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Selection of an ureolytic yeast from straw treated by urea and molasses

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ABSTRACT

Selection of an ureolytic yeast from corn straw treated with urea and molasses. Six isolated yeasts of a mixture of corn straw added by 5% of urea then by 10% of molasses were studied in fermentation thirty days on the same substratum at initial pH of 5.50.

Three strains presented an urease production but only one, had a marked ureolytic activity. The yeast strain in question is LVyc1 being identified.

KEYWORDS

Molasses;
Straw;
Urea;
Urease;
Yeast.

INTRODUCTION

The region of Gharb in Morocco is subject to difficult weather conditions : catstrophic floods in 2008, 2009 and 2010, prolonged drought. Livestock are particularly affected. Death of livestock, the spread of diseases related to poor nutrition are causing a fall in the yield of meat and some collection centers reported a drop in milk production from 60 to 70%.

Straw enter in the basic diet of producters from the region, mainly consisting of small farmers. It is very rich in parietal carbohydrates, poor in sugars and in digestible crude protein and include, but it is not efficient, nutritional needs of animals[11]. The addition of nitrogen (ammonia, urea) to the straw improves weight gain of cattle[11]. We were interested to the straw's enrichement in nitrogen by incorporation of urea. Nous nous sommes intéressés à l'enrichissement de la paille en azote par incorporation d'urée et transformation microbienne.

Degradation of urea into ammonia and carbon dioxide depend on the prensence of enzymes allowing the ureolysis, of the moisture content, temperature, the duration of treatment[6]. The microbial flora of departure plays a very important role in the process of enzyme synthesis ensuring ureolysis[12]. Different microorganisms have a urease : Eubactcerias including Cynaobacteria, Actinobacteria, yeasts, filamentous fungi[5].

From a base mixture of straw, urea and molasses, we isolated yeast strains[1], that have been studied for their ability to degrade urea in thirty day of fermentation.

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MATERIAL AND METHODS

Isolation of straw strains

40 kg of wheat straw in th region of Ghard in Morocco) crushed using a hammer mill were added from 5kg of urea purchased from a dealer and 55kg of well water. Only 10 kg of molasses mixture were then added to the above mixture. The environment is contained in plastic drums with an ambient air at a temperature of 20°C. No parameter was set to begin with.

Every five days, samples were taken for isolation of yeast strains on PDA environment sterilized for 15 min at 121°C. The cultures were incubated 24 h at 30 ° C in Petri dishes 90 mm in diameter containing 20 ml of medium. After five successive rounds of subculture/ transplanting, isolated yeasts are kept in test tubes on PDA environment inclined at 4°C in the dark.

Characterization of strains

Six yeast strains, LVcy1 to LVcy6, were followed on semi-synthetic environment (10 g of the same chopped and sterilized straw, 1 g urea, 1 g sucrose, initial pH set at 5.5 with 1 N NaOH) in Petri dishes of 90 mm diameter containing 15 ml of medium. Sterilization occurs at 105°C for 15 min. Incubation is carried out at 24 h at 30°C. We are seeking to get at least one strain of correct growth at low pH and capable of hydrolyzing urea.

Three parameter were monitored every 5 days for 30 days : pH biomass and acidity.

The pH of cultures was measured using a pH meter Orion Research's kind previously calibrated at pH 7 and 4.

Growth of yeast strains was monitored by measuring the absorbance of the medium using a spectrophotometer type UV-2004 at 600 nm against the control (semi-synthetic environment).

The organic acids are able to retaining the proton H + on some pH, consequently it is necessary to couple the monitoring of the pH to that of acidity. Ten ml of the supernatant is transferred into a 100 ml beaker. A few drops of 1% phenolphthalein are added into it. The titration is carried out by a solution of NaOH N / 9 until the indicator changes color and become pink. The acidity is expressed as a percentage of lactic acid (MW = 90.08 g) by 100 ml of culture.

The determination of strains is performed by microscopic observation. The LVyc1 strain is a round yeast of small colony, 2 to 4 microns, with budding well noticed.

