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Effect of aeration and manganese concentration on pigment production by *Yarrowia* yeast

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INTRODUCTION

Natural and synthetic pigments are widely used in the food industry, the textile industry, the paper industry, agriculture and water science. Natural pigments not only increase the marketability of products but also have beneficial biological activities as antioxidant and anti-cancer agents, while synthetic pigments have harmful toxicological side effects (Malik et al., 2012). *Y. lipolytica* is known for its ability to produce melanin pigments from L-tyrosine naturally. The pigments produced by *Y. lipolytica* are presumably pyomelanins, which are formed via the homogentisic acid (HGA) intermediate. HGA accumulates outside the cell, where it undergoes autooxidation and polymerization to form pyomelanin (Tahar et al., 2019).

OBJECTIVES

The aim of the research program is to investigate the pigment production of *Yarrowia* yeasts. Less studied *Yarrowia* yeasts were used in the work. The focus was on increasing pigment production, and the effect of manganese and oxygen concentration were studied.

MATERIALS AND METHODS

Strains: *Yarrowia lipolytica* 6/3 , *Yarrowia divulgata* 445/4, *Yarrowia porcina* 859/4

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 Sörensen buffer pH 8.

Condition of inoculum optimasition: 28 °C, 7 days, 100-160 rpm.

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

Determination of pH: using Mettler Toledo SevenMulti™ pH meter

RESULTS

Effect of aeration

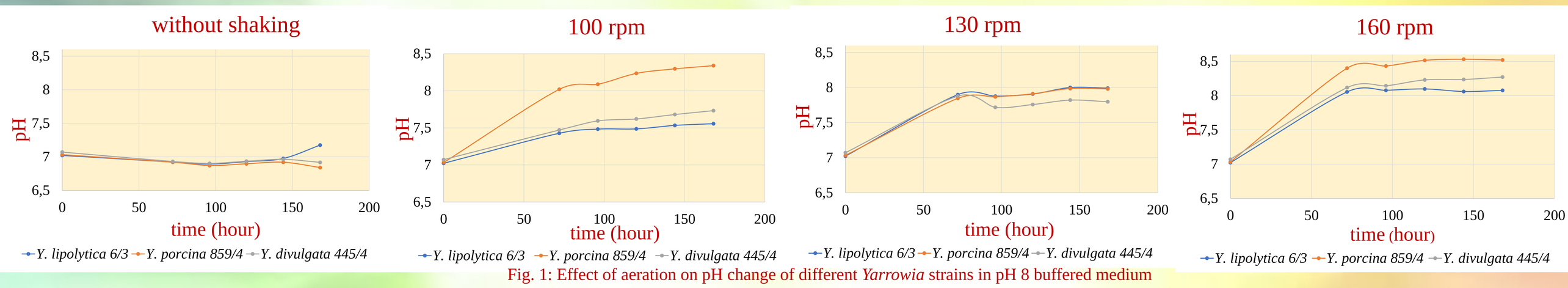

Fig. 1: Effect of aeration on pH change of different *Yarrowia* strains in pH 8 buffered medium

The effect of aeration on pigment production was studied at three different shaking speeds - 100 rpm, 130 rpm and 160 rpm - during one week of fermentation. From the evolution of pH (Figure 1) it can be stated that without shaking some decrease is observed over time, only *Y. lipolytica* 6/3 has an increase at 168 hours, however, at the other three shaking rates there is a continuous increase in pH in all three strains.

