EFFECT OF pH AND TEMPERATURE ON THE FERMENTATION AND FLOCCULATION CAPACITY OF STRAINS OF *Saccharomyces* STORED AT MYCOTHECA-URM .II.

Efecto del pH y la temperatura en la capacidad de fermentación y floculación de cepas de Saccharomyces conservadas en la micoteca -URM .II.

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SUMMARY

The combined effect of pH medium (10% aqueous solution of molasses) and incubation temperature in the expression of fermentation capacity and flocculation of 12 strains of ***Saccharomyces*** *stored at the Micoteca-URM were studied. Of these , 11 strains belong to* ***S. cerevisiae*** *and 1 to* ***S. kluyveri.*** *They were tested at pH 4,5 and 5 and incubation temperatures of 28° C ± 1° C (room temperature), 25°C and 37° C. All the strains fermented in media with both pH rates and in the 3 temperature of incubation. However period of fermentation and flocculation varied according to different pH and temperature.*

RESUMEN

Se determinó el efecto conjunto del pH del medio de cultivo (solución acuosa al 10% de melaza) y de la temperatura de incubacion, en la expresión de la capacidad de fermentación y floculación de 12 cepas de ***Saccharomyces*** *conservadas en la Micoteca-URM. De estas, 11 pertenecen a* ***S. cerevisiae*** *y 1 a* ***S. kluyveri.*** *Se utilizaron 2 valores de pH (4,5 y 5) y 3 temperaturas: 28°C ± 1°C (temperatura ambiente), 25°C y 37°C. Todas las cepas fermentaron en los dos pH y a las tres temperaturas, variando en relación al período de fermentación. Sin embargo, los períodos de floculación y fermentación mostraron variación frente a ambos parámetros.*

INTRODUCTION

Biotechnology has used yeasts in the industrial processes of alcoholic fermentation on account of the high productivity and output rates of such microorganisms (3, 6, 13, 21, 25).

Various factors may act on the fermentation and flocculation of yeasts. They come under three groups: physical factors (temperature, agitation) (5, 8, 16, 18, 20, 26, 27, 30, 33, 34, 35), chemical factors (pH, chemical compounds) (1, 2, 7, 8, 9 ,12, 19, 24, 28, 29, 31, 32) and biological factors (yeast strain, cell concentration) (4, 7, 10, 17, 22, 25).

Sugarcane molasses are an excellent raw material for the fermentation industries since they offer a vast energetic source of nutrition (water, sugar, nitrogenous compounds and others) to the microorganisms responsible for fermentation processes (13, 17, 23).

Thanks to the importance of yeasts for industry as regards the capacity of fermentation and flocculation this paper deals about the combined effect of the pH of the culture medium (a 10% molasses aqueous solution) and incubation temperature, as expressed in the fermentation and flocculation capacity of strains of ***Saccharomyces*** stored at the Mycotheca of the Department of Mycology, Center of Biological Sciences, at Federal University of Pernambuco, registered at the Commonwealth Mycological Institute (ICMI) as URM.

MATERIAL AND METHODS

a) Strains of *Saccharomyces.* From the stock kept in mineral oil, a total 12 strains were supplied by the Mycotheca-URM as follows: 11of ***S. cerevisiae*** Hansen (1337, 1338, 1460, 1807, 1820, 2624, 2658, 2659, 2689, 2690, and 2716) and 1of ***S. kluyveri*** Phaff Muller & Shifrine (1814), which were reviewed taxonomically according to the methods indicated in the general monographs (11, 14, 15).

b) Medium of culture for conservation and growth of strains. Sabouraud agar plus yeast extract (YE), final pH 6.5, distributed in tubes.

c) Molasses aqueous solution (10%). Once ready, this solution (10g of molasses, 100 ml distilled water) was

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Table 1.
Fermentation and flocculation of strains of *Saccharomyces* in 10% molasses aqueous solution with pH 4,5 in different incubation temperatures.

STRAINS	INCUBATION TEMPERATURES											
	RT (28° C ± 1° C)				25° C				37° C			
	INCUBATION PERIODS											
	6h	24h	30h	48h	6h	24h	30h	48h	6h	24h	30h	48h
S. cerevisiae 1337	-	*	*	*	-	*	*	*	-	*	*	*
S. cerevisiae 1338	-	*	*	*	-	*	*	*	*	*	*	*
S. cerevisiae 1460	-	*	*	*	✦	□	*	*	*	*	*	*
S. cerevisiae 1807	-	*	*	*	-	*	*	*	-	*	*	*
S. kluyveri 1814	-	-	*	*	-	□	□	□	-	□	□	□
S. cerevisiae 1820	-	*	*	*	-	*	*	*	-	*	*	*
S. cerevisiae 2624	-	✦	□	□	✦	✦	✦	□	-	✦	*	□
S. cerevisiae 2658	-	*	*	*	-	*	*	*	*	□	□	□
S. cerevisiae 2659	-	*	*	*	-	*	*	*	-	*	*	□
S. cerevisiae 2689	-	*	*	*	-	□	□	□	-	□	□	□
S. cerevisiae 2690	-	*	*	*	-	*	*	*	*	□	□	□
S. cerevisiae 2716	-	*	*	*	✦	*	*	*	*	*	*	*

Table 2.
Fermentation and flocculation of strains of *Saccharomyces* in 10% molasses aqueous solution with pH 5 in different incubation temperatures.

