Exploring Pigment Production Potentials of Some Ascomycete Fungi in Varying Physical Conditions

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Abstract

Synthetic colors have been widely used in various industries including food, textile, cosmetic and pharmaceuticals. However, the long term toxicity issues related to the synthetic pigments have triggered intense research in natural colors and dyes. Plants, animals and microbes are the sources of natural pigments. Microbial pigments have some advantages over plant and animal based pigments as microbes are fast growing and could be standardized commercially. The production of many currently authorized natural food colorants has a number of disadvantages, including a dependence on the supply of raw materials and variations in pigment extraction. Fungi provide a readily available alternative source of naturally derived food colorants that could easily be produced in high yields.This work aims to the optimization of the fermentation media composition for growth of fungi namely *Aspergillus* and *Fusarium* spp. to achieve maximum pigment production potential of these fungi and comparison of the isolated pigments.

KEYWORDS:*Aspergillus clavatus*, *Fusarium equiseti*, natural colorants, synthetic colors toxicity andtoxicity. And

INTRODUCTION

Hazardous effects of artificial food colorants imply to find out an alternative way of getting these colorants i.e. eco- friendly pigments. Fungi are commonly known to produce a range of natural products called secondary metabolites that have been utilised in the medical, industrial and agricultural fields (Calvo, 2002). Like plants, certain fungi also produce natural pigments or dyes such as betalains, carotenoids, and terpenoids. The fungal pigments have become a major area of research since diverse types of chemically different pigments are being isolated from different classes of fungi.The phylum Basidiomycota has been explored for useful pigments and colourless precursors of these pigments. These findings will allow the extensive study of phylum Ascomycota concerned with isolation of these active compounds, elucidate the structures, and evaluate them as replacements for artificial food colorants. Some fungi that belong to Ascomycota and Basidiomycota have been reported to produce useful pigments and colourless precursors of these pigments (Velisek and Cejpek, 2011).

The growth and metabolic activities are somehow related to the production of secondary metabolites and other useful products in fungi. The temperature and pH and varying nutrient levels have significant effect on the biomass of fungi

(Sanyukta and Srivastava, 2015).Moreover, their antioxidant and antimicrobial nature further adds to their positive effects. In this study the commonly occurring fungi was investigated for its pigment production potential at varying physical conditions i.e. pH and temperature.

METHODS

Acquisition of fungal strains: Fungal strains namely *Aspergillus clavatus* and *Fusarium equiseti*were acquired from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, for pigment production.

Revival of fungal cultures: Fungal strains acquired, were revived and cultured in the medium and at temperature as prescribed by MTCC, namely Czapek Yeast Extract Agar medium (CYEA- K_2HPO_4 1.0g/L, Yeast Extract 5.0g/L, Sucrose 30.0g/L, Agar 15.0g/L and 10 ml of czapek concentrate containing $NaNO_3$ 30.0g/100ml, KCl 5.0g/100ml, $MgSO_4.7H_2O$ 5.0g/100ml and $FeSO_4.7H_2O$ 0.1g/100ml) for *A. clavatus* and Potato Dextrose Agar (PDA- Potato infusion 200g/L, Dextrose 20g/L and Agar 20 g/L) for *F. equiseti* and maintained for further research.

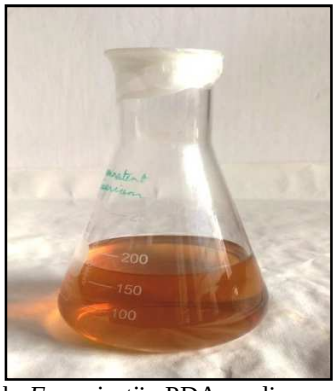
Finding whether the strains are pigment producers: The acquired fungal strains were cultured in the prescribed broth medium to check whether they produce coloured products or not i.e. in 250 ml of Czapek Yeast Extract Broth medium (CYEB) for *A. clavatus* and Potato Dextrose Broth (PDB) for *F. equiseti*respectively,at 25^0C in dark condition.

Effect of pH and temperature: Submerged fungal cultureto optimize the media for pigment production based on effect of physical parameters i.e. temperature of 25^0C and 30^0C and pH ranging from 3 to 9 was done. Each set of experiment was done in triplicates.

