in their formulation ingredients that inhibit the development of certain microorganisms, while contain some substances that promote the growth of others.

Use of culture media: In many fields of science and in different domains of development, culture media are used directly or indirectly. In the opinion of (Rodríguez and Zhurbenko, 2018) its main applications are:

Clinical diagnosis in human and veterinary medicine: Detection of pathogenic microorganisms from fluid, tissue and excreta samples and their disease-causing toxins.

Pharmaceutical and biotechnological industry: Mass cultivation of metabolite-producing microorganisms, and quality control.

Food industry: Mass cultivation of microorganisms that produce nutrients and food, obtaining fermented foods (yogurt, other dairy and food products), and quality control.

Other industries and productive sectors: Mass cultivation of microorganisms, and quality control.

Environmental control: Control of water and supply sources, environmental control, and residual control.

Research: Morphological, functional, systematic and other studies, aimed to understand the microbial flora, research related to the development of substances or products in the aforementioned industries and sectors.

From the point of view of (Rodríguez and Zhurbenko, 2018) and (Barba and López, 2017) culture media in general, are restored in distilled or deionized water. The water must be freshly obtained, free of toxic metals with a pH of 5.5 to 7. If the pH is less than 5.5, it must be boiled to eliminate the CO_2 and check the pH again. The glassware to be used must be made of neutral glass, washed with detergents, avoid the use of a chromic mixture whose residues can be inhibitory to microorganisms, rinse at least twice with running water and then with distilled or deionized water, drain and dry. The container where the medium is prepared must have a capacity that doubles the volume of the medium to be prepared and must guarantee adequate agitation. As a rule, volumes of media greater than 1 L should not be prepared. The powdered medium should be weighed on a scale with adequate precision, preferably using "weighing pots" made of materials inert to the medium, or weighing directly into the container.

The spatulas must be completely dry and must be used only once. Their material must be stainless steel or another material that is inert to the medium. The bottle is opened and handled in a place that does not present high relative humidity and temperature. Handling must be fast and precise, exposing the product to the environment as little as possible. Make sure when you cover the bottle that there are no residues of the medium on the edges of the entrance.

The fundamental aspect to consider is to develop a single microorganism and specifically, the mycelium of the mushroom, without the growth of other microorganisms. To develop the mycelium and obtain the cultivation of various species of *Pleurotus* and other mushrooms, the ideal is to have a small environment or laboratory, isolated from insects, with restricted access, also having specific sites, places or spaces that can be easily cleaned and disinfected. The use of a clean lab coat and a mask is recommended to increase precautions; as well as caps (hair covers), shoes or boots that resist the use of disinfectant.

Equipment such as a pressure cooker or, if possible, an autoclave, a laminar flow chamber or, alternatively, a transfer chamber made with two holes for gloves on the front, with a thick glass top cover and a small lamp or fluorescent tube of ultraviolet light inside, to previously disinfect by irradiation before carrying out the operations.

Constituents and conditioning of culture media for edible mushrooms: As expressed by (Barba and López, 2017), the usual constituents that serve as nutrients or culture media, which are frequently used for the growth and development of edible mushrooms, are:

Agar: It is used as a gelling agent and provides solidity to culture media. In bacteriological agar, the dominant component is a polysaccharide that is obtained from certain marine algae and that has the undoubted advantage of serving as a support for the growth of microorganisms, providing the necessary humidity. An agar gel at a concentration of 1 to 2% liquefies around 100 ºC and gels around 40 ºC, depending on its degree of purity.

Extracts: Its preparation consists in extracting certain animal or vegetable organs or tissues with water and heat, and subsequently concentrating them to the final form of paste or powder. These dehydrated preparations are often used in the preparation of culture media. The most used are meat, yeast and malt extract.

Peptones: Are complex mixtures of organic, nitrogenous compounds and mineral salts, which are obtained by enzymatic or chemical digestion of animal or vegetable proteins (meat, soy, casein). Peptones are very rich in peptides and amino acids, but may be deficient in certain vitamins and mineral salts.

