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NEW ISOLATED SINGLE CELL BIOMASS PRODUCING YEAST STRAINS FOR FOOD AND FEED INDUSTRY

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ABSTRACT

Biosynthesis of single cell biomass on an industrial level is closely related to biomass multiplication process, namely to the fermentation stage. This requires solving major technological problems, including those related to the choice of optimal conditions leading to higher rates and yields in biomass and to nutrient medium composition optimization. For the fermentation of different raw materials, specific yeast that grows fast, has high yields in biomass, is accommodated to the specific conditions of technological process and is more resistant to infections is required. Despite the large amount of studies performed on biomass production, the theme continues to be of interest due to the new yeast strains with increased performance in fermentation. In this study, 52 new yeast strains were isolated from fruits and their juices and from special media. Their identification at species level was performed by classical methods. After the preliminary experiments, seven strains of *Saccharomyces cerevisiae* were selected and tested both at laboratory and pilot scale for their performances. Based to their ability to ferment the molasses, these strains could be used at industrial level in order to obtain an increased production of single cell biomass. The tests conducted in pilot station point out that *S. cerevisiae* DFP strain produced the highest quantity of biomass in the fermented medium.

Key words: biomass production, molasses, *Sacharomyces cerevisiae* age.

INTRODUCTION

Microbial protein is known in the literature with the generic term of single cell protein (SCP), a term indicating that the microbial-origin protein comes from unicellular organisms (bacteria, yeasts, moulds, algae etc.) (Anghel et al., 1993).

Tracking of the influence of each growing factor both on the yield and on the quality of the biomass produced is very important in the technological process of obtaining the yeast SCP. The identification of a newly isolated yeast strain requires accurate characterization and description, for allowing a comparison with already known yeast strains. The main characteristics used for identifying yeasts are the morphological characteristics of vegetative cells, the way of reproduction, the biochemical characteristics and the physiological characteristics (Tofan et al., 2002).

For these reasons, the isolation and characterization of new yeast strains, able to produce higher biomass yields during fermentation in specific conditions, continue to be of great interest from practical and industrial points of view.

The aim of this study was to isolate and select yeast strains, mainly belonging to *Saccharomyces* genus. After isolation and selection, the yeast strains were tested, studied and verified under laboratory and pilot conditions, having as objective to identify yeast strains with optimum activity and high multiplication capacity. To obtain single cell biomass, experiments were conducted using as culture medium beet molasses. Molasses is a by-product from sugar beet industrialization, its use as raw material being convenient primarily for economical reasons (in terms of low cost price).

Track of evolution of biomass parameters during fermentation process was performed during pilot experiments and finally the yield in biomass and specific consumptions were calculated, in order to establish the process effectiveness.

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MATERIAL AND METHODS

Biological material. Fruits and juice fruits as well as sugar beet molasses were used for yeasts isolation. Dilutions of suspensions obtained from these materials were placed on complete media (YEPD) containing 1% yeast extract, 2% peptone, 2% glucose and 2% agar. The Petri plates were then incubated at 27°C for 48 to 72 hours. From the isolated yeasts, a number of 7 strains were studied to determine their abilities in biomass production. A medium with molasses was used as a special medium (molasses was diluted to 3°Bx, salts with phosphorus and nitrogen were added, and pH was fixed at 4.5-4.8 with H_2SO_4).

Identification of yeast isolates. Isolated colonies with distinct morphological appearance and with enhanced abilities of biomass production were picked up aseptically and subjected to several tests for identification. The classical methods described by Barnnet et al. (1978) based on examination of morphological and physiological properties of yeasts isolated in pure cultures were applied. The DBB test (Diazonium Blue B) was used to differentiate ascomycetous yeasts from basidiomycetous yeasts. Determination of culture character of yeasts was made on medium based malt mash of 8% d.m. concentration sown with an eye loop of each strain selected. The tubes were incubated 72 hours at 28°C and then 10 days at 20°C. Ring formation, film appearance and sediment formation, after three and ten days, respectively were observed. Finally, the selected strains were identified at species level using API ID32C system (Biomerieux).

Fermentation technology. Overnight pre-inoculum cultures were prepared in YEPD and the optical density (OD) of the culture was determined at the wavelength of 600 nm using a UVD-3200 UV-Vis spectrophotometer. Each aliquot (50 mL) of the media containing 20% glucose and 0.67% nitrogen base was inoculated with 1.2×10^8 cells based on the conversion factor of 0.50 OD being equal to 1×10^7 cells.

The pilot installation used in the experiments consists of fermentation vessels with different volumes (total volume of 20 L, 100 L and 140 L), fitted with pH monitoring and controlling equipment and dosage equipment (raw material, nutrient salt solutions).

