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FLOCCULATION AND FERMENTATION CAPACITY OF STRAINS OF *Saccharomyces* STORED AT MYCOTHECA-URM. I.

Capacidad de floculación y fermentación de cepas de ***Saccharomyces*** *conservadas en la micoteca-URM. I.*

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Palabras clave: ***Saccharomyces***, fermentación, floculación
Key words: ***Saccharomyces,*** fermentation, flocculation

RESUMEN

Cepas de ***Saccharomyces*** *mantenidas en la Micoteca-URM, fueron estudiadas en relacion a su capacidad de fermentar y flocular en una solución acuosa de melaza a 18° Brix. De estas, 11 pertenecían a* ***S. cerevisiae*** *y 1 a* ***S. kluyveri.*** *El mayor contenido de etanol (6,02% v/v) fue obtenido con la cepa 1460 y el menor (1,39% v/v) con la 2624. Los menores porcentaje de células que permanecen en suspensión(R%), 65,82%, 65,31%, 41,66% y 55,55%, fueron obtenidos respectivamente con las cepas 2659, 2624, 2716 y 1337, indicando una mayor intensidad de floculación.*

Las cepas 2659 y 2716 produjeron una mayor concentración de etanol. Los resultados indican que una cepa de ***Saccharomyces*** *puede o no expresar conjuntamente una buena capacidad de fermentación y floculación.*

SUMMARY

Strains of ***Saccharomyces*** *stored at the Micotheca-URM were studied as regards the capacity to ferment and flocculate in aqueous molasses solution at 18¦ Brix. Of these, 11 belong to* ***S. cerevisae*** *and 1 to* ***S. kluyveri.*** *The highest ethanol content (6,02 % v/v) was obtained with strain 1460 and the lowest (1.39% v/v) with 2624. The lowest percentage of cells which remained in suspension (R%) ,65.82%, 65.31%, 41.66% and 55.55% were obtained respectively with strains 2659, 2624, 2716 and 1337; such results show a greater intensity of flocculation. The strains 2659 and 2716, produced higher concentration of ethanol. The results show that a strain of* ***Saccharomyces*** *may express or not also a good capacity of fermentation and flocculation.*

INTRODUCTION

The Micoteca-URM of the Mycology Department of the Center of Biological Sciencs (CCB) at the Federal University of Pernambuco (UFPE), registered at the Comnonwealth Mycological Institute (CMI) under the abbreviature URM (University of Recife Mycology), has presently 313 strains of yeasts, of which 12 belong to the genus ***Saccharomyces*** Meyen emend. Reess; all of them are preserved in mineral oil and some by lyophilization according to the methods of Sherf (39) and Raper & Alexander (33), respectively.

Due to the increasing utilization of microorganisms in industrial processes and the advances in the area of Biotechnology, the importance of culture collection has been acknowledged at the international level (5, 16).

The genus ***Saccharomyces*** has been widely used by the bread-making industries, animal feed, alcoholic liquors, ethyl alcohol, milk-derived products and other (21, 37).

The different species and even the different samples of a same species may present or not the same morphological and /or physiological properties. Among them: capacity of alcoholic fermentation and flocculation; tolerance to ethanol and carbon dioxide; osmosensitivity and others (3, 11, 17, 23).

Alcoholic fermentation was studied by Pasteur in 1858, but only in 1876 he did discovered the anaerobic facultative character of the beer yeasts. In 1897, the Buchners were pioneers in enzymology obtaining an extract of yeast which fermented sugars and produced ethanol and carbon dioxide ($C0_2$), discovering the role of enzimes (endocellular substances) in the alcoholic fermentation which includes a sequence of enzimatic reactions triggered when a yeast with a fermentative capacity makes contact with the substratum (6, 7, 37, 49).

