Production and stability of pigment by *Yarrowia*

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Gizella Sipiczki, Olga Lúcia Kovács, Erika Bujna

Hungarian University of Agriculture and Life Sciences, Institute of Food Science and Technology, 1118 Budapest, Villányi út 29-43

INTRODUCTION

Y. lipolytica is the most commonly studied species of the genus *Yarrowia*, initially thought by researchers to be the only representative of its genus. This yeast is known for both its positive and negative effects. Among biological pigments, plant, animal and microbial pigments can be distinguished in terms of their origin. It is known that *Y. lipolytica* synthesizes brown pigment, pyomelanin. Pyomelanin pigment is formed by the accumulation of homogentisic acid around *Y. lipolytica* cells from tyrosine degradation and its subsequent auto-oxidation and polymerization in an alkaline environment (Carreira et al., 2001). Both natural and synthetic pigments are widely used in, for example, the food industry, the textile industry, the papermaking industry, agriculture, and water science. Natural pigments not only increase the marketability of products, they also have beneficial biological activity, such as antioxidant activity and anti-cancer agents, while synthetic pigments have harmful toxicological side effects (Malik et al., 2012).

OBJECTIVES

The aim of the research was to investigate the stability of the pigments produced by each *Yarrowia* strain. The following subtasks were set: optimization of the inoculum in terms of both age and quantity, investigation of the stability of the pigments during boiling, freezing and storage.

MATERIALS AND METHODS

Strains:

Yarrowia lipolytica 6/3
Yarrowia lipolytica 854/4
Yarrowia divulgata 2062
Yarrowia divulgata 5257

Medium: The medium used in the pigment production test contained 4 g / l KH_2PO_4, 2.5 g / l $MgSO_4$ * 7 H_2O, 0.106 g / l $MnSO_4$ * 5 H_2O, 0.27 g / l tyrosine, 1 g / l glycine, 1 g / l L-glutamine, 1 g / l L-asparagine. The medium was prepared with distilled water.

Condition of inoculum optimasition: 28 °C, 6 days, 130 rpm.
Condition of boiling: 100 °C, 30 min
Condition of freezing: -18 °C, 5 month
Condition of storage: light or dark room temperature, light or dark cold, 5 month

Determination of pigment production: the absorbances were determined spectrophotometrically at 400 nm using the supernatant after centrifugation .