RESULTS

The acquired fungal strains cultured in the prescribed broth medium showed colour production in the broth on 6 weeks of incubation at 25^0C. (Fig. 1)

a.*A. clavatus* in CYEA medium



b. *F. equiseti*in PDA medium

Figure 1.Culture filtrates of: a. MTCC 9969 (*A. clavatus*) and b. MTCC 9658 (*F. equiseti*)

Effect of pH and temperature on fungal biomass: A significant change in the colour of the broth medium was observed. Color change from pale yellow to brownish pigmentation was observed.The fully grown culture with mycelia was then separated from the broth on 42nd day to obtain a cell free broth. The mycelia were dried at 60^0C, overnight in hot air oven and weighed for each set of pH and temperature and average mycelial weight was calculated.

Table 1: The dry weight of mycelia (at 25^0C)

Fungal strain *Aspergillus clavatus (MTCC9969) in*	***Mycelial weightin g/L***
pH3	10.160
pH4	8.390
pH5	**11.186**
pH6	9.006
pH7	**7.442**
pH8	7.740
pH9	8.666

Table 2: The dry weight of mycelia (30^0C)

Fungal strain *Aspergillus clavatus (MTCC9969) in*	***Mycelial weight in g/L***
pH3	**21.540**
pH4	13.568
pH5	7.860
pH6	7.950
pH7	7.742
pH8	7.65
pH9	8.0

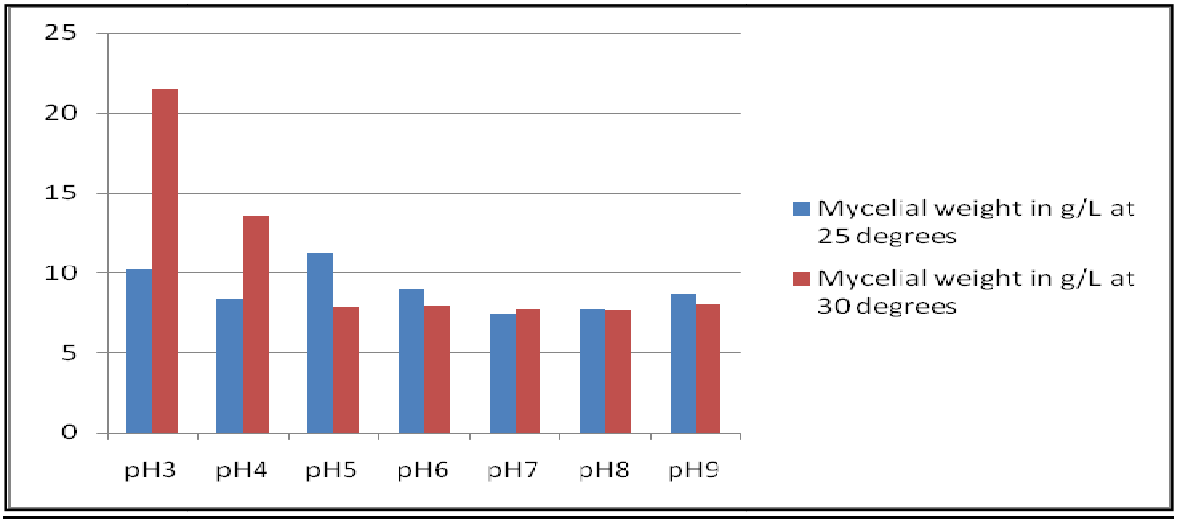


Figure 2. Comparing the Fungal biomass production by MTCC 9699 at pH ranging from 3 to 9 due to temperature (in degree Celsius)

Optimization of media for pigment production: For pH optimization pH was set ranging from 3 to 9 for both the temperatures each in triplicates. The cultures were left for 6 weeks in the incubator set at 25^0C and 30^0C separately. And 2ml of filtrate was collected on completion of every 7 days i.e. on 7th, 14th, 21st, 28th,

35th and 42nd days respectively from each flask and in duplicate and absorbance was measured.