Buffer systems: Are used to maintain the pH within the optimal range for bacterial growth. Sometimes it is necessary to add some components to the culture medium. Because most fungi are not neutrophils, salts such as disodium or dipotassium phosphates, or other substances such as peptones, are often used to prevent sudden changes in pH.

pH indicators: Are compounds incorporated into the culture medium, which, when metabolized, are intended to detect variations in pH, causing a change in color due to acid-base variations. This allows detecting the growth of microorganisms in some area of the culture medium and, on some occasions, to identify characteristic and specific microorganisms. Acid-base indicators such as methyl orange, methylene blue, phenol red, basic fuscin, biliary salts and lactic acid, are the most used.

Reducing agents: With the objective of creating conditions in the culture media that allow the development of microaerophilic or anaerobic germs, these reducing agents are added, being the most used cysteine and thioglycolate, among others.

Selective agents: The addition of certain substances to a culture medium can make it selective. Thus, for example, the addition of crystal violet, biliary salts, sodium azide, potassium tellurite, antibiotics, etc., at the appropriate concentration will act as selective agents and inhibit the growth of certain microorganisms, which can grow and act as competitors.

According to (Ardón, 2007) the cultivation of edible mushrooms requires control over the conditions of the medium. The more environmental factors that can be controlled, the more costs will have to be incorporated into the investment for the crop. Crops are more stable and productive when the cultivation site is air-conditioned, but then production costs rise. However, the conditions of artisanal cultivation are relatively cheap, although they depend on the environmental conditions and are productive at a low level, sufficient for self-consumption and sale of the surplus obtained.

In the artisanal cultivation of mushrooms, a certain regulation can also be implemented over the environmental factors, conditioning the sites in such a way to allow the flow of air streams and the application of irrigation. For the development of the fungal mycelium in the laboratory, solid culture media are used to provide the mushroom with the necessary nutrients for its development. Obtaining an

axenic culture (pure culture) consists of isolating a single microorganism (strain) under laboratory conditions through tissue culture, to produce a clone of descendants.

Tissue culture is a technique used for strains isolation and, therefore, it's also a way of conserving the fungal resource. Conservation refers to the maintenance in optimal conditions of genetic material from wild clones, hybrids, commercial strains, or any other valuable material. It requires a reliable, simple and cheap methodology that ensures not only the conservation of the genetic characteristics of the strains, but also their vitality
.
Below are some simple methods for preparing the most common culture media used to preserve *Pleurotus* strains. It is recommended to consider the following according to (Gaitán *et al*., 2006):

Materials:
- Scale.
- Aluminum foil.
- Distilled or purified water.
- Pressure cooker or autoclave.
- Flasks.
- Erlenmeyer.
- Petri boxes.
- Burners.

Malt Extract Agar.
Ingredients:
- Malt extract: 10 g.
- Agar-Agar: 15 g.

Procedure: The ingredients are weighed and mixed in the flask with 1000 mL of distilled water. The suspension is heated and stirred until the ingredients are completely dissolved.

Potato Dextrose Agar.
Ingredients:
- Potato: 200 g
- Agar-Agar: 15 g
- Dextrose or Glucose: 20 g
- Yeast: 2 g

Procedure: Peel and boil the potato in 500 ml of distilled or purified water for 10-15 min. The extract is filtered and more water is added to adjust to 1000 mL to restock the water that evaporated. Add the other ingredients and heat over low heat, stirring constantly for 1-2 minutes until they are completely dissolved.

Wheat Straw Malt Extract Agar.
Ingredients:
- Wheat seeds: 400 g.
- Malt extract: 10 g.
- Wheat straw: 100 g.
- Agar-Agar: 15 g.
- Dextrose: 10g.

Procedure: Boil the wheat seeds in 1 L of water, letting the liquid consume up to approximately 500 mL; the same are done with the wheat straw. Both extracts are filtered and mixed in a container, the other

ingredients are added and water is added to adjust to 1 L. The suspension obtained is heated until all the ingredients dissolve.

