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ORANGE PIGMENT PRODUCTION BY FUNGI *MONASCUS RUBER TIEGHEM IOC 2225*

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ABSTRACT

In this study, the aim was to select a stable strain of *Monascus ruber* capable of producing orange pigment by modifying the pH of the culture medium. A liquid medium was prepared with pH adjusted to 2, seeds were cultured, and five iterations of seeding and repetition were carried out. The results showed robust growth and distinctive orange pigmentation in the colonies cultured on potato dextrose agar (PDA) plates, as well as a notable production of orange pigments in the culture media. These findings suggest the viability of this artificial selection strategy to obtain stable strains with consistent production of only orange pigment which will facilitate the subsequent downstream process.

Keywords: *Monascus ruber Tieghem IOC 2225*; Orange Pigment; membrane filtration; pH adaptation.

1 INTRODUCTION

The global market for natural pigments was valued at around $2.5 billion in 2022, with a projected growth rate of 4.5% by 2026. Pigments can be produced by microorganisms such as fungi, and *Monascus* strains have been used for pigment production. The pigments of *Monascus*, a filamentous fungus, are active ingredients used in various industries such as food, cosmetics, textiles, and medical applications[1]. In the literature, six pigments produced by Monascus have been identified: rubropunctamine and monascorubramine for red pigments, rubropunctatin and monascorubrin for orange pigments, and monascin and ankaflavin for yellow pigments[2]. Although the production of these pigments used to be mainly carried out in solid media, submerged cultivation has emerged as a promising technique for their production[3], as it allows modification of fermentation parameters like pH to aim for yellow, orange, or red pigments. Orange-colored pigments have low solubility in culture media, but upon interaction with amino groups, they turn red and become readily soluble[4]. pH plays a fundamental role in pigment production, with lower pH levels below 3.5 showing increased production of orange pigments[5]. Therefore, in this work, the effect of pH was evaluated through a selection and sequential adaptation at low pH aiming to obtain a modified strain capable of producing only orange pigment.

2 MATERIAL & METHODS

Fungal Strain and cultivation medium

The *Monascus ruber* Tieghem strain IOC 2225 was utilized in this study. The fungi were cultivated on potato dextrose agar (PDA) until use. Initially, the spores were harvested and inoculated into a liquid medium with a pH of 6, composed of glucose (20 g/L), peptone (2.5 g/L), $CaCl_2$ (0.1 g/L), and KH_2PO_4 (5g/L). After cultivation, the fungi were transferred to PDA agar plates and incubated for 7 days at 28 °C. Following this incubation period, the spores were harvested and inoculated into 100 mL of the same medium for fermentation, but with a pH of 3, which was adjusted using sulfuric acid (0.25N). Fermentation was performed at 150 rpm, 28 °C for 7 days. Samples were collected during fermentation for the analysis of orange pigment.

After 7 days, the fungi were transferred to PDA agar for spore production. The spores were then cultivated in liquid fermentation at pH 2, following the previously indicated procedure, and this process was repeated five times (Fig. 1). Subsequently, spores from the 5th cycle were inoculated into liquid media with pH values of 2, 3, 4, and 5. pH 3 exhibited the highest production of orange pigment after 10 days. Under this condition, no red pigment was detected when measured with a spectrophotometer.

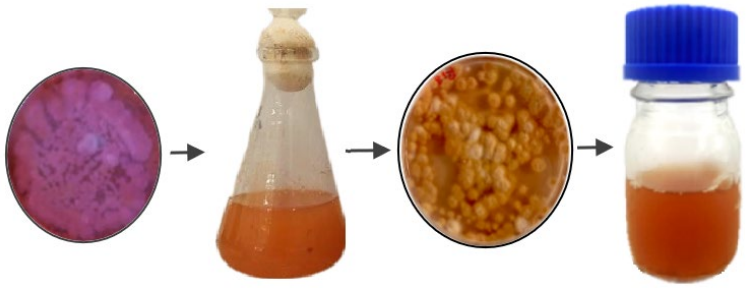

Figure 1. Representative scheme of the study

Concentration of pigment using polymeric membranes

After the fermentation process (10 days), the liquid fraction was recovered by filtration using Whatman No. 4 filter paper. The filter paper was weighed, folded, and placed in a funnel to slowly add the culture medium. Once the liquid culture medium was filtered, the filter paper with the recovered biomass was removed and dried at 50°C overnight. Subsequently, the filtered liquid containing the pigment underwent separation at different pH values through ultrafiltration using a TriSet flat sheet membrane with a molecular weight cutoff of 5000 Dalton; it was then subjected to concentration via nanofiltration using a TriSet flat sheet membrane with a molecular weight cutoff of 150 Dalton; for both processes, a high-pressure stirred cell Sterlitech HP4750 (USA) was utilized.