RESULTS

Effect of inoculum amount on pigment production

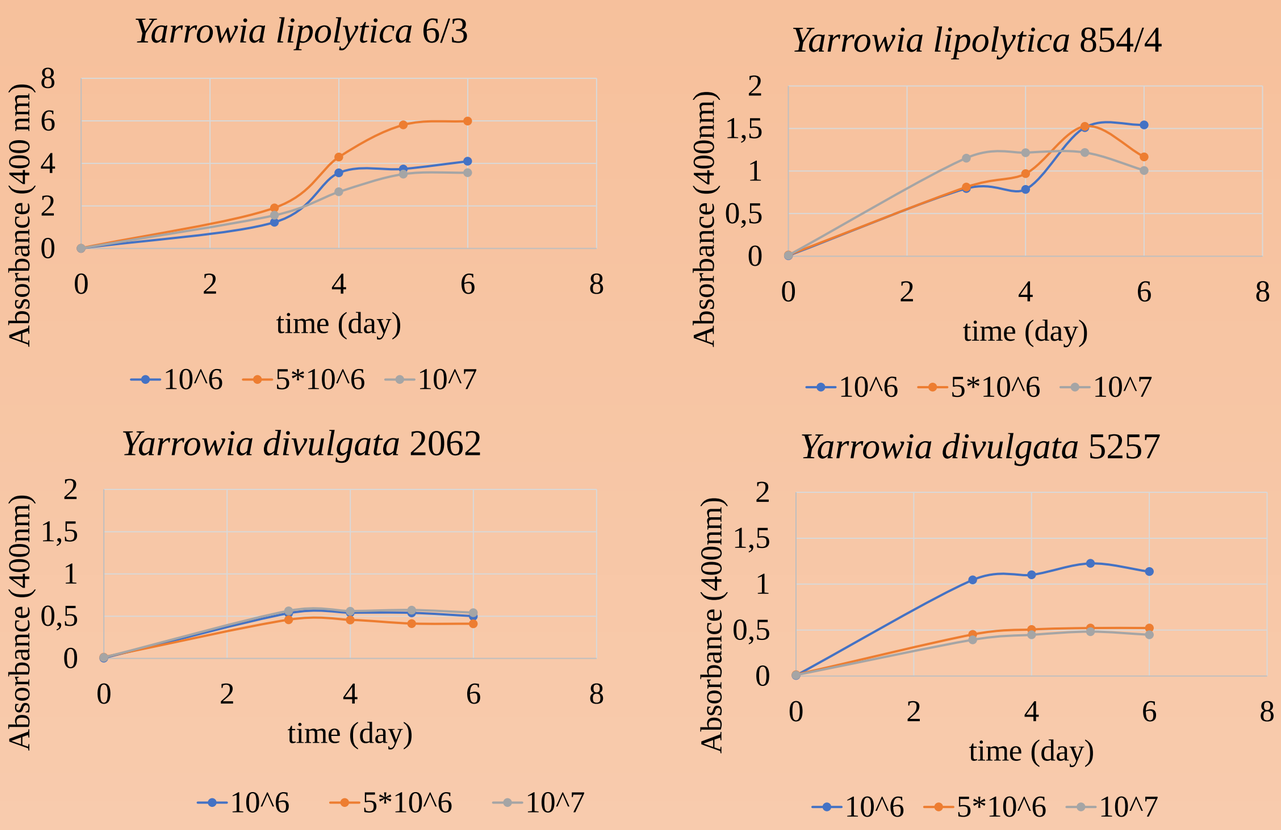

Figure 1. Effect of inoculum amount on pigment production

Based on the results (**Figure 1**), it can be observed that there was significant dye production, and the inoculated cell concentration greatly influences its effectiveness. Different inoculum amounts proved to be most favorable for each strain**, Table 1** shows the recommended cell count for effective pigment production.

Table 1. Optimal cell count of inoculum for pigment fermentation

***Y. lipolytica* 6/3**	***Y. lipolytica* 854/4**	***Y. divulgata* 2062**	***Y. divulgata* 5257**
5 * 10^6 CFU /ml	10^6 CFU /ml	10^7 CFU /ml	10^6 CFU /ml