Determination of solid content, crude protein and ash. The solid content in pressed biomass was determined using the method of 16 hours drying (AOAC 961.06) (AOAC, 2000).

The protein content in pressed biomass was determined by Kjeldahl method (AOAC 920.53), using 0.5 g test portion (AOAC 962.10). Digestion was performed for 30 min after solution cleared (AOAC, 2000).

The ash content was determined by the gravimetric method, using 2 - 3 g test portion. Add 2 mL of conc. H_2SO_4 were added, the product was heated on hot plate until was well carbonized, and than calcinated in furnace at ca. 650°C to constant weight. Result was expressed as percent ash.

The ash content was calculated by using the formula given below:

Ash (g/l00 g) = [(weight of dish with sample after calcination - weight of empty dish/weight of dish with sample before calcination - weight of empty dish) x 0.9] x 100; 0.9 = coefficient for transformation of sulphated ash into conventional ash.

RESULTS AND DISCUSSION

1. Isolation of new yeast strains

A total of 52 strains of yeast were isolated during our experiments. Among them, seven strains proved increased abilities to produce high biomass content and they were used in the fermentation tests. Their morphological characteristics are presented in table 1.

The particularities of these strains at microscopic level were presented in table 2 and figure 1.

The application of API ID32C system for identification of selected strains allowed the conclusion that all seven isolates belong to *Saccharomyces cerevisiae*.

Table 1. Morphological characteristics of selected yeast strains

Strain	Culture characters		
	On liquid medium	On solid medium	
		In sloping tube	In Petri plates (colonies)
DPP	Colony white to cream, moderate growth after 24 h, growth at medium surface is absent, with ring of foam, the deposit presents a fine appearance, with complete disintegration. Gas bubbles all over the medium mass.	Abundant growth type “H”, route seeding thin and wet, spread out aspect, opaque culture.	Large uniform colonies, round, with full edge and convex profile, smooth aspect type "S", opaque, with glossy surface and sticky consistency.
DPM			
DPF			
DPE5			
BF37			
D1	Colony white to cream, moderate growth after 24 h, growth at medium surface is absent, with ring of foam, the deposit presents a fine appearance, with complete disintegration. Gas bubbles all over the medium mass.	Abundant growth type “H”, route seeding thick and wet, spread out aspect, opaque culture.	Large uniform colonies, round, with full edge and convex profile, smooth aspect type "S", opaque, with blind surface and sticky consistency.
D2	Colony white to cream, moderate growth after 24 h, growth at medium surface is absent, with ring of foam, the deposit presents a fine appearance, with complete disintegration. Gas bubbles all over the medium mass.	Abundant growth type “H”, route seeding thin and wet, spread out aspect, opaque culture.	Large uniform colonies, round, with full edge and convex profile, smooth aspect type "S", opaque, with blind surface and sticky consistency.

Table 2. Microscopic characteristics of selected yeasts

Strain	Cells			Pseudomycelium	Sporulation
	Shape	Group	Budding mode		
DPP	Round cells	Isolated or grouped in small clusters	Unipolar bud	Does not form pseudomycelium	1 - 2 spors
DPE5					
DPM	Round cells	Isolated or grouped in small clusters	Unipolar bud	Does not form pseudomycelium	1 - 4 spors
DPF	Big round cells	Isolated or grouped in small clusters	Unipolar bud	Does not form pseudomycelium	1 - 3 spors
BF37	Round-oval cells	Isolated or grouped in small clusters	Unipolar bud	Forms pseudomycelium	1 - 4 spors
D1	Round-oval cells	Isolated or grouped in small clusters	Unipolar bud	Does not form pseudomycelium	1-3 spors
D2					

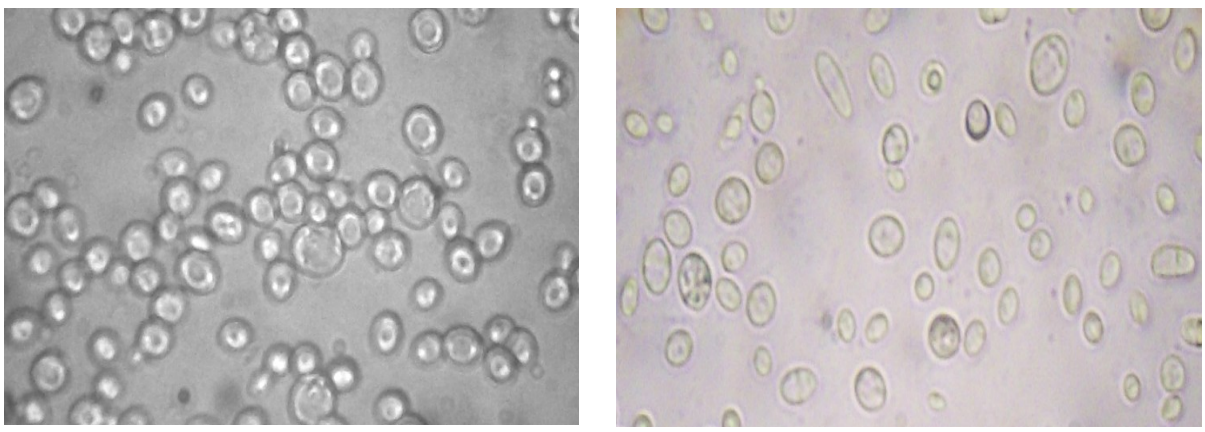

Figure 1. Cell shape of strains designated as DPP (left) and DPF (right)

