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FILAMENTOUS FUNGI AS PRODUCERS OF NATURAL PIGMENTS

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Abstract

The filamentous fungi are among the most dominant phylum in the nature and great due to their significant contributions to biotechnological applications for the welfare of human being. Filamentous fungi are widely distributed in diverse ecological niches and produce extraordinary range of secondary metabolites. The pigments from filamentous fungi were used as food colorants, natural dyes in textile industry, cosmetics and pharmaceutical industry. Natural pigments from filamentous fungi serve as a green alternative to synthetic dyes in food colorants, cosmetics and are biodegradable in nature.

Filamentous ascomycetes fungi are known to produce an extraordinary range of colours. There is a wide selection of non-pathogenic strains of filamentous fungi that are non-toxin producers and can be used as potential sources of natural food colorants with improved functionality. The ability of these fungi to grow on residuals of different complexity (e.g., starch-based, lignocellulose-based residuals) is well-documented, showing versatility regarding different processes that can be built around the filamentous fungi. Unlike the use of pigments from vegetables and fruits, the cultivation of ascomycetes does not compete with agricultural land for food production, and therefore, the synthesis of pigments is faster due to time-efficient and simple fermentation processes. The fermentation processes generate high yields of biomass together with value-added products such as pigments, organic acids and alcohols. Interest in food-grade pigments is because of the pigments' ability to enhance the products' natural color in order to indicate freshness, appearance, safety, and sometimes even to add a novel sensory aspect to attract consumers.

A few strains of ascomycetes filamentous fungi being considered as potential pigment producers include, some strains of Talaromyces (e.g., T. purpurogenus and T. atroroseus producing red pigments), Cordyceps unilateralis (deep blood red pigment), Herpotrichia rhodosticta (orange), Curvularia lunata and several species of Drechslera (many different pigments). Strains of these species are promising because they are non-mycotoxigenic and non-pathogenic to humans. Nevertheless, the individual mycotoxin profiles of these strains remain to be explored. Some other pigment producing fungi for their use in the production of potential food colorants are species of Eurotium and Fusarium oxysporum (yellow and red pigments, respectively), Fusarium fujikuroi (red and orange pigments) and strains of Penicillium such as P. citrinum, P. islandicum, P. aculeatum and P. pinophilum. However, several species of Penicillium are able to produce known toxic metabolites and Eurotium spp. and F. oxysporum have been shown to produce mycotoxins as well. The potential production of mycotoxins is a major problem which limits the commercial application of these strains of fungi. This problem, together with the increasing demand for natural coloring alternatives from both customers and regulators, has triggered investigations and screens for other potential pigment-producing genera of fungi.

In this paper we present the isolation from the environment of some new strains of filamentous fungi on PDA agar culture medium, their cultivation in laboratory conditions for the production of pigments in a liquid medium in a submerged system in incubators with orbital agitation – rotary shaker. We also present the optimization of pigment production on different liquid culture media. The subsequent studies will aim the taxonomic identification of each newly isolated fungal strain, the production of mycotoxins, which could prohibit the use of these fungi as potential producers of useful pigments in the food industry, and last but not least, the isolation and biochemical identification of the molecular structure of pigments produced by these fungi.

Key words: *new strains of filamentous fungi, natural pigments, submerged fermentation.*

INTRODUCTION

The filamentous fungi are among the most dominant phylum in the nature and great due to their significant contributions to biotechnological applications for the welfare of human being. Filamentous fungi are widely distributed in diverse ecological niches and produce extraordinary range of secondary metabolites. The pigments from filamentous fungi were used as food colorants, natural dyes in textile industry, cosmetics and pharmaceutical industry.

Natural pigments from filamentous fungi serve as a green alternative to synthetic dyes in food colorants, cosmetics and are biodegradable in nature.

Filamentous fungi are used in numerous biotechnological applications to obtain fermented foods, enzymes, antibiotics, biological control agents, stimulators for plant growth and development, etc.

Recent studies underline the current interest in the use of filamentous fungi in order to obtain the natural pigments produced by them.

This interest comes as a response to the increased demand of consumers from all over the world for natural products, to replace chemical synthesis products or plant and animal products that are currently used to obtain natural pigments used in the food, textile, leather industry, furniture.