Effect of inoculum age on pigment production

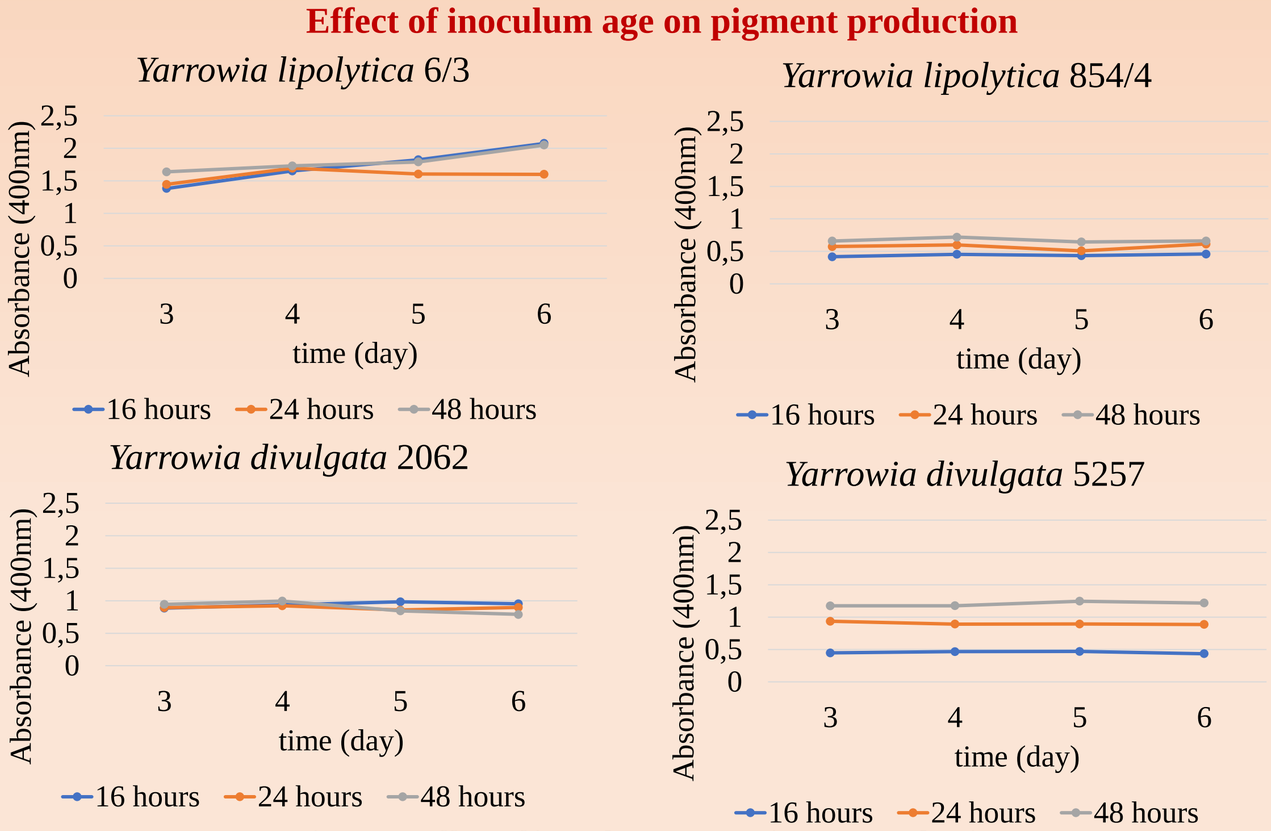

Figure 2. Effect of inoculum age on pigment production

The effect of 16, 24, and 48 h inocula on pigment production was compared during the experiment. Based on the results of the previous experiment **(Table 1)**, the fermentation medium was initiated with the optimal cell count of inoculum for effective pigment production. It can be observed that the older the inoculum, the more efficient the dye production is for most strains, however, there is no significant difference in the efficiency of the 48-hour and 24-hour inocula for either strain (**Figure 2**).

Effect of boiling and freezing on pigment production

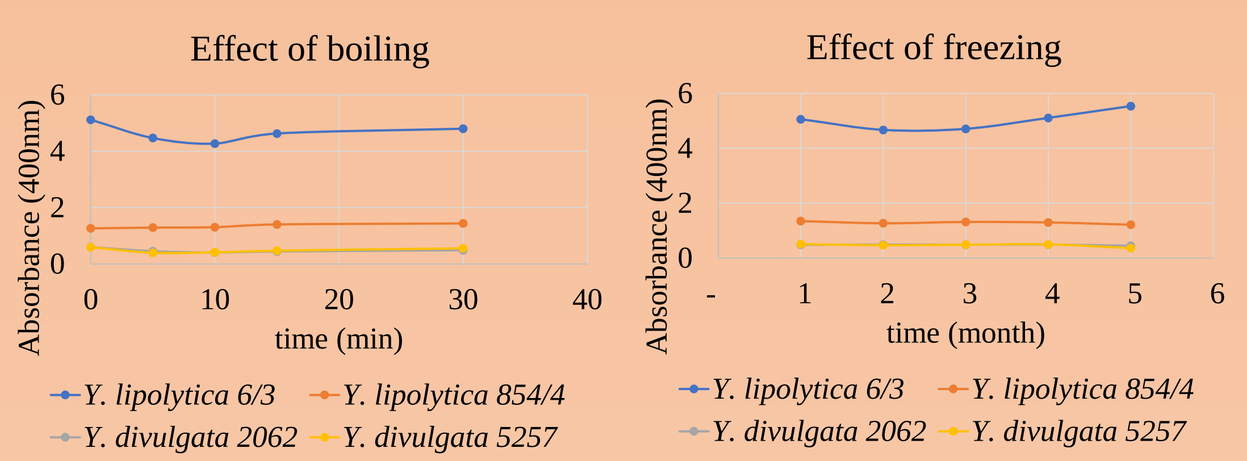

Fig. 3. Effect of boiling on pigment production **Fig. 4. Effect of freezing on pigment production**

From the results shown in **Figure 3**, it is clear that for all strains tested, the pigment proved to be extremely stable throughout the boiling period. In the case of *Y. lipolytica* 6/3 strain a slight non-significant decrease in the values can be seen, in the case of the other strains it can be said that the values are almost unchanged, no decomposition is observed.
The study was performed for 5 months with monthly sampling. Based on the results, it can be concluded that the pigment produced by both *Y. lipolytica* and both *Y. divulgata* strains is very stable (**Fig 4**). No significant change was observed over the months for either strain.

Effect of storage on pigment stability

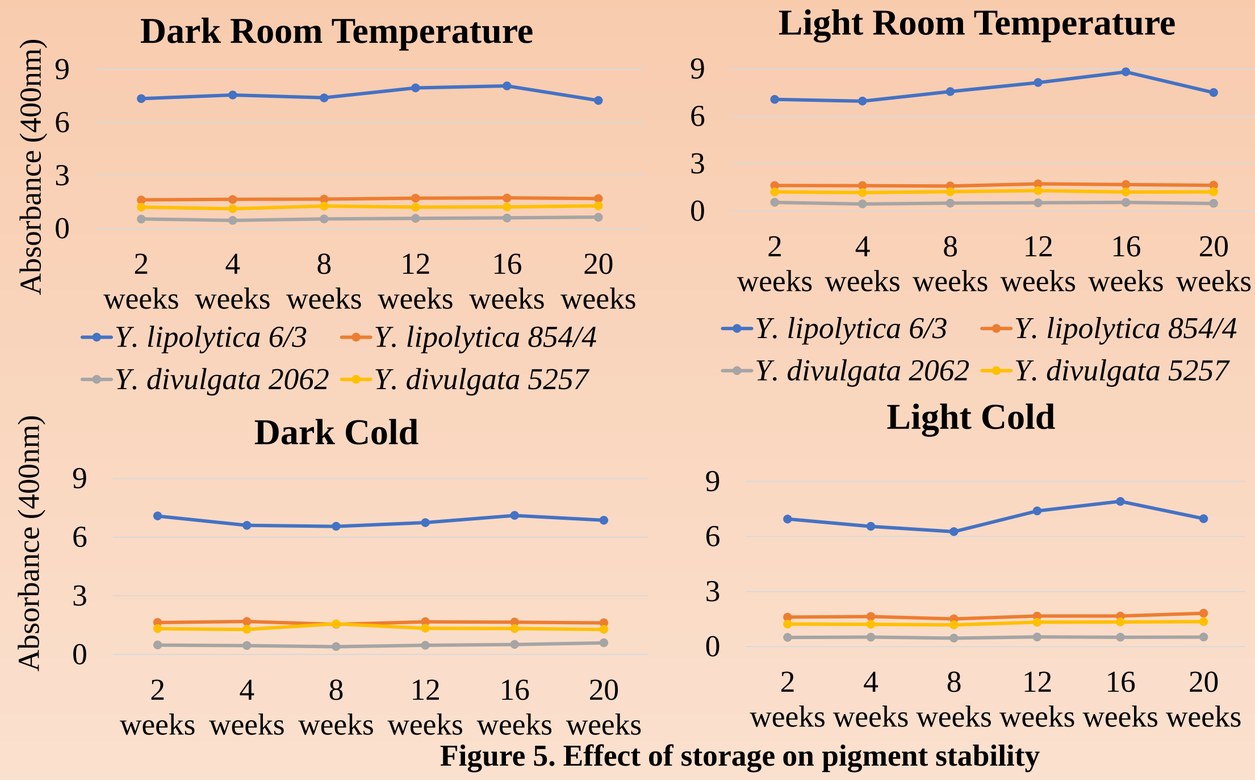

Figure 5. Effect of storage on pigment stability

Storage was performed for 20 weeks under 4 different conditions. Each sample was stored at dark or light room temperature and under dark and light refrigerated conditions, respectively (Figure 5). It can be concluded that the pigment produced by *Yarrowia* remained stable under all storage conditions, with no slight change in color intensity over the weeks.

CONCLUSIONS

- Different inoculum amounts were most effective for each strain
- The 24-hour inoculum is most optimal
- Boiling did not cause decomposition in the pigments produced by any of the strains tested
- The pigments produced by all four examined *Yarrowia* strains during storage at -18°C for
- 5 month is very stable
- The pigments stored at dark and light conditions at room temperature and +4°C fot 5 month are also very stable

References

Carreira, A., Ferreira, L. M., Loureiro, V. (2001): Brown pigments produced by *Yarrowia lipolytica* result from extracellular accumulation of homogentisic acid. *Appl. Environ. Microbiol., 67* (8), 3463-3468.

Malik, K., Tokkas, J., Goyal, S. (2012). Microbial pigments: a review. *Int J Microbial Res Technol, 1* (4), 361-365.