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PIGMENT PRODUCTION BY *Monascus purpureus* CECT2955 IN SUBMERGED CULTURE USING *Aloe vera* HYDROLYZATE

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Introduction. *Monascus purpureus* is a filamentous fungus producing natural pigments widely used in Asian countries like China and Japan (1). It is well documented that *Monascus purpureus* strains can produce three general types of pigments: orange, yellow and red; which are considered as natural dyes. These pigments in comparison with other microbial colorants are highly resistant to high temperatures and extreme pH changes, making them attractive in food, cosmetic, pharmaceutical and textile industries (2). However, few studies have focused on the optimization of the operating conditions to maximize the production of pigments using fungus strains.
The aim of this work was to produce and optimize the production of pigments by *Monascus purpureus* CECT2955 in submerged culture using *Aloe vera* leaves hydrolyzate.

Methodology. *Monascus purpureus* CECT2955 was used. A Taguchi's orthogonal design (L_9, 3^4) was used for the optimization of four factors at three levels. The factors and levels tested were pH (3, 5 and 7), inoculum concentration (5, 10 and 15% v/v), stirring speed (150, 200 and 250 rpm) and nitrogen source concentration (0, 2.5 and 5.0 g/L of NH_4NO_3). For culture media, dehydrated *Aloe vera* was subjected to acid hydrolysis (0.5% H_2SO_4/ 90 min). Subsequently the hydrolyzate was sterilized and inoculated with *Monascus purpureus* CECT2955 mycelium. Pigment production was conducted in 125 mL Erlenmeyer flasks with 25 mL of hydrolyzate enriched with glucose to a final concentration of 20 g/L of carbon source and then were incubated at 30 °C for 7 days. Pigment production was evaluated at the end of the fermentation. Pigment was quantified by optical density (410, 450 and 500 nm for yellow, orange and red pigments respectively) expressed as absorbance units (AU) (3). The results were analyzed using the Qualitek-4 software (Nutek Inc., MI, USA).

Results. *M. purpureus* was able to grow and produce pigments in all treatments analyzed. Different colors were notably observed for each treatment (yellow, orange and red). The individual influence of each factor is shown in Figure 1. It can be seen that for the three pigments produced the pH and agitation are the factors that exert greater influence in pigments production. In contrast, inoculum concentration and nitrogen source were factors with minimum impact on pigment production. The optimum operating conditions for pigments production are described in Table 1. The pigment predicted values were 3.798 UA_{410nm}, 2.832 UA_{450nm} and 3.027 UA_{495nm} for yellow, orange and red pigments respectively. Validation experiment at the optimum conditions (3.175 UA_{410nm}, 2.259 UA_{450nm} and 2.928 UA_{495nm}) showed a good agreement with the theoretical pigments levels.

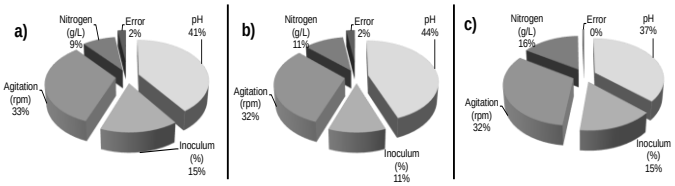

Fig. 1. Influence of each factor on the production of pigment by *M. purpureus* using *Aloe vera* hydrolyzate.
a) Yellow pigments b) Orange pigments c) Red pigments

Table 1. Optimum conditions for total pigment production by *M. purpureus* using *Aloe vera* hydrolyzate.

Optimal conditions	
Factor	Level
pH	7
Inoculum (%)	15
Agitatión (rpm)	200
NH_4NO_3 (g/L)	0

Conclusions. Agitation and pH were the factors with high influence on pigments production by *M. purpureus* CECT2955 using *Aloe vera* leaves hydrolyzate in submerged culture. The maximum levels of pigments were observed at pH value of 5, inoculum of 15% v/v and agitation of 200 rpm.

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