3 RESULTS & DISCUSSION

In Figure 2, the sugar consumption profile by the fungus during the production of orange pigment in the first fermentation stage at pH 3 is shown. As can be observed, the fungus slowly consumes the available sugar in the medium, reaching a 50% consumption within 10 days, with a production of orange pigment of 1.5 AU at 470nm.The production of orange pigment by this fungus at pH 3 was previously reported by [6], where, using glucose as a carbon source, they managed to obtain orange pigment thanks to acidic conditions.

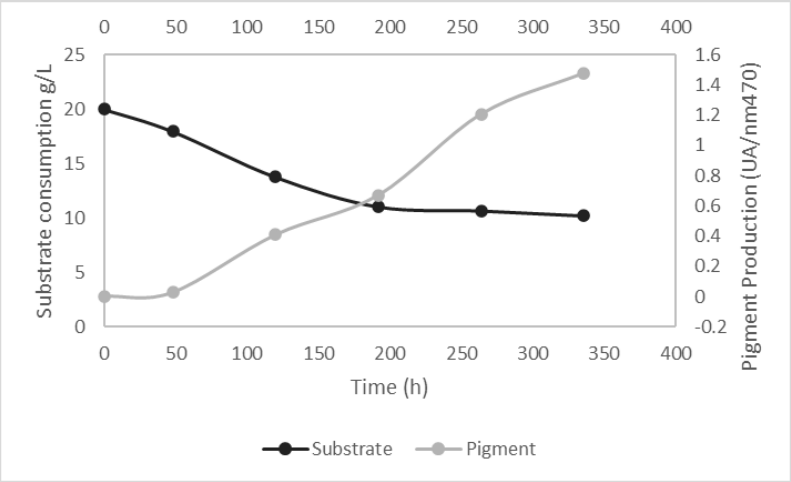

Figure 2 Profile of sugar consumption at pH 3 and production of orange pigment (red strain) by the fungus *Monascus ruber Tieghem* IOC 2225.

Figure 3 shows the sugar consumption profile and pigment production during the 6th fermentation period of 10 days for the orange strain subjected to an acid medium pH 3. In this case, it consumes less than 50% of the substrate and produces 0.505 AU of orange pigment at 470 nm. However, in Figure 2, the pigment produced contains traces of visible red pigment, while in Figure 3, the pigment produced does not show any visible presence of red pigment, this may be due to the fact that a stable strain is being obtained under acidic conditions as this is necessary for the accumulation of orange pigments avoiding amination as reported by 6, which is favourable for the purification of the product.

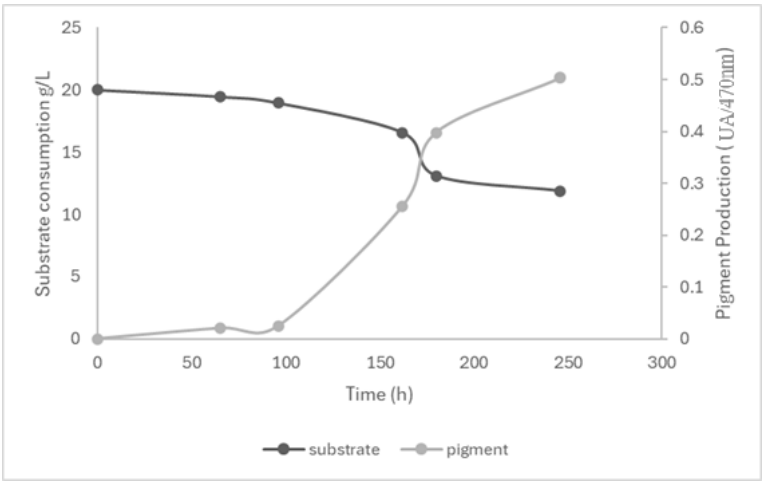

Figure 3 Consumption Profile of Sugars at pH 3 and Production of Orange Pigment (Orange Strain) by the Fungus *Monascus ruber Tieghem* IOC 2225.

Figure 4 Visual appearance of *Monascus ruber* colonies cultivated on PDA (A) and Production of pigments in flasks agitated for 7 days of cultivation at 150 rpm, 28°C (B)

Figure 4 depicts the image of culture media adjusted to pH 2, highlighting the production of orange pigment. Research shows a favorable response in the production of orange pigments at 300 rpm[5]. Furthermore, quantitative PCR techniques demonstrated that the fungus *Monascus ruber* positively responds to the production of orange pigments at a more acidic pH compared to neutral pH[6].