2. Fermentation studies with selected strains

The biomass production from raw materials of agricultural origin, with lower price, is the aim of most of the studies performed in many laboratories. In our experiments, molasses was used as raw material.

The molasses used in the experiments had the following quality parameters: dry matter – 76.8%; total sugar content – 50.04%.

It was worked using the technological version with molasses dilution until ca. 40% dry matter, adjustment of pH value to 4.5 by adding conc. H_2SO_4, followed by sterilization and clarification throughout decantation and filtration.

The first step was to use a simple feeding diagram. This paper presents the result of fermentation tests using a more elaborated feeding diagram in three successive stages (Begea et al., 2009). For all three fermentation stages the air flow-rate was 0.6 l/l slurry/ min (Tables 3-5).

Table 3. First fermentation stage

Hour	Molasses 40% d.m. (litres)	Ammonium sulphate 30% (litres)	Superphosphate 16°Bx (litres)	pH	Temperature (°C)	Biomass 30% d.m. (g)
1	0.640	0.036	0.082	4.5	30	1.0
2	-	-	-	4.5	30	-
3	-	0.021	-	4.5	30	-
4	0.128	0.021	0.042	4.5	30	18.0
5	0.128	0.021	0.042	4.5	30	-
6	0.128	0.021	0.042	4.5	30	92.0
7	0.340	0.021	0.042	4.5	30	-
8	0.340	0.021	0.042	4.5	30	-
9	0.680	0.021	0.084	4.5	30	210.0
10	0.680	0.021	0.084	4.5	30	375.0
11	0.340	0.021	0.042	4.5	30	-
12	0.340	0.021	0.042	4.5	30	580.
13	0.128	0.021	0.042	4.5	30	-
14	0.128	0.021	0.042	4.5	30	645
15	-	0.021	0.042	4.5	30	687
16	-	-	-	4.5	30	-
17	-	-	-	4.5	30	759
Total	4.0	0.330	0.670	-	-	750

Table 4. Second fermentation stage

Hour	Molasses 40% d.m. (litres)	Ammonium sulphate 30% (litres)	Superphosphate 16°Bx (litres)	pH	Temperature (°C)	Biomass 30% d.m. (g)
1	3.2	0.27	0.54	5.0	30	120
2	-	-	-	5.0	30	-
3	-	-	-	5.0	30	-
4	0.64	0.05	0.1	5.0	30	280
5	0.64	0.05	0.1	5.0	30	-
6	0.64	0.05	0.1	5.0	30	840
7	1.28	0.08	0.18	5.0	30	1400
8	1.28	0.08	0.18	5.0	30	-
9	1.5	0.16	0.38	5.0	30	2150
10	1.5	0.16	0.36	5.0	30	-
11	1.5	0.08	0.18	5.0	30	2940
12	1.5	0.08	0.18	5.0	30	-
13	1.04	0.08	0.18	5.0	30	3240
14	0.64	0.05	0.1	5.0	30	-
15	0.64	0.05	0.1	5.0	30	3680
16	-	-	-	5.0	30	4120
17	-	-	-	5.0	30	4800
Total.	16.0	1.32	2.68	-	-	4800

Table 5. Third fermentation stage

Hour	Molasses 40% d.m. (litres)	Ammonium sulphate 30% (litres)	Superphosphate 16°Bx (litres)	pH	Temperature (°C)	Biomass 30% d.m. (g)
1	8.0	0.660	1.320	5.0	30	260
2	-	-	-	5.0	30	-
3	-	-	-	5.0	30	-
4	1.6	0.125	0.250	5.0	30	810
5	1.6	0.125	0.250	5.0	30	-
6	1.6	0.260	0.520	5.0	30	2100
7	3.2	0.260	0.520	5.0	30	3200
8	3.2	0.400	0.800	5.0	30	-
9	3.74	0.400	0.800	5.0	30	6400
10	3.73	0.390	0.880	5.0	30	8100
11	3.73	0.260	0.520	5.0	30	-
12	3.2	0.180	0.360	5.0	30	10600
13	3.2	0.120	0.240	5.0	30	-
14	1.6	0.120	0.240	5.0	30	12150
15	1.6	-	-	5.0	30	14250
16	-	-	-	5.0	30	-
17	-	-	-	5.0	30	16160
Total	40.0	3.30	6.70	-	-	16160

At the end of multiplication process, the biomass was separated from fermentation medium by centrifugation (10 minutes at 4000 rpm) and filtration using a vacuum pump. The wet biomass was used in order to establish the

specific consumption, biomass production and biomass yield for all tested yeast strains. Wet biomass, standardized at 30% d.m., was used for the determination of the main parameters of biomass.