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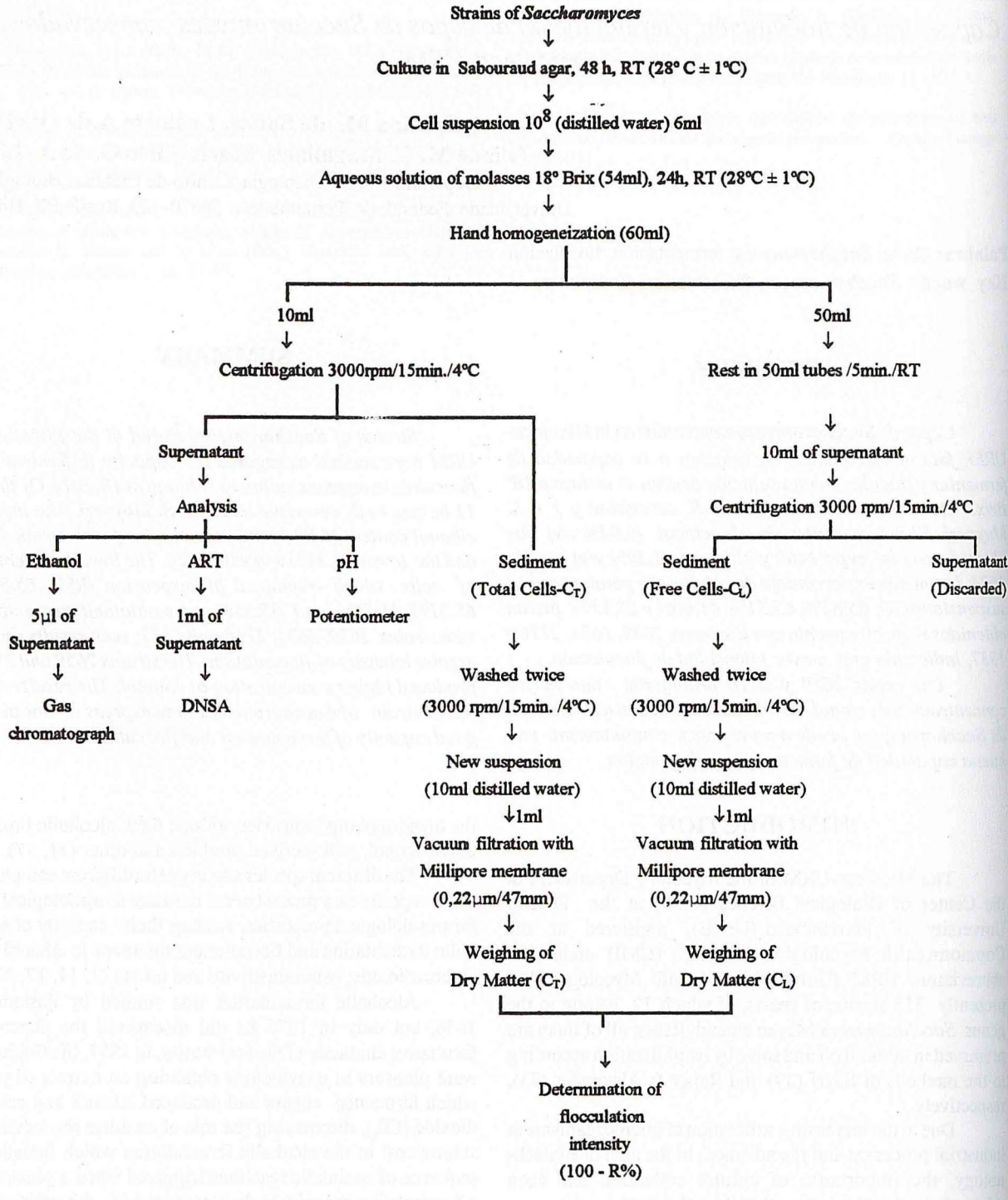
SCHEME 1