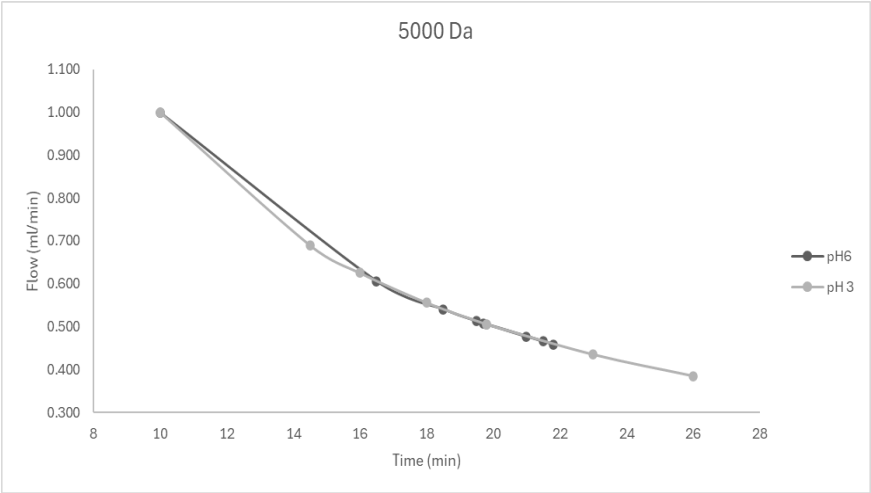

Figure 5 Separation Flow at Different pH Levels by Ultrafiltration Using a TriSet Flat Sheet Membrane with a Molecular Weight Cut-Off of 5000 Daltons

In Figure 5, a separation was conducted at different pH levels set at 3 and 6. As observed, there is no significant difference; however, this process was also used in the purification of the orange pigment, resulting in approximately 42.5 UA at a wavelength of 470 nm in the retentate.

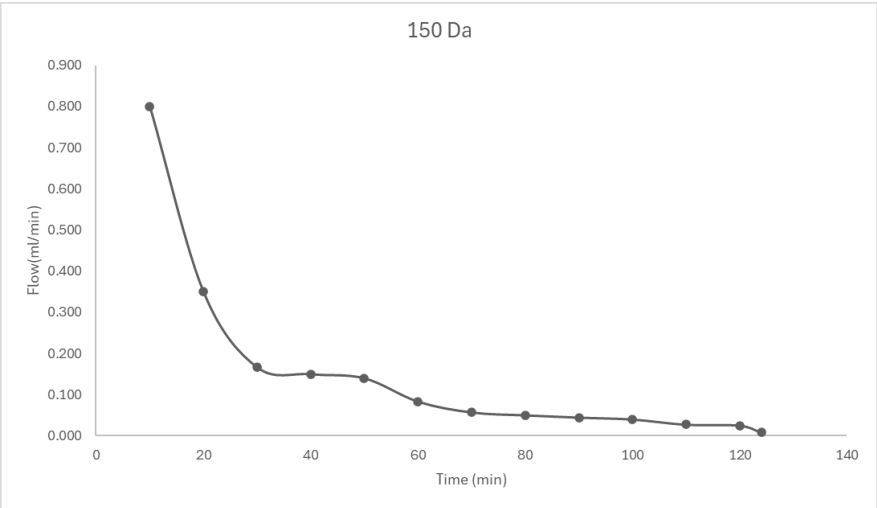

Figure 6 Concentration Flow by Nanofiltration Using a TriSet Flat Sheet Membrane with a Molecular Weight Cut-Off of 150 Daltons.

In Figure 6, the concentration of the pigment was achieved through nanofiltration, resulting in an absorbance of 94.2 AU at a wavelength of 470 nm. This concentration is suitable for subsequent textile dyeing.

4 CONCLUSION

The modification of the pH in the culture medium to acidify it significantly influenced the production of both extracellular and intracellular orange pigments. Qualitative preliminary results show that repeating the process for five cycles, aiming to select

strains that maintained the ability to produce orange pigment, demonstrated the viability and effectiveness of this artificial selection strategy. We can also highlight the efficacy of microfiltration and nanofiltration as methods for the purification and concentration of biomolecules of interest, obtaining 94.2 UA at a wavelength of 470 nm. However, it is recommended to complement these findings with molecular analyses to identify any molecular-level changes.

REFERENCES

1 HE, J., JIA, M., LI, W., DENG, J., REN, J., LUO, F., BAI, J., & LIU, J. 2022. Crit Rev Food Sci Nutr. 62 (26). 7139-7153
2 GONG, P., SHI, R., LIU, Y., LUO, Q., WANG, C., CHEN, W. 2023. Lebenson Wiss Technol. 185 (0023-6438). 115-162
3 OLIVEIRA, C.F.D., CARDOSO, L. A. DA C., VENDRUSCOLO, F. 2022. Front Sustain Food Syst. 6 (2571-581X).
4 VENDRUSCOLO, F., SCHMIDELL, W., DE OLIVEIRA D., NINOW, J. L. 2017. Bioprocess Biosyst Eng. 40 (1). 115-121.
5 CHOE, D., SONG, S. M., SHIN, C. S., JOHNSTON, T. V., AHN, H. J., KIM, D., KU, S. 2020. Foods. 9 (7). 858.
6 LI, L., CHEN, S., GAO, M., DING, B., ZHANG, J., ZHOU, Y., LIU, Y., YANG, H., WU, Q., CHEN, F. 2019. Appl Microbiol Biotechnol 103 (20) 8393–8402.

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