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# LACTIC ACID PRODUCTION BY BACTERIA ISOLATED FROM RHIZOSPHERIC SOIL OF DAHLIA TUBERS

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**Abstract**: *We isolated lactic acid bacteria from the rhizospheric soil of dahlia tubers. Lactic acid was produced by batch fermentation of dahlia hydrolysate. The selection of the best homolactic acid productive strain was made by biotechnological characteristics: pH tolerance, lactic acid yield and lactic acid productivity. The selected strains produced lactic acid at maximum yield after 48 hours of fermentation. The productivity was better after 48 hours of fermentation. For all investigated strains, the Lbd2 strain showed the best lactic acid production yield of 6.30 ± 0.02 after 96 hours of fermentation and was the most acid-tolerant bacteria, due to the pH value which was 3.3±0.06. The Lbd2 lactic acid productivity after 48 hours of fermentation was 0.10 g· $L^{-1}$· $h^{-1}$, being the highest value obtained of all the strains studied.*

***Keywords****: inulin, polyfructans, fructose, acid hydrolysis.*

## 1. Introduction

Polyfructans (fructosans or fructans) are found as reserve carbohydrates in plants, being in high amounts in Jerusalem artichoke, dahlia and chicory [1]. Inulin, levan and graminan are polyfructans, the difference between those chemicals being due to the type of bonds between the fructose [2].

Dahlia sp. is a decorative plant that contains polyfructans in the tubers.

Polyfructans, and especially inulin, can be chemically or enzymatically hydrolyzed to fructose and sucrose. These are fermentable sugars that can be transformed into lactic acid or other substances by fermentation.

Lactic acid is a substance with high importance in human life. It has applications in food, pharmaceutical, textile, leather, and other chemical industries. It is recognized as safe for human consumption and in food industry is used as acidulant, flavoring, buffering agent or inhibitor of bacterial spoilage in a wide variety of processed foods. The advantages of the biotechnological and chemical production are the optical purity of isomers, low cost of substrates, low production temperature, and low energy consumption [3, 4].

*Lactobacillus acidophilus, L. amylovorus, L. casei, L. fermentum, L. johnsonii, L. gasseri, L. paracasei, L. plantarum, L. rhamnosus, L. delbrueckii* can use fermentable sugars (especially fructose) as carbon source [5, 6]. These bacteria are called lactic acid bacteria (or LAB).

The technique of lactic acid fermentation is important because it can reduce the time of fermentation or

increase the yield of lactic acid. So, it can be used in the separate hydrolysis and fermentation, the simultaneous saccharification and fermentation or combined hydrolysis and fermentation.

Our goals are to isolate the homolactic acid bacteria from the rhizospheric soil of dahlia tubers and to test the isolated strains for lactic acid production.

## 2.Materials and methods

### *Materials*

The polyfructans rich feedstock used in this study was dahlia flour. Dahlia Singer tubers were purchased from S.C. Agrosel S.R.L., Romania. Dahlia flour was produced in the laboratory by cleaning and cutting the roots followed by freezing at -70°C in Platinum 500 freezer (AS Biomedical, Germany), drying using Alpha 1-4 LD Plus lyophilizer (Martin Christ, Germany) and finally grinding with VC2011 grinder (Victronic, PRC).

### *Hydrolysis*

3% (w/v) suspension was prepared by mixing the dahlia flour with distilled water. For chemical hydrolysis the pH of dahlia flour was subsequently adjusted to 2 using 98%, 1N and 0,1N sulphuric acid solutions, then heated at 100 ± 2°C for 30 minutes. The pH was measured using a pH meter S20 (Mettler Toledo, USA). To reduce sugars, the hydrolysate was cooled at 23 ± 2°C and the pH was increased until 6.0 ± 0.2 using 33%, 1N and 0.1N sodium hydroxide solutions. The hydrolysate was then inoculated with bacteria.

### *Lactic acid bacteria isolation*

The lactic acid bacteria strains were isolated from rhizospheric soil of dahlia tubers. Soil samples were collected with sterile spoons, and saved into clean bags. Two grams of soil sample were inoculated in 100 ml MRS (deMan, Rogosa and Sharpe) broth and then incubated at 37°C, for 48 hours in a BF 4000 incubator (Binder GmbH, Germany). Samples were serially diluted and plated onto MRS agar supplemented with 1% calcium carbonate using the double-layer method, as described in Barbu [7]. The MRS agar and MRS broth and calcium carbonate were purchased from Sigma-Aldrich, Germany. Only the colonies showing a clear halo on MRS agar were selected. The isolates were tested for characteristics of cell and colony morphology to certify the existence of lactic acid bacteria, according to Tofan [8]. Their isolation was carried out in pure cultures in MRS broth, according to Tofan [8].

### *Preservation of isolates*

All strains were stored in the laboratory freezer Platinum 500 (AS Biomedical, Germany), at -70°C in MRS broth supplemented with 10% (v/v) glycerol.

### *Biotechnological characterization of isolated lactic acid bacteria*

The type of fermentation has been determined using the liquid Mac Cleskey medium according to Barbu [7]. The lactic acid bacteria that showed homolactic fermentation were isolated from the rhizospheric soil of dahlia roots and were identified as Lbd1 to Lbd3.