Strains of *Saccharomyces*
Culture in Sabouraud agar, 48 h, RT (28° C ± 1°C)
Cell suspension 10^8 (distilled water) 6ml
Aqueous solution of molasses 18° Brix (54ml), 24h, RT (28°C ± 1°C)
Hand homogeneization (60ml)
10ml
50ml
Centrifugation 3000rpm/15min./4°C
Rest in 50ml tubes /5min./RT
Supernatant
Analysis
10ml of supernatant
Centrifugation 3000 rpm/15min./4°C
Ethanol
ART
pH
Sediment (Total Cells-C_T)
Sediment (Free Cells-C_L)
Supernatant (Discarded)
5µl of Supernatant
1ml of Supernatant
Potentiometer
Gas chromatograph
DNSA
Washed twice (3000 rpm/15min. /4°C)
Washed twice (3000 rpm/15min. /4°C)
New suspension (10ml distilled water)
New suspension (10ml distilled water)
1ml
1ml
Vacuum filtration with Millipore membrane (0,22µm/47mm)
Vacuum filtration with Millipore membrane (0,22µm/47mm)
Weighing of Dry Matter (C_T)
Weighing of Dry Matter (C_L)
Determination of flocculation intensity (100 - R%)

Various works conducted with strains of ***Saccharomyces*** have shown that alcoholic fermentation may be influenced by various factors such as microorganisms, concentration of inoculum, substratum, pH, fermentative processes, aeration, nutritional elements, tolerance to ethanol and CO_2 and by other factors (4, 7, 10, 11, 14, 18, 21, 29, 31, 32, 38, 42, 48, 49, 51).

Various kinds of raw material may be used in the alcoholic fermentation process. Sugarcane molasses are commonly used by fermentation industries. Usually contents are as follows: 20% water, 62% sugar, 8% ashes, 3% of nitrogenous materials and 7% of other components. As an average, sugars are made up of 32% saccharose, 16% levulose and 14% dextrose. As a rule it may be safely said that molasses are a rich residue which contains 50% of fermentable sugars. Thus the microorganisms which are agent of the fermentative processes make a satisfactory use of it as an energetic source of nutrition (21, 27, 36).

The flocculation of yeasts is defined as the capacity of spontaneous aggregation of the cells with the formation of flocs which separate from the substratum by sedimentation (7, 35, 43, 47). This fenomenon is being used in the biological production of ethanol ,beer and yeast biomass(8, 9, 24, 25, 41).

Different factors may act on the flocculation. They make up three groups: **physical factors** (temperature, agitation) (8, 9, 12, 15, 25, 40, 46, 52), **chemical factors** such as, pH, and chemical compounds (1, 2, 8, 9, 12, 19, 25, 28, 34, 41, 44, 47, 50) and **biological factors** (yeast samples, cellular concentration) (8, 13).

In industrial processes of fermentation use is made of flocculating or non floculating species of the genus ***Saccharomyces***. Along that line and with the intention to set at the Micoteca-URM a Bank of Yeast of Biotechnological Interest, this paper has as its objectives to characterize the strains o f***Saccharomyces*** stored , as regards to their capacity to ferment and flocculate in an aqueus solution of molasses al 18° Brix.

MATERIAL AND METHODS

a) Strains of *Saccharomyces*. From the stock kept in mineral oil, 12 strains were supplied by the Mycoteca-URM of the Departement of Mycology (CCB-UFPE) as follows: 11 (1337, 1338, 1460, 1807, 1820, 2624, 2658, 2659, 2689, 2690, 2716) were of ***S. cerevisiae*** Hansen, and 1 (1814) of ***S. kluyveri*** Phaff Muller & Shifrine . These strains were revised taxonomically according to the methods found in the general monographs (17, 22, 23)

b) Culture medium for maintenance and growth. Sabouraud agar plus 0.5% of yeast extract (YE) in tubes and final pH 6.5. Aqueous solution of molasses at 18° Brix. Molasses (24 g), $(NH_4)_2 S0_4$ (0.1 g), K_2HP0_4 (0.1 g) distilled water (q.s.q. 100 ml), final pH 5.3. The aqueous solutions of these components were autoclaved separately at 120°C/15 min, and after cooling mixed in 250 ml Erlenmeyers, the end solutions was left room temperature (R.T 28° ± 1°C) for 72 hours for sterilization control.

c) Determination of concentration of the ethanol and ART(total reductor sugars), R % (percent of cells which remained in suspension) in an aqueous solution of molasses at 18° Brix, with 15,62 % w/v of ART and ph final 5.3.