The level of biomass production by the selected yeasts strains with molasses as raw material and the physical – chemical technological parameters of biomass are presented in tables 6 and 6a.

Table 6. The main parameters of yeast biomass

Yeast strain	Wet biomass (g/l)	Solid content (%)	Crude protein (N x 6.25) (% d.m.)	Ash (% d.m.)
DPP	69.98	31.80	51.69	7.64
DPM	56.40	32.16	52.45	8.14
DPF	57.40	35.61	57.86	8.22
DPE5	55.20	38.52	53.16	8.78
BF37	64.10	33.34	57.03	7.46
D1	59.90	39.11	52.39	8.19
D2	65.80	38.67	54.36	7.38

Table 6a. The main parameters of yeast biomass

Parameter	Variation range (Dv)	Average value (Vm)
Wet biomass, g/l	55.20 - 69.98	61.25
Solid content, %	31.80 - 39.11	35.60
Crude protein (N x 6.25), % d.m.	51.69 - 57.86	54.03
Ash, % d.m.	7.38 - 8.78	7.97

The technological indicators calculated for the pilot experiments are presented in figures 2 and 3.

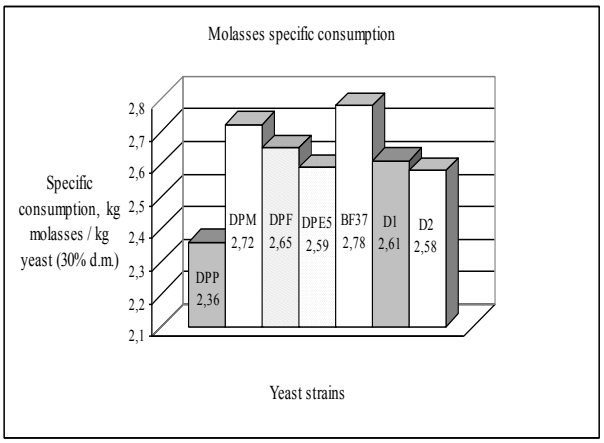

Figure 2. Molasses specific consumption for pilot tests

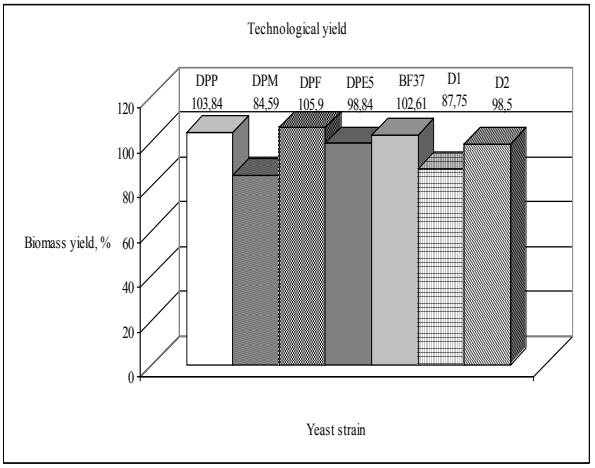

Figure 3. Biomass yield for pilot tests

The results for pilot tests for the selected yeast strains show the following:

- the molasses specific consumption was between 2.36 - 2.78 kg molasses (standardized at 40% d.m.)/ 1 kg active single cell biomass (30% d.m.);
- the yield in biomass was between 84.59 - 105.9%.

The best results were obtained with the strain DFP that proved to produce the highest biomass concentration, with a relatively low specific consumption. This strain produced also the biomass with highest protein content. Due to these properties, the strain was used in specific fermentations, in order to establish the performance in pilot installation.

CONCLUSIONS

The screening experiments for identification of new natural yeast strains with increased abilities of biomass production allowed the selection of seven strains. The cell morphology of the yeast cells under microscope, the colony morphology and biochemical characteristics (according API ID32C system) for all the isolates were specific to *Saccharomyces cerevisiae*.

All the isolates from various natural substrates had different fermentation features even though they belong to the same genus.

Selected yeasts belonging to *Saccharomyces* genus had the ability to produce high biomass yield.

The best results (the highest biomass yield) were obtained with the strain DFP. This strain could be used at industrial level for fermentation of various sugar based raw materials in order to obtain an increased production of biomass.

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