As fermentation medium, we used the dahlia flour chemical hydrolysate prepared as described above. The hydrolysate was then pasteurized at 80°C for 30 minutes using Stericell 111 oven (MMM, Germany) and cooled at 21 ± 2°C. The hydrolysate was inoculated with 1% of each isolated lactic acid bacteria and immediately incubated at 37°C using a BF 4000 incubator. The fermentations were conducted in 100 ml flat bottom flasks. The lactic fermented samples were taken every 24 hours and analyzed for pH, reducing sugars and acidity.

The conversion yield of substrate to product and the productivity were

determined according to Pirt [9]. The determinations were made in triplicate.

***Physical and chemical analysis***

The dry matter was determined by a standard drying method in an oven at 105°C to constant mass, according to AOAC Official Methodology of Analysis [10].

The concentration of fructose was estimated by 3,5-dinitrosalicylic acid method using 6505 UV-VIS spectrophotometer (Jenway, UK), according to Miller [11]. Fructose was used to establish a standard curve.

The polyfructans amount was determined using Fructan Assay Procedure for the measurement of Fructo-Oligosaccharides (FOS) and Fructan Polysaccharide (Megazyme International, Ireland), according to McCleary and Blakeney [12].

The Romanian standard SR 90:2007 [13] was used for pH determination.

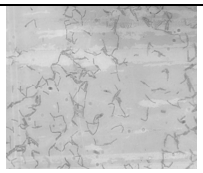
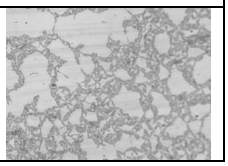
The lactic acid was determined at $\lambda$ = 470 nm with hydroquinone using the spectrophotometer 6505 UV-VIS, according to the method described in Banu [14].

All the chemicals used were of analytical grade. All the determinations were made in triplicate.

## 3.Results and Discussion

Three lactic acid bacteria strains were isolated from rhizospheric soil of dahlia tubers. The colonies distinguished clearly because they had around them a clear halo, while the rest of the medium in the plate was opalescent. The halo, that is a transparent surface around the colonies, is due to the production of lactic acid by the isolated bacteria. Table 1 presents different characteristics of the isolates.

**Table 1.**
**Characteristics of the isolated bacteria**

| Bacteria | Colony characteristics | Cell morphology | Cells image |
|---|---|---|---|
| Lbd1 | 3 mm cream circular colony, convex, opaque, smooth | Long curved rods, rounded ends, single, pairs, short chains |  |
| Lbd2 | 2-3 mm cream circular colony, convex, opaque, smooth | Long rods, rounded ends, single, pairs, in palisades |  |
| Lbd3 | 2 mm white-cream colony, convex, glistening, opaque | Short rods, rounded ends, single, pairs, in palisades |  |

The separate hydrolysis and fermentation technique was used for the lactic acid production, which consisted in 30 minutes of acid hydrolysis, followed by 72 hours of lactic acid fermentation. Fructose, glucose

and sucrose formed after hydrolysis were fermented into lactic acid by bacteria isolated from the rhizospheric soil of dahlia tubers. The composition of the dahlia flour and the yield of hydrolysis are presented in Table 2.

**Table 2.**

**Chemical composition of the dahlia flour before and after hydrolysis (values presented as mean ± standard deviation)**

| Product | Dry matter (%) | Flour composition | | Flour hydrolysate | Polyfructans hydrolysis yield (%) |
|---|---|---|---|---|---|
| | | Fructose (% dry weight) | Polyfructans (% dry weight) | Fructose (% dry weight) | |
| Dahlia flour | 94.36 ± 2.17 | 1.20 ± 0.24 | 74.27 ± 3.78 | 37.90 ± 1.92 | 51.03 |

The initial dahlia flour polyfructans (inulin and oligosaccharides) amount is 74.27 ± 3.78 g/100 g flour. The amount of fructose after chemical hydrolysis increased from 1.2 to 37.9 g/100 g flour. Also, the hydrolysis yield was of 51.03 %. Usually, the yield of the acid hydrolysis of polyfructans from tuber plants to fructose is around 50%. Razmovski [15] stated that 30 minutes and a pH = 2.0 were needed for 52 % hydrolysis of Jerusalem artichoke inulin. Our findings comply with the ones from the scientific literature due to the identical hydrolysis parameters used.

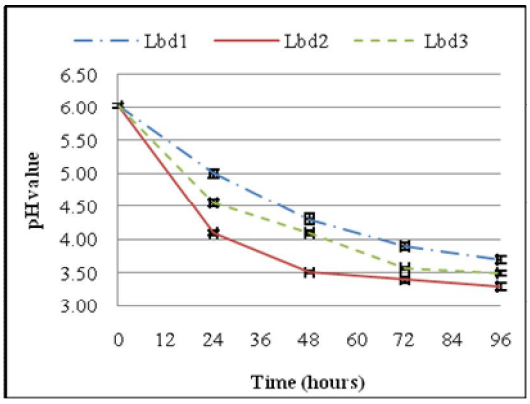



**Figure 1.pH variation of lactic acid fermented dahlia hydrolysate**

After 96 hours of lactic acid fermentation with bacteria isolated from rhizospheric soil of dahlia tubers we observed a pH variation as presented in figure 1. All the studied microorganisms are resistant to low pH value, but Lbd2 are produced after 24 and 48 hours of fermentation in a more acidic media compared with the other microorganisms. Also, it is tolerant to lowest pH value of the fermentation medium (