Spectrophotometric analysis: The absorbance of pigment containing broth (crude form) was measured using UV- Vis spectrophotometer at the wavelengths ranging from 200 nm to 700 nm taking the pure sterile broth as reference sample for comparisons to find absorbance λ_{max}. At370nm the filtrate showed maximum absorbance. The absorbance was measured for each experimental set up.

a. Day 1 of inoculation b. Day 42 of inoculation

Figure 3. Increase in color production with time in pH 3 (a. Day 1 of inoculation b. 42nd day from inoculation)

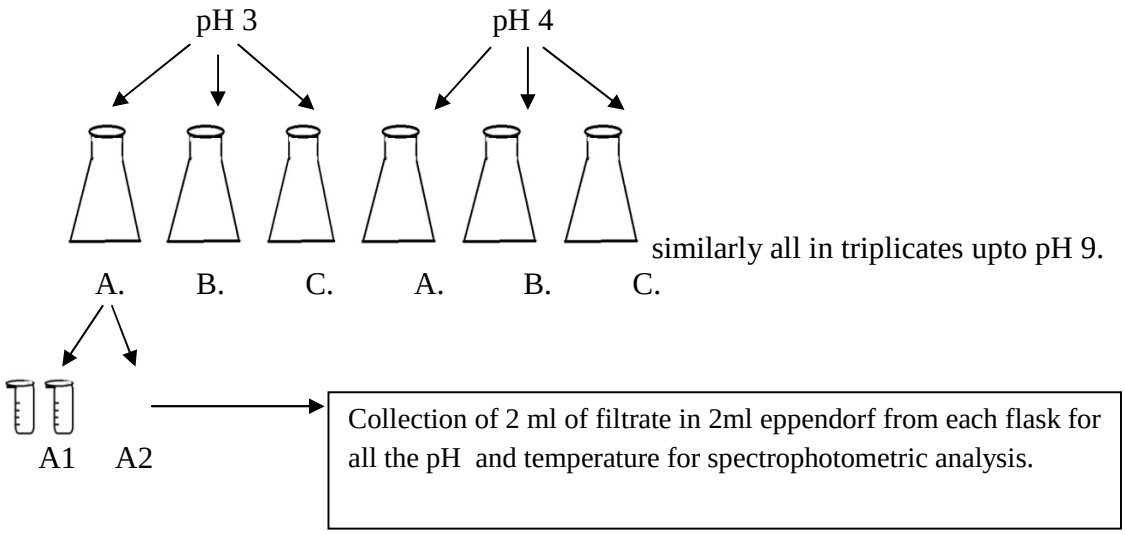


Figure 4. Experimental set up to study the effect of pH and temperature on pigment production(25^0C and 30^0C)

DISCUSSION

At 25^0C, maximum biomass production observed was 11.186g/L at pH 5.At 30^0C maximum biomass production observed was 21.540g/L in the medium having pH 3.Therefore, overall analysis showed that maximum biomass of 21.540g/L was produced at pH 3 incubated at 30^0C by MTCC9699. And this infers that low pH might have resulted in higher biomass production. The absorption maximum was found at 370nm for the cell free filtrates produced from *Aspergillus clavatus.*The pH change in each flask was measured at the end of 42^{nd} day, where all of them have pH increased upto pH 8, which shows that the secreted metabolites make the medium alkaline. This shows that the metabolites or, colorants if any produced in the broth altogether results in increased pH. The spectrophotometric analysis showed that the filtrate had an absorption maximum at 370nm, which reflects that the content might possess an UV active compound. The results show that temperature and pH have significant effect on the growth of fungi as well as pigment production, which further needs the concentration and purification these filtrates to prove them as potent producers of useful pigments.

REFERENCE

- Calvo A, Wilson RA, Bok JW, Keller NP. Relationship between secondary metabolism and fungal development. *Microbiology and Molecular Biology Reviews.* 2002;**3(66)**: 447-459.
- Dufosse L. Microbial Production of Food Grade Pigments, *Food Technol. Biotechnol*. 2006;**44(3)**: 313–321.
- Velisek J, Cejpek K. Pigments of higher fungi – a review. *Czech Journal of Food Science.* 2011;**29**: 87–102.
- Sanyukta and Srivastava AK. Study of Effect of Various Carbon Sources on Biomass and Extracellular Protein of *Aspergillus Fumigatus Strain* (MTCC 1811). *Indian Journal Of Applied Research.* 2015; **5(10)**: 171- 173.