STRAINS	INCUBATION TEMPERATURES											
	RT (28° C ± 1° C)				25° C				37° C			
	INCUBATION PERIODS											
	6h	24h	30h	48h	6h	24h	30h	48h	6h	24h	30h	48h
S. cerevisiae 1337	-	*	*	*	-	*	*	*	-	□	*	*
S. cerevisiae 1338	-	*	*	*	-	*	*	*	-	*	*	*
S. cerevisiae 1460	-	*	*	*	-	*	*	*	*	*	*	*
S. cerevisiae 1807	-	*	*	*	-	*	*	*	-	*	*	*
S. kluyveri 1814	-	-	*	*	-	□	□	*	-	□	□	*
S. cerevisiae 1820	-	*	*	*	-	*	*	*	*	*	*	*
S. cerevisiae 2624	✦	✦	□	□	✦	□	□	□	-	✦	✦	□
S. cerevisiae 2658	-	*	*	*	-	□	□	□	*	□	□	□
S. cerevisiae 2659	-	*	*	*	-	*	*	*	*	□	□	□
S. cerevisiae 2689	-	*	*	*	-	*	□	□	-	□	□	□
S. cerevisiae 2690	-	*	*	*	-	*	*	*	*	□	□	□
S. cerevisiae 2716	-	*	*	*	✦	*	*	*	*	□	*	*

RT = Room Temperature
- = negative
* = fermentation
✦ = flocculation
□ = fermentation and flocculation

filtered twice through hydrophile cotton to retain solid particles in suspension. Next, 2 ml were transferred to test tubes (12 x 120 mm) holding Duhran tubes (7 x 30 mm). The pH 4.5 and 5 were used.

Both Sabouraud agar + 0.5 % of YE and the 10% molasses aqueous solution were autoclaved at 120° C/ 15 min and left at room temperature (RT) 28° C (± 1° C) for 72 hours to control sterilization.

d) Test of fermentation and flocculation. The strains of ***Saccharomyces*** were seeded in Sabouraud agar + 0.5 % YE and left at room temperature for 48 hours. Suspensions with 10 ml of sterilized distilled water were prepared and their concentration adjusted to 104 cells/ml. Of that suspension, 0.3 ml was transferred to 2ml of the 10% molasses aqueous solution for each pH, held in test tubes with Duhran tubes. Incubation took place for 48 hours at RT, 25° C and 37°C and readings made at 6 h, 24 h, 30 h and 48 h.

All the tests were made in triplicate.

RESULTS AND DISCUSSION

All the strains tested fermented in the 10% molasses aqueous solution with both pH 4.5 and 5, at temperatures of RT, 25° C and 37° C, varying in relation to the period of fermentation (Table 1and 2). Alcoholic fermentation was identified thanks to the presence of CO_2 in the Duhram tube and flocculation evidenced by the microscopic observation of flocs. With the strains of ***S. cerevisiae*** 1460, 2658, 2690, 2716, fermentation started after 6 hours of incubation at 37° C with both pH rates 4.5 and 5.0.

With ***S. cerevisiae*** 1338, this occurred with pH 4.5 and for 2659 and 1820 with pH5. With strains 2689, 1337,1338, 1807 at 37° C in both pH tested, fermentation occurred after 24 h; with strain 2624, fermentation started after 30 h with pH 4.5 and 48 h with pH 5.

With strain 2624, pH 4.5 and RT, fermentation took place in 30 hours from start of experiment with an empty Duhran tube; at 25° C fermentation started after 48 hours. With pH 5.0 fermentation took place at 24 h at 25° C, 30 h at RT and at 48 h at 37° C. This suggests that pH 5.0 at 25° C is suitable for fermentation.

With 25° C and RT, with both pH, strains 1460, 2658, 2659, 2689, 2690, 2716, 1820, 1337, 1338, and 1807 of ***S. cerevisiae*** fermented at 24 h.

The strain 2624 at both pHs 4.5 and 5.0 was the only which flocculate at RT; flocculation also occurred at 25°C and 37° C.

With both pH tested strain 2659 and 2690 flocculated only at 37° C; ***S. cerevisiae*** 2689 and ***S. kluyveri*** 1814 flocculated at 25° C and at 37° C.

With pH 4.5 the strains 1460 and 2716 flocculated only at 25° C and 2658 only at 37° C. With pH 5.0 strains 2658 and 2716 flocculated only at 25° C and at 37° C and not at RT.

S. kluyveri (1814) with both pH tested, fermentation started at 24 h at 25° C and at 37° C, and at 30 h at RT.

Strains of ***S. cerevisiae*** 1820, 1337, 1338 and 1807 did not flocculate at both pHs 4.5 and 5.0 at all temperatures .

The findings in this paper show that according to the strain the pH and temperature of incubation may act or not on the capacity of fermentation and flocculation of yeasts. In the case of some strains higher temperatures (37° C) induce to fermentation and flocculation. Also no great variations occurred in the capacity of fermentation and flocculation of the strains with the different pH tested.

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