Strains of ***Saccharomyces*** were seeded in Sabouraud agar + 0.5 % of YE and left at R.T./48 h; next the cells were suspended in 10 ml of sterilized distilled water; the concentration was adjusted for 10^8 cells/ml. From each suspension 6 ml were inoculated in 54 ml of aqueous solution of molasses at 18° Brix, held in a 250 ml Erlenmeyer, and left at room temperature for 24 hours. Next the 60 ml solution was agitated by hand and 10 ml were centrifugated in a cooled centrifuge at 3.000 rpm/15 min./4°C. The supernate was analyzed for concentration of ethanol for liquid-gaseous chromatograph and of the total reductor sugars (ART) by the DNSA (dinitrosalicylic acid) method (Miller (26)), as well as of the pH values as potentiometer. The sediment was used for the determination of total cells (C_T). The other fraction (50ml) of the agitated solution was used for the determination of free cells (C_L).From the know values of C_T and C_L, those of R % were calculated according to the method of Pereira Jr. & Bu' Lock (30) (Scheme 1).

RESULTS AND DISCUSSION

Different results were achieved with the strains of ***Saccharonyces*** as regards the capacity of fermentation and flocculation in an aqueous solution of molasses al 18° Brix. The concentration of ethanol produced by the strains of ***Saccharomyces*** after 24 hours of fermentation varied from 1,39 % v/v with strain 2624 to 6.02 % v/v of ethanol after a 24 hours fermentation. Lima (20), operated with this same strain in a fermentation tube with sugarcane molasses at 15° Brix , with fermentation times from 1 to 12 hours and obtained ethanol concentrations which varied from 1,18 % v/v (1 hour fermentation) to 5,53 % v/v (11 hours fermentation). The variation of results of fermentation with different strains of ***Saccharomyces*** is stated in the literatures as evidenced by different works (4, 14, 31, 38, 51).

To determine the percentage of ART the glucose standard curve was used, the correlation rate was r = 0.9974 showing a good relation between the variables. The equation Y= 1.041 X - 0.153 was used to obtain glucose concentrations of the supernate.

At time 0 the concentration of ART was 15,62 % w/v an and pH 5,30 ; after 24 hours of fermentation, ART concetration varied from 11,60 % w/v to 2,90 w/v and pH from 4.29 to 5 (Fig. 1). ART concentration and pH values were inversely proportional to the ethanol produced by the strains.

Values of R % were calculated to determine indirectly the intensity of flocculation which is represented by 100 - R %; thus lesser values of R %, indicate higher rate of flocculation. The Fig. 1, shows that the rates of R % derived

from strains of ***Saccharomyces*** varied from 41,6 to 92, 41 %.

Strains 2659, 2624, 2716, 1337, have the lowest values of R %, respectively 65,83 %, 65,31 % 41,66 % and 55,55 %, indicating higher intensity of flocculation (100-R %) in relation to the other strains (Fig. 1). The 2716 showed greater capacity of flocculation. Lima (20), considered that yeast to be highly flocculation since it flocculated in about 5 seconds. ***S.cerevisiae*** (strain 1820) and ***S. kluyveri*** (1814), were responsible for the formation of small flocs which behaved as dispersed cells since the values of R %, respectively 92,41 % and 82,31%, indicate that these flocs did not sediment in 5 minutes (Fig. 1).