3.2 Obtaining strains

In (Gaitán *et al*., 2006) is declared that the mycelium of a fungus (cotton-like form) that develops on a nutritious culture medium is called a strain. Its isolation can be done through tissue (fragment of the fungus) or through spores, requiring the following materials:

Materials:
- Scalpel or razor.
- Filter paper or sterile bond paper.
- Dissecting forceps.
- Laminar flow chamber or burners.
- Dissection clamps.
- Alcohol or benzol as disinfectants.
- Pipettes.
- Petri dishes with culture medium.
- Beakers.

a) Isolation by tissue
This type of isolation is one of the simplest ways to obtain a strain and the result is an identical copy of the fungus from which the tissue was obtained. In an environment of absolute asepsis, including previously sterilized materials, the fungus is placed, which must be in good condition and free of soil and/or insects. The fungus is cut longitudinally with a knife; with the help of cold, sterile clamps, fragments of the mycelium or fungus meat are placed in Petri dishes containing the culture medium. The boxes with the isolates are incubated between 25-28 °C, preferably in the dark or half-light. About 2 or 3 days later, mycelial growth will be observed in a cotton-like aspect on the surface of the medium. The color will be white or yellowish white, indicating that the isolation was successful. Cultures with the best appearance should be selected and transferred to new boxes containing culture medium.

b) Isolation by spores
To carry out this isolation, you must have a fungus spore, which is obtained by placing the fungus cap with the sheets down on sterile paper for 6 to 8 hours. To avoid contamination and favor a humid environment so that the discharge of spores is carried out properly, the fungus and the paper are covered with a clean and sterile container. After the time has elapsed, the fungus is removed from the paper, leaving it printed with the spores in the form of a radial imprint. It is dried preferably in an incubator for 24 hours at 28-30 °C. Once the spore has been obtained on the paper, a small fragment of approximately 1 cm is cut with a sterile knife or scissors, which is then submerged in 100 mL of cold sterile distilled water, stirring so that the spores dissolve in the liquid.

From this dilution, 0.5 mL are extracted with the help of a pipette and placed in Petri dishes with culture medium. The box is moved slightly to evenly distribute the water with the spores throughout the medium. The dishes are incubated under the same conditions used in tissue isolation, and 5 or 8 days later, the development of the cottony mycelium will be observed. This process must be performed under conditions of absolute sterility.

Table 2 shows the possible defects that could be encountered in culture media, and its possible causes, according to (Rodríguez and Zhurbenko, 2018).

Table 2. Possible defects in the culture media caused by incorrect handling.

Defect	Possible cause
Compacting of the dehydrated product	The container was not closed properly after use. The container remained open for a long time. The container was handled in a place with excessive environmental humidity. The product used exceeded the expiration date.
The pH value does not match the indicated	No deionized or distilled water was used. The deionized or distilled water used was not prepared recently, or its pH was not adequate. The medium was overheated during its preparation. An alkaline glass container was used. The container used was contaminated or dirty. The container was not closed properly after use. The pH was not determined correctly. The product used exceeded its expiration date The dehydrated medium acquired moisture.
Apparition of cloudiness or precipitates	The medium was not evenly distributed before sterilization. The medium was not heated enough. The medium was overheated. Deionized, distilled water was not used. Freshly purified water was not used. The medium was not shaken enough during the preparation. The container used was dirty. The pH value was not adjusted correctly. The prepared medium lost water by evaporation. The medium used acquired moisture. The recommended amount of medium or supplement was not used. The medium was overheated during its preparation. The pH value was not adjusted correctly (in case the medium contains indicators).
The color of the medium does not match the indicated	The container used was dirty. The sugars contained in the medium were caramelized. The recommended amount of medium was not used. The total dissolution of the agar was not guaranteed during the preparation of the medium. The medium was not mixed correctly during its preparation.
The gel is obtained softer than what is characteristic for the medium.	The recommended amount of medium was not used, or excess water was added. The medium was overheated during its preparation. Media preparation with acidic pH values was incorrect. The medium was molten on more than one occasion. The medium was not mixed correctly during its preparation.
The growth of microorganisms is poor	Residues of growth inhibitory substances remained in the containers where the medium was prepared or distributed in water, or in the test material The pH value was not adjusted correctly. The germs in the test material were damaged. In the basal media, an inadequate amount of supplements was added, or the addition was carried out at excessive temperature. The medium used acquired moisture. The culture medium was prepared incorrectly. The product used exceeds its expiration date.