Determination of pH and biomass of the three strains LVyc1-LVyc2 and LVyc3 according to the concentration of urea

The three strains from straw treated with urea and molasses were grown on a semi syntactic environment to different concentrations of urea as nitrogen sources and incubated at 30°C for 24 hours.

The pH was measured using a pH meter Orien Research kind. The values †of the measure are taken after calibration of the device. The stallions used are pH 7 and 4.

Growth of yeast strains LVyc1-LVyc2 and LVyc3 is monitored by measuring the optical density of the medium using a type spectrophotometer (UV-2004 POWER: 110/220V-50/60Hz). The reading of the density is made at 600 nm against an indicator (semisynthetic environment) uninoculated.

(a) Urease test

Young yeast suspensions were collected from the semi-synthetic mid and then cultured in tubes containing 5 ml of mid Tryptophan Urea (3 g L-tryptophan, 20 g urea, 1 g potassium hydrogen phosphate, potassium dihydrogen phosphate 1 g, NaCl 5 g, 95 ° ethanol 10 ml, 25 mg phenol red, distilled water 1 L).

The strains said with a high hydrolytique potential of urea are able to ensure its lysis by the following reaction:

$$(NH_2)_2CO + H_2O + H^+ \xrightarrow{\text{Urease}} 2NH_3 + HCP_3^-$$

A red color reflects an alkalinization of the mid, following a hydrolysis of urea (urease +) ; whereas a yellow or orange color indicates the absence of hydrolysis (urease -).

The results were verified by culture on semi-synthetic environment containing only nitrogen source as urea, added to the environment at 1, 2, 3, 4 and 5 g / l, and without carbon source (neither sucrose or straw). The incubation was carried out 24 h at 30 ° C.

(b) Preparation of the fermenting mash

5 g of urea and 55 g of water were added to the 40 g of chopped straw. To this mixture, the molasses is

added at 0, 5, 10, 15 and 20%. The five lots are subjected to spontaneous fermentation at room temperature, in laboratory, away from sunlight. The PH, the acidity and the total flora (via the absorbance) were measured after thirty days. Molasses which is a source of sugars and minerals, is added to promote the growth of microorganisms such as yeasts and lactic acid bacteria

RESULTS

We can see a slight increase of the final pH for the six cultures of yeast (TABLE 1). The pH varies between 6.47 and 6.65

TABLE 1 : Variation of pH and of the absorbance A of the culture environment of the six yeasts at 30 ° C and initial pH of 5.50

Strain	pH_{final}	$A_{initial}$	A_{final}	Acidity (mg/100 ml)
LVcy1	6,65	0,65	0,92	0,13
LVcy2	6,64	0,60	0,93	0,22
LVcy3	6,61	0,53	0,92	0,18
LVcy4	6,48	0,62	0,89	0,16
LVcy5	6,54	0,51	0,72	0,24
LVcy6	6,47	0,61	0,75	0,18

The organic acid content varies between 0,13 et 0,24 mg/100.

For biomass, approximated by measuring the absorbance, there are two different evolutionary trends (TABLE 1) strains LVyc1, LVyc2 and LVyc3 have a final absorbance almost identical (0.92 to 0.93) followed by LVyc4 (0.89). The absorbances are lower (0.72 to 0.89) for yeasts LVyc5, LVyc6 and LVyc4.

The strains LVyc1, LVyc2 and LVyc3 were selected for the test of confrontation with urea (TABLE 2). They have shown a healthy growth and interesting values for the pH and acidity of the previous tests.

The final pH varies between 6.42 and 7.16. LVyc1 and LVyc2 could not lower the pH to low values †when they are cultured in the presence of high concentrations of urea (4-5 g / l).

The neutralization of ammonia molecules still seems difficult. LVyc1 gives the best growth. It reached a high absorbance (0.98).

Only the LVyc1 strain showed a clear production of urease (Figure 1).