According to different authors (7, 25, 35, 43), the results of this work allowed the tested strains to be tested as regards flocculation as follows:

Class I.- Wholly dispersed: 1460, 2658, 2689, 2690, 1338 , 1807.

Class II.- Flocculating in small flocs: 1820, 1814.

Class III.- Flocculating in denses masses: 2716 (Fig.4).

Class IV.- Flocculating by the non separation of buds: 2659, 2624, 1337 (Fig. 2, 3, 5).

Among the strains wich flocculated better, strains 2659 and 2716, produced high concentrations of ethanol (Fig.1). According to Seiko et al.(38), yeasts with such characteristics are suitable for continuous fermentations in the production of ethanol with cell recycling. Strains 1337 and 2624, produced low concentrations of ethanol (Fig.1)and are not suitable for processes of alcoholic fermentation.

S.cerevisiae 2624, produced flocs made up of pseudomycelium exclusively (Fig.3) and low concentrations of ethanol(1.39% v/v)(Fig.1). These results confirm data in Rose & Harrison (35), where strains which form flocs by pseudomycelium are the best for industrial fermentations.

S.kluyveri(1814), showed a poor capacity of fermentation and flocculation (Fig.1).

Among the strains which did not flocculate, the 1460, 2658, 2689,2690, produced higher concentrations os ethanol (Fig.1) and this recomends their use in fermentation processes.

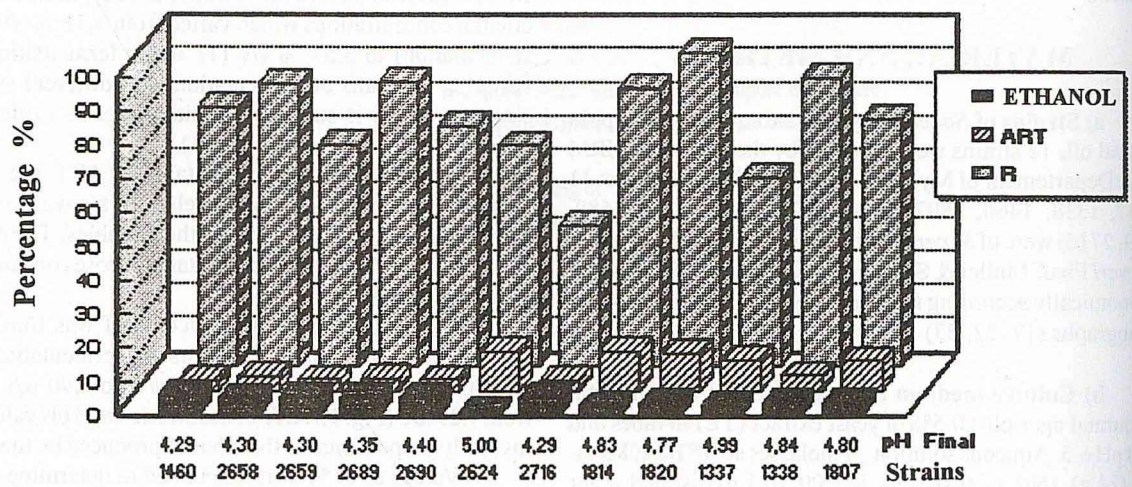
The results of this work already tested back the information in Rose & Harrison (35), which state that the capacity to ferment and to flocculate may be expressed or not jointly for the same strain of ***Saccharomyces***.

