Studies on Obtaining a Probiotic Product Based on Mixed Microbian Biomass

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Abstract

Biotechnological studies on obtaining the probiotic product consist in the growth of the 3 microorganism strains in the specific micropilot conditions, followed by the separation of the respective biomasses in the broth, the drying of the biomass of Saccharomyces cerevisiae, Lactobacillus acidophilus and Bacillus subtilis.

Pharmaceutical studies consisted in establishing the specific effect of the saprophytic intestinal flora as a biological normalizer, and in the ability of the 3 microorganism strains to survive at low pH values and under the activity of the gall salts, as well as in establishing the level of the resistance to antibiotics.

Keywords: *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, *Bacillus subtilis*, biomass, probiotic.

Introduction

Probiotics are defined as microorganisms capable of colonizing the intestine and of preserving or of triggering an increase in the digestive natural flora, preventing the stability of the pathogenic microorganisms and assuring the safety of the best use of food [1, 2].

In order to eliminate the negative effects of the therapy and to restablish the balance of this ecosystem, we should administrate the so-called probiotic strains (bacteria, yeast) which pass alive through the digestive tract, colonize it temporarily and lead to the regeneration of the local flora in structure and number both directly (through bacterial antagonism and enzimic action) and indirectly (by stimulating of the host's immunity) [3, 4].

The probiotic products obtained with mixed biomass (bacteria and yeasts) could play an important role in the therapy of different diseases, taking the advantage of natural products which have no side effects.

Materials and Methods

Strains:

- *Saccharomyces cerevisiae* ICCF 224, 225, 226
- *Bacillus subtilis* ICCF 77
- *Lactobacillus acidophilus* F. B.

Fermentation Experiments

The three types of microorganisms have been grown in batch system, in a LKB bioreactor at an 8l working volume.

Growth parameters were in each case, as follows: *Saccharomyces cerevisiae*: temperature 28^0C, pH 4, agitation speed 600 – 900 rpm, aeration rate 0,8–1L/L/min; *Bacillus subtilis*: temperature 32^0C, pH 7 – 8, aeration rate 1 L/L/min, agitation speed 500 rpm; *Lactobacillus acidophilus*: temperature 37^0C, pH 5,5, agitation speed 50 rpm, anaerobic conditions. The formed biomass was separated from the cultivation environment by centrifugation and then lyophilized.

Analytical Methods

The development of microorganisms during cultivation was determined by means of dry cell weight, correlated with the optical density, determined at λ = 570 nm for yeast and λ = 550 nm for bacteria.

Results and Discussions

I.

The evolution of the growth parameters in the batch system as well as the results obtained in the process are presented in **Table 1** and **2** and **Figure 1** of the *Saccharomyces cerevisiae, Bacillus subtilis, Lactobacillus acidophilus* strains.

Table 1. The specific evolution of the *Bacillus subtilis* ICCF – 77 strains development and sporulation process

Cultivation time [hrs]	pH	Optical density (λ = 550 nm)	Disolved oxygen (%)	Microscopic observations
0	6,6	0,070	100	-
4	6,3	0,080	90	Bacilli in division
8	7,3	0,280	87	Bacilli with endospores
12	7,8	0,360	85	Bacilli with endospores: about 70% free spores
16	8,2	0,370	82	Bacilli with endospores: about 80% free spores
20	8,3	0,330	70	Bacilli with endospores: about 90% free spores
24	8,2	0,320	75	Bacilli with endospores: about 98% free spores

Table 2. The development of *L. acidophilus* strain at micropilot scale

Cultivation time [hrs]	pH	O.D. (λ = 550 nm)	WCW g/l
0	6,5	-	-
24	4,3	5,200	1,2
48	4,2	10,825	2,9
72	4,0	14,900	5,1

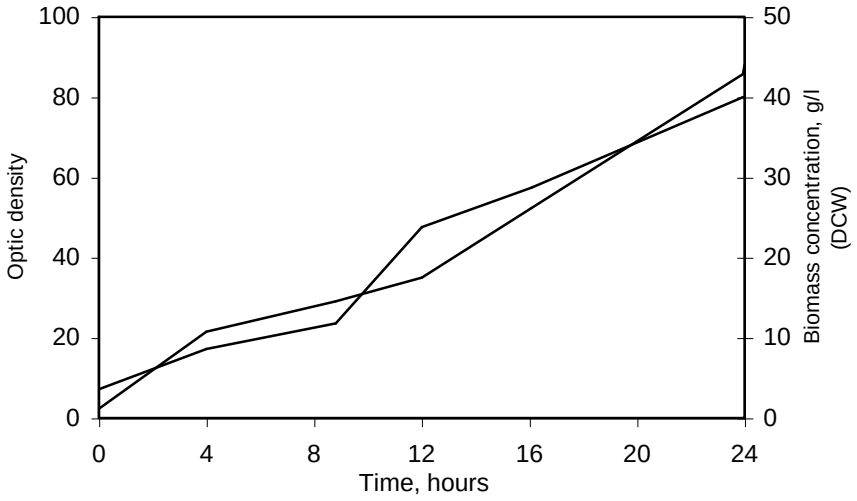

Figure 1. Fermentation profiles of a batch cultivation experiment with *Saccharomyces cerevisiae* 224, 225, 226