MATERIAL AND METHODS

Petri dishes sterilization

It's made in the oven at 180°C for 1 h (from the moment when the oven reached the temperature we fixed).

Preparation of culture media PDA

- Balance;
- Magnetic stirrer;
- pH-meter;

PDA extract:

- Extract of 200 g potatoes;
- 1 liter water;
- 20 g glucose;
- 1.5 g agar (for solid shape);
- pH 5.6 ± 0.2

Sterilization of culture media

It's made in autoclave for about 15 minutes at 115°C 1.2 atm.

Pouring of solid media culture

At the microbiological hood in Petri dishes.

Taking samples of air

To obtain new strains of fungi filamentous producer of pigments.

Obtaining filamentous fungi

From various biological source (seeds and beans) flaxseed, wheat, chickpea, beans.

Optical microscopy studies

Stereomicroscopic and optical analysis for morphological identification of fungi.

The inoculation

Of selected strains in the screening stage for liquid media culture PDA (at microbiological hood).

The cultivation

It's made in orbital shaker incubator at 150 rpm (rotation per minute) at 28°C for 5 days, followed for another 5 days at the room temperature (22-25°C).

pH determination

Of culture media where the fungi grew for the working parameters optimization which affect the production of pigments.

RESULTS AND DISCUSSIONS

The method for sterilizing laboratory glassware by dry heat in a Memmert oven. Sterilization process – 1 hour at 180 degrees C.

Figure 1. Memmert oven

-Preparation of culture media

-The method for using the Precisa electronic laboratory balance

-The method for using the Heildoph magnetic stirrer hot plate

-The method for determining the pH of culture media with electronic WTW pH meter

PDA medium

extract from 200 g potatoes

1 liter of tap water

20 g glucose

1.5 g agar

pH 5.6 +/- 0.2

Figure 2. Laboratory balance and magnetic induction hub

Figure 3. Magnetic stirrer hot plate, bench pH meter

The method for sterilizing culture media by moist heat and pressure in an autoclave – steam sterilizer Raypa.

Figure 4. Sterilization process
15 minutes at 115 degrees C, 1.2 atm

Gravity method for taking air samples – outdoor and indoor

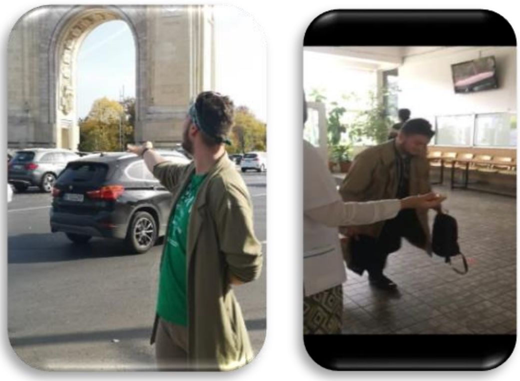

Figure 5. Sampling of outdoor and indoor air samples

Figure 6. Microscopic analysis of the samples

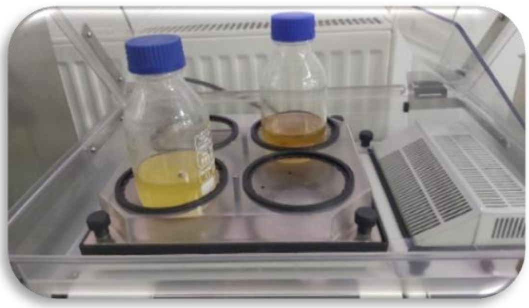

Figure 7. The cultivation

Figure 8. pH determination

CONCLUSIONS

In this paper we present:

- the isolation from the environment of some new strains of filamentous fungi on PDA agar culture medium;
- their cultivation in laboratory conditions for the production of pigments in a liquid medium in a submerged system in incubators with orbital agitation – rotary shaker;
- the optimization of pigment production on different liquid culture media.

The subsequent studies will aim:

- the taxonomic identification of each newly isolated fungal strain;
- the production of mycotoxins, which could prohibit the use of these fungi as potential producers of useful pigments in the food industry, and last but not least,
- the isolation and biochemical identification of the molecular structure of pigments produced by these fungi.

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