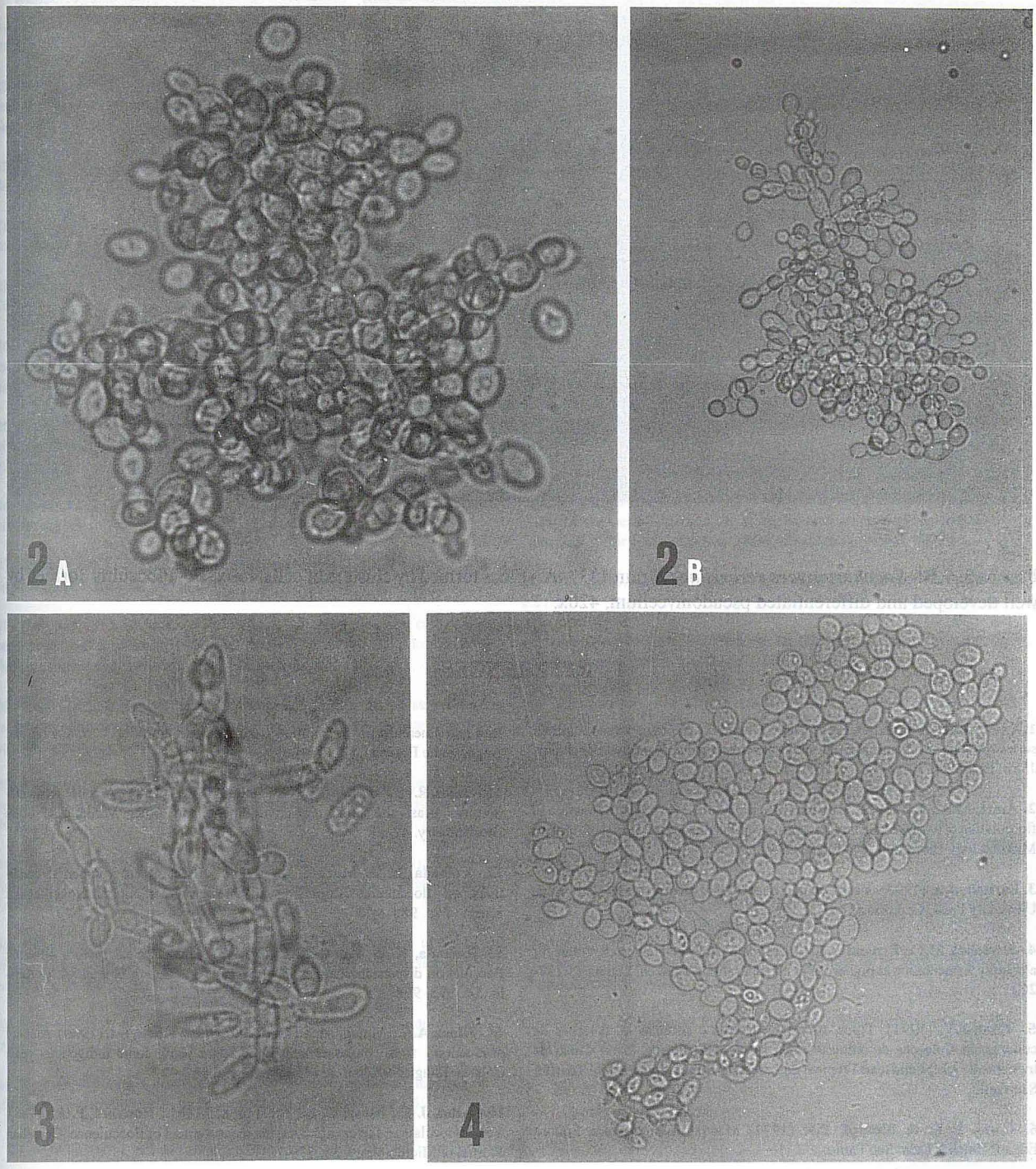
Fontana et al. (9), noticed that the pH range from 5 to 5,5 and the temoperature from 15 to 28°C, are conditions suitable for flocculations. Conditions of R.T.(28°C ± 1°C) and pH 5,3 of the aqueous solutions of molasses at 18° Brix, in which this work took place, are the same indicated by those authors for the flocculations.

The results of this work show that there is no relations between fermentation an flocculations; strains of ***S.cerevisiae*** may or may not present joining capacity to ferment and flocculate;strains of ***S.cerevisiae*** 2659 and 2716, are known as yeast with a good fermentation and flocculation capacity; strains 1460, 2658, 2689, 2690, are yeasts with a good fermentation capacity but do not flocculate; the types of flocs differ from one ***S.cerevisiae*** to other.