The cultivation process of *Saccharomyces cerevisiae* strains lasted 24 hours. We obtained 370 g biomass by consuming 612 g ethanol. The specific consumption of ethanol was of 1.65, and the bioconversion rate was 60.5%. The specific growth speed of the 3 yeast strain during the 24 hours of growth was 0.1 h^{-1} and cell productivity reached the value 0f 5.77 $g \times L^{-1} \times h^{-1}$, with T_D = 4.95 h.

The experimental results show a good rate of survival for *B. Subtilis* spores during the first 3 hours of cultivation, in a medium with an acidic pH. The cells' viability is affected only after 5 hours of incubation. *L. acidophilus* strain remains alive in an acid environment of pH 2,5 during the first 3 hours of incubation, having a titre of $1,7 \times 10^6$.

Both the yeast strain *S. Cerevisiae* and the bacteria strain, *B. subtilis* and *L. acidophilus*, growth on a standard culture medium supplimented with bile acid salts (**Table 3**). At the end of 24 hours incubation they reach a titre having the same order of magnitude as the reference.

Table 3. The effect of incubation of *B. subtilis* ICCF 77 strain in buffer solutions of lower pH

Cultivation time [hrs]	**pH – cell development CFU/ml**		
	1,5	**2,5**	**4,5**
0	9×10^2	1×10^3	1×10^3
3	7×10^2	$8,5 \times 10^2$	$9,5 \times 10^2$
5	10	6×10^2	1×10^3

II.

Pharmaceutical Tests

a) Resistance to antibiotics of *Saccharomyces* strains

The qualitative analysis, using the microtablet kits has revealed

- complete resistance (inhibition zone ∅ = 0 mm for: bacitracin, polymyxin, neomycin, nolidixic acid, streptomycin, tetracylline, penicillin, cloramphenicol, kanamycin, ampicillin, erytromycin, furazolidone, nitrofurantoid, neoxazole)

- sensitivity (inhibition zone ∅ = 23 mm) for colistin.

b) The study revealed a high antagonistic activity of the *Saccharomyces cerevisiae* ICCF 224, 225, 226 strains (inhibition zone 20 – 40 mm) on several enteropathogenic strains (*Salmonella typhymurium* TA 100, *Proteus vulgaris* NCTC HK 11, *Yersinia sp.*) and the Gram positive strain *Sthaphylococcus aureus.*

c) Resistance to antibiotics of *Lactobacillus acidophilus* F.B.

- complete resistance (streptomycin, kanamycin, neomycin, colistin, polymyxin, ampicillin, oxacillin, nolidixic acid, neoxazol)
- sensitivity (tetracycllin, erythromycin, penicillin K, cloramphenicol)

d) Resistance to antibiotics of *Bacillus subtilis* ICCF 77

- complete resistance (ampicillin, cloramphenicol, oxacillin, penicillin, tetracycllin, polymyxin, nolidixic acid, colistin)
- sensitivity (doxyciclin, ciprofloxacin,norfloxacin)

e) Microbial antagonism (*Yersinia enterocolitica* Paris IP76, *Staphylococcus aureus* ATCC 25923, *Streptococcus salivarius* IP 55126, *Salmonella thyphimurium* TA 100, *Pseudomonas aeruginosa* ATCC 27853).

Conclusions

Our experimental studies resulted in the obtaining of liophilized biomass from yeast (*Saccharomyces*) and bacteria (*Bacillus* and *Lactobacillus*), necessary for the preparation of a new probiotic product at micropilot stage.

The studies carried out at micropilot level have led to setting up of new processes for obtaining, separating and the lyophilizating of the three kinds of biomass. We found out the length of the bioprocess, the parameters for the biosynthesis process and the phases of development/sporulation for the three microorganisms types.

The pharmaceutical tests referred to : the level of resistance to antibiotics, microbial antagonism, the cell viability and the survival capacity in a medium with acidic pH and with lile acid salts.

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