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Research Article

Methods for Separation and Analysis of a Lactobacillus Biomass, Able to be Used for Health Improvement and Convalescence

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Abstract

A set of chemical and microbiological analyses was established, in order to substantiate scientifically the valuable content of a *Lactobacillus* biomass, in order to use it as a component of a probiotic preparation playing an important role on health improvement and convalescence. The best results concerning the optical density of the cultures and the quality of the biological material were obtained using a culture of *Lactobacillus* paracasei on media with honey, pollen and raffinose.

Keywords: *Lactobacillus*; Biomass Analyses; Health Improvement

Abbreviations

L. Paracasei: Lactobacillus paracasei; L. rhamnosus: Lactobacillus rhamnosus; L. Plantarum: Lactobacillus plantarum.

Introduction

A set of biochemical and microbiological analyses was established, in order to substantiate scientifically the lactic-acid biosynthetic potential and the valuable content of a pure *Lactobacillus* biomass, in order to use it as a component of a probiotic preparation. This paper presents the selection of the methods most appropriate by which the characteristics of the bacterial biomass can be verified and used. Further the conditioned formula of the final probiotic product was represented by a cream and the best composition of this product was found by using some strains resistant to high sugar content, like a culture of *Lactobacillus paracasei*.

There are many other studies carried out all around the world, in order to obtain different probiotic preparations, depending on the local raw materials and the microbial strains adapted to the specific environmental conditions.

The conclusion of this study was that probiotic potential of the isolated probiotic *L. rhamnosus* and *L. Plantarum* is highest (100%) as compared to commercially available probiotic preparations, such as Pre-Pro kid, Sporlac powder, LactoBacil plus, P-Biotics kid and Gastroline containing probiotics. According to this research way, a better bacterial strain (*L. Paracasei*) was discover by our works.

Materials and Methods

The *Lactobacillus* probiotic strains selection was made in according with the following parameters: the strain development, the lactic-acid biosynthesis level and the utilization of sugar or its final concentration. In order to obtain a pure biomass preparation, 3 steps at centrifuge (each of them with increased speed) were used.

The next work of our studies was represented by the experiments concerning the highest rate of the cellular multiplication and the most valuable biomass composition (traced by the level of the present microelements) which may be obtained. The further operation was carried out in order to prepare the *Lactobacillus* probiotic biomass as necessary for the trace elements determination. A three- step scheme was selected for the biomass Microwave Digestion, depending on the organic compounds types and by using pure nitric acid as reagent. Ultrapure water with a specific resistance of 18 MΩ or greater was obtained using a Milli-Q™ water purification system (Millipore Corporation, USA).

Nitric acid (65%, m/v, Ultrapure® grade, Merck, Darmstadt, Germany) was used.

Standard solutions for calibration curves were prepared by dilutions of 10 mg L^{-1} of Standard 3 (trasable to NIST) procured from Perkin Elmer, Inc. Shelton, USA.

Microwave digestion of the *Lactobacillus* probiotic biomass samples for ICP-MS analysis was carried out using a model Multiwave™ 3000 microwave system (Anton Paar).

The copper, zinc, cobalt, cadmium and iron total concentrations were determined by A Perkin Elmer Elan DRC-e inductively coupled plasma spectrometer ICP-MS and by FAAS [1-14].

Results and Discussions

The first studies, carried out on a laboratory scale, concerning the multiplication of some *Lactobacillus* and *Bifidobacterium* probiotic strains on media supplemented with honey and milled/non-milled pollen, indicated the best results on media with honey supplemented also with other different oligosaccharides. Then, our studies were carried out in order to obtain a higher rate of the cellular multiplication and a more valuable biomass composition (traced by the level of the present microelements). Therefore, the copper, zinc, cobalt, cadmium and iron total concentrations were determined and the obtained results are presented in the next Table and Graphics and picture.

Element	Sample 1 *Lactobacillus plantarum* (ppm)	Sample 2 *Bifidobacterium* (ppm)	Sample 3 *Lactobacillus paracasei* (ppm)
Cu	17,62	17,02	45,54
Zn	38,59	37,98	62,18
Co	5,05	5,02	Under 1,0
Cd	0,06	0,05	0,30
Fe	668,96	664,24	183,1

Table 1: The Cu, Zn, Co, Cd and Fe Determination.

Conclusions

The data obtained by our researches indicate the best optical density (the parameter used for the strains development's determination) and the highest yields of lactic acid and some microelements, in the case of some bacterial strains initially selected on a fermentation media containing honey, human milk and raffinose.

The analyses of the biomass preparation obtained on a culture medium based on glucose, peptone and yeasts extract indicate a greater presence of the iron and zinc (important elements involved in health improvement and for accelerating the convalescence) mostly in the case of *Lactobacillus paracasei* selected strain.

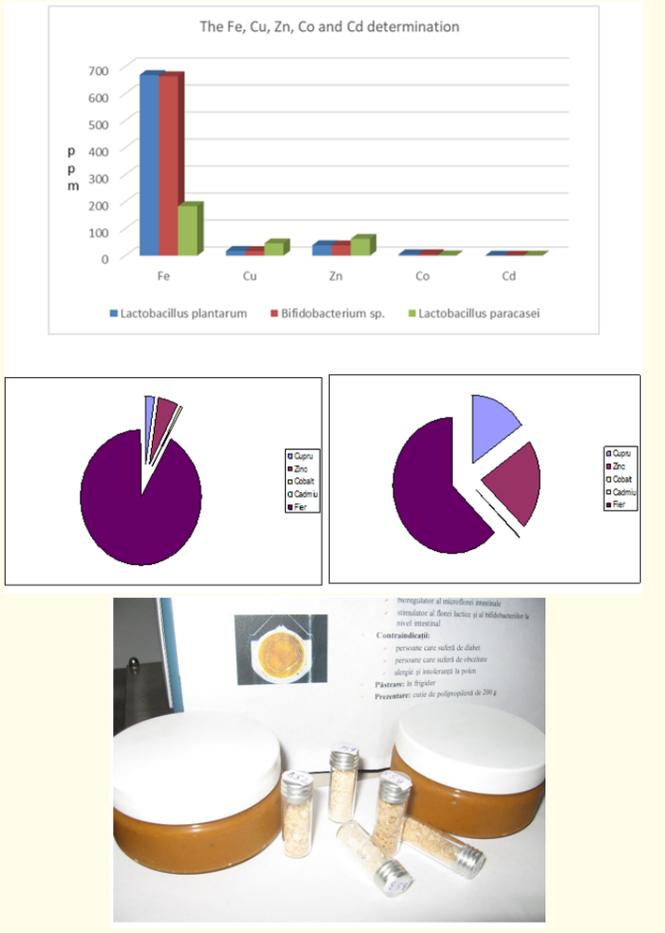

Figure: Biomass of Lactobacillus and the probiotic preparation.

The repeated analyses indicate a good stability of our pure biomass of Lactobacillus preparation.

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