The characterization of yeasts from the standpoint of their capacity to ferment and to flocculate shows how important they are for applied research in alcoholic fermentation industries. This in itself plainly justifies the need for the implementation of the yeast bank of biotechnological interest at URM-Mycotheca.

Figure 1.- Concentration of ethanol produced by samples of *Saccharomyces*, ART, R% and final pH in an aqueous solutions of molasses at 18°Brix with initial pH at 5,3





Figures 2,3,4. ***Saccharomyces cerevisiae***. **Fig 2 A-B.** strain 2659, **A.**- Flocs formed by clusters of cells,740x, **B.** Flocs formed by both cell, clusters and well developed and differentiated pseudomycelium, 420x, **Fig 3.**- Strain 2624. Flocs formed by well developed and differentiated pseudomycelium, 590x. **Fig.4.**- Strain 2716. Flocs formed by clusters of cells,720x.

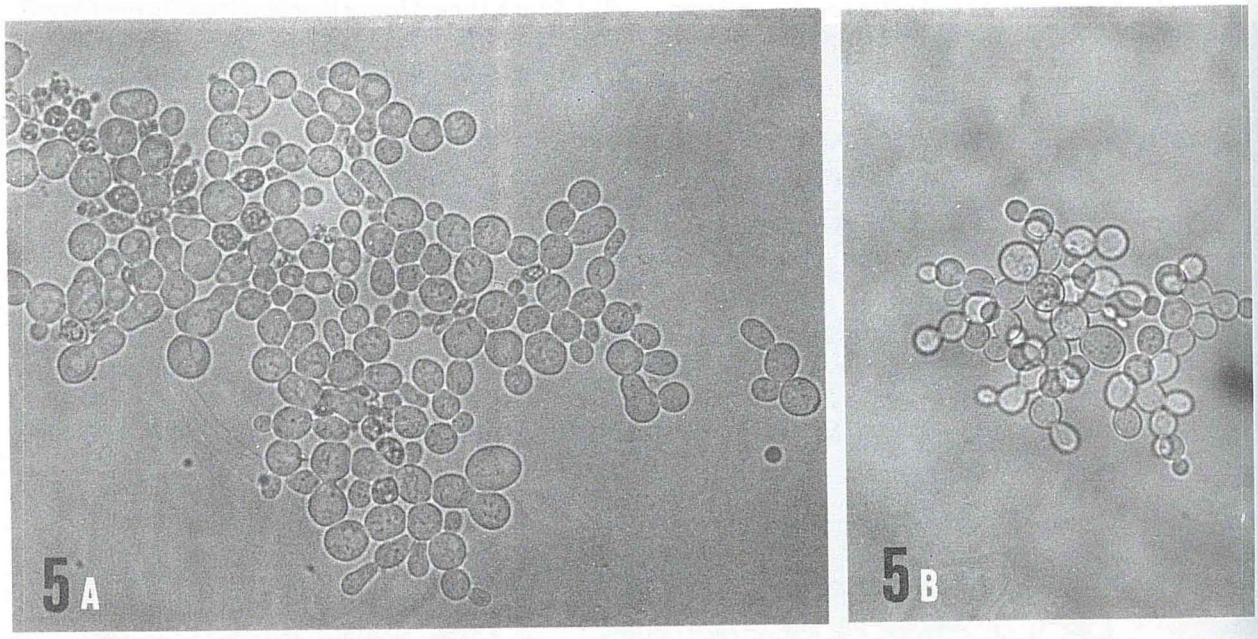


Figure 5.A,B.- ***Saccharomyces cerevisiae.*** Strain 1337. **A.-** Flocs formed by clusters of cells,460x. **B.-** Flocculus formed by well developed and differentiated pseudomycelium, 420x.

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