TABLE 2 : Evolution of pH and biomass of three yeast strains in the presence of five concentrations of urea on semi-synthetic at 30°C and initial pH of 5.50

Urea	Strain	Final pH	Initial A	Final A
	LVcy1	6,66	0,51	0,75
1g/l	LVcy2	6,66	0,39	0,73
	LVcy3	6,42	0,50	0,74
	LVcy1	6,84	0,58	0,99
2g/l	LVcy2	6,81	0,53	0,83
	LVcy3	6,77	0,52	0,78
	LVcy1	6,80	0,57	0,79
3g/l	LVcy2	6,71	0,36	0,75
	LVcy3	6,77	0,58	0,69
	LVcy1	7,09	0,57	0,98
4g/l	LVcy2	7,02	0,63	0,72
	LVcy3	6,93	0,78	0,72
	LVcy1	7,16	0,29	0,79
5g/l	LVcy2	7,31	0,49	0,72
	LVcy3	6,84	0,51	0,81

LVyc1 LVyc2 LVyc3 LVyc4

Figure 1 : Visual assessment of urease activity of yeasts after three days of culture on the tryptophan urea environment at 30°C and pH 6.8. yeasts LVyc5 and LVyc6 behave like LVyc4

The strains tested are yeasts isolated of the wort of fermentation prepared beforehand. TABLE 3 gives the origin of the six isolated strains.

TABLE 3 : Origin of the yeast strains tested on the trea tryptophan environment at 30 ° C and at pH 5.50

Strain	Origin
LVyc1	Straw + urea (5%) + molasses (10%)
LVyc2	Straw + urea (5%) + molasses (15%)
LVyc3	Straw + urea (5%) + molasses (20%)
LVyc4	Single straw
LVyc5	Single straw
LVyc6	Single straw

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DISCUSSION AND CONCLUSION

Only the LVyc1 strain presents a marked urease activity, this activity is mainly due to the presence of the urease's enzyme. This shows the important interest of the incorporation of this ureolytic strain in the process of straw treatment with urea. These results are consistent with those of

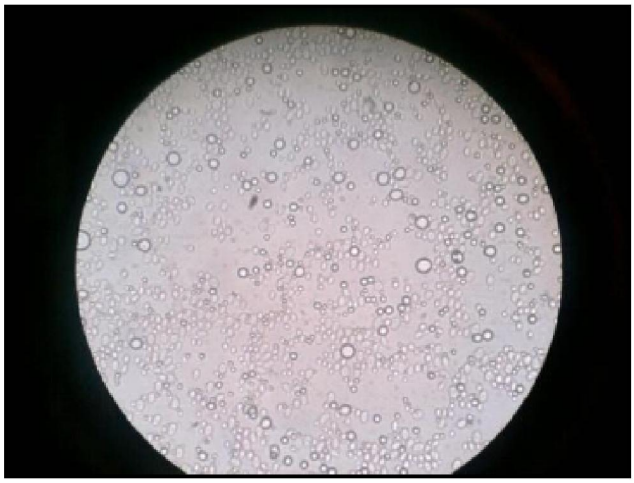

Figure 2 : Cells of the LVyc1 yeast isolated from the straw treated with urea and molasses

The use of urease of microbial origin (LVyc1) is a beneficial alternative for farmers who use soy flour, the most known product for its urease property for the treatment of straw for cattle feed. Williams et al. (1984) and Hassoun et al. (1990)

The nitrogen molecule which result from the hydrolysis of urea by the urease's enzyme; Sahnoune (S.) et al 1991; is a major source for the synthesis of proteins of the animal which is directly involved in the compensation of the need of production and maintenance. M.C.N.Jayasuriya, G.R.Pearce 1983.

The total and rapid degradation of urea into ammonia is possible by using strains of the LVyc1 genus. Work for optimizing the processing conditions are in progress to determine the humidity rates, the amount of the enzyme, the concentration of the urea and the molasses for the successful treatment of straw. M.Chesnot 1994.

In the end, we can say that the straw is a vegetable carrier which contains microorganisms with urease properties capable of multiplying in a biotope rich in urea as a nitrogen source, and therefore the complete hydrolysis of urea. This is confirmed by R.Cordesse et al (1994).

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