The growth of microorganisms is atypical	The culture conditions were not the recommended ones. Residues of undesirable growth-promoting or inhibitory substances remained in the containers where the medium was prepared or distributed in the water or in the test material The culture medium was overheated

Source: (Rodríguez and Zhurbenko, 2018).

3.3. Contaminants, pests and diseases in culture media

The authors (Gaitán *et al*., 2006) considered that contaminants, pests and diseases in culture media are the main problems faced by mushroom producers. Contaminants generally appear in the incubation phase and this is mainly due to poor substrate pasteurization, poor handling or lack of hygiene at the time of seeding. The main contaminants encountered are fungi (molds), bacteria and yeasts, the most important being fungi such as *Trichoderma*, *Penicillium*, *Aspergillus*, *Neurospora*, *Mycogone* and *Coprinus*, among others.

These fungi appear in the form of green, yellowish, black and/or orange spots on the substrate, rapidly invading it and preventing mycelial growth of the mushrooms. Its presence is favored by the high humidity present in the environment and in the substrate, as well as by high temperature, direct light and poorly pasteurized substrate.

Pests are insects that attack incubating crops and in the production area, attracted by the smell of the substrate. These insects are called "fungus flies" such as dipterans of the genus *Lycoriella*, which place their eggs in the substrate where, at first, they feed on the mycelium of the fungus and later on the adult fructifications. Other common insects in mushroom crops are the so-called "ladybugs": small beetles of the genera *Mycotretus* and *Pseudyschirus* that eat the developing mushrooms.

Diseases that manifest in fruiting bodies are largely caused by bacteria and viruses. These microorganisms spread quickly through water, insects or dirty utensils, so their treatment and control is really difficult. Diseases are favored by excessive humidity, heat and poor ventilation, causing yellow, orange or brown areas to appear in the mushroom caps, which rot quickly and emit a bad smell, thus affecting production yields.

One of the main bacteria that cause these spots on fruiting bodies is *Pseudomonas*. It is worth mentioning that the control of contaminants, pests and diseases depends to a great extent on the hygiene of the personnel and the facilities, that is, periodic cleaning of floors, walls, work tables and utensils must be done.

3.4. Consumption and marketing of mushrooms

From the point of view of (Gaitán *et al.*, 2006) once harvested, the mushrooms can be consumed, marketed fresh or stored. If the objective is fresh marketing, this should be done immediately after harvest, paying special attention to packaging: choose a method that avoids mistreatment, since this reduces quality and thus costs. Also, pay special attention to the size of the mushroom at the time of cutting it (it should be approximately 10 cm in diameter from the pileus) and observe that the edge is smooth and folded downwards.

The type of packaging can be varied: small Styrofoam trays covered with sticky paper, plastic boxes or baskets, Styrofoam boxes, etc. Because it is a perishable food just like vegetables, it is recommended to refrigerate them at 5 °C for no more than 4 days inside a plastic basket at 2/3 of its capacity and covered with adhesive paper with small perforations. The harvested mushrooms should not be placed in plastic bags for any reason, as this promotes its decomposition.

It should be considered that mushrooms lose 1% to 2% of their initial weight per day, so rapid commercialization is important. If it's desired to keep them for a longer time, they can be dehydrated using hot air at a temperature of 35 - 45 °C, or canned (brine, vinegar, etc.). When harvesting mushrooms, avoid throwing them forcefully into the collection container. Eliminate any residue that the mushroom may have, such as straw, dust or some insect, likewise inspect it to eliminate those that may have insect larvae inside the mushroom.

4. CONCLUSIONS

To obtain axenic cultures, several types of media can be used. Listed below are some of the culture media that are frequently used for the preservation and propagation of fungal strains. For the edible mushrooms of the *Pleurotus* genus in Cuba, different culture media are used, the most frequent are: Potato, dextrose and agar (PDA); Agar, potato-dextrose and yeast (APDY); Agar, yeast extract, glucose (AYEG); Malt Extract Agar (MEA) and Sabouraud Dextrose Agar (SDA), one of the most widely used in the country.

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