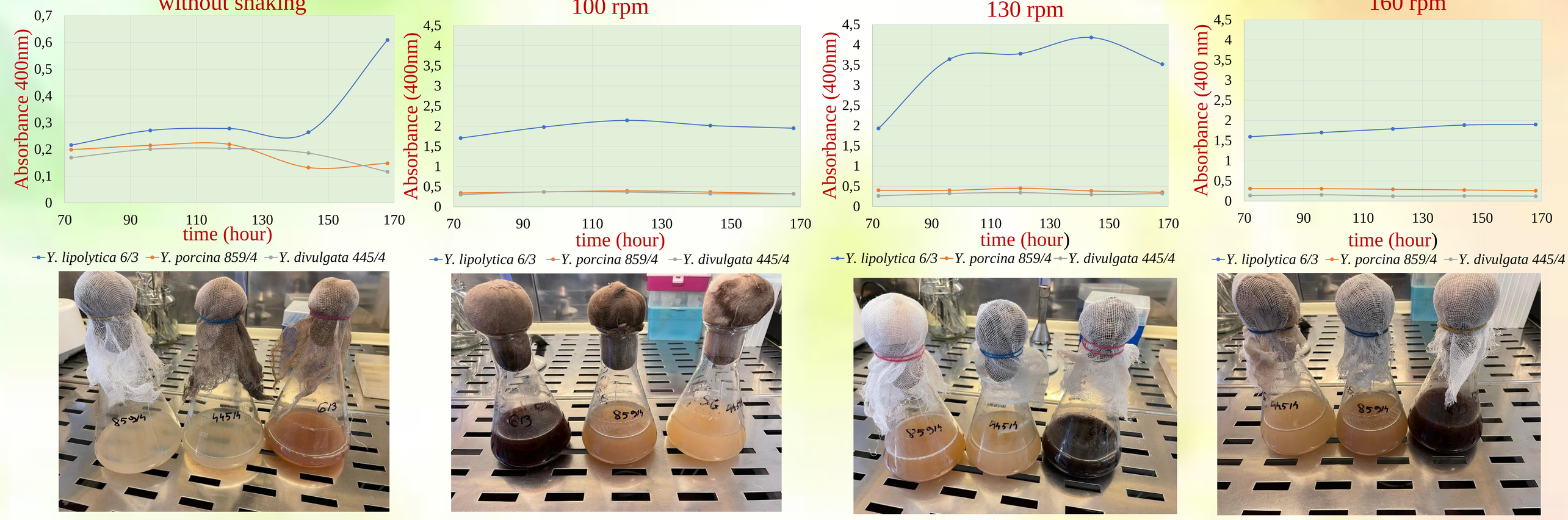

Fig. 2: Effect of aeration on pigment production in different *Yarrowia* strains in pH8 buffered medium

The results of one week fermentation's pigment production (Figure 2) show that the best producing strain was *Y. lipolytica* 6/3 in all settings, however, there is a significant difference between the values of each setting. While the maximum absorbance value did not reach 1.0 for fermentation without shaking, the maximum value was around 2.0 for fermentations at 100 rpm and 160 rpm. This may be because the pH did not reach an optimal pH 8 based on the previous experiment without shaking.. There was only a slight increase in absorbance values after 96 hours at both 100 rpm and 160 rpm shaking speeds. For 130 rpm, the maximum value was measured at 144 hours, which is about double the maximum for 100 and 160 rpm. For *Y. porcina* and *Y. divulgata* the maximum values remained below 0.5 regardless of the shaking rate, however, *Y. porcina* 859/4 proved to be a better pigment producer.

Effect of manganese concentration

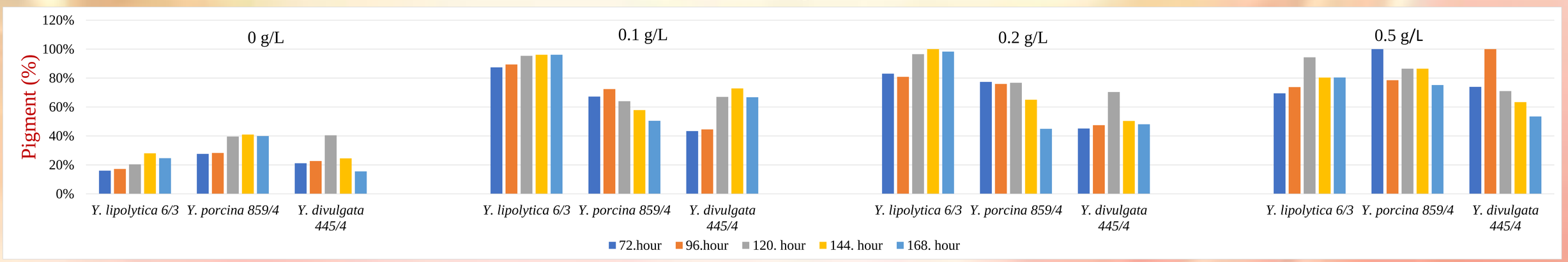

Fig. 3: Effect of manganese on pigment production of different *Yarrowia* strains at 130 rpm shaking speed in pH8 buffered medium

Based on the data in Figure 3, it can be stated that *Y. lipolytica* proved to be the best pigment producer at all four concentrations, the highest absorbance value was measured at 0.2 g/L of manganese for this strain. For *Y. porcina* and *Y. divulgata*, the maximum was given by the use of 0.5 g/L concentration, but at different fermentation times. Of these two strains, *Y. porcina* proved to be a more efficient pigment producer, but both strains significantly lag of *Y. lipolytica* pigment production.

CONCLUSIONS

The degree of shaking affects the pigment production, as all three strains showed higher values during shake fermentation than in stationary cultures. Different shaking rates were most effective for each strain. The presence of manganese has a positive effect on the pigment production of all three *Yarrowia* strains, as yeasts produced less dye in the absence of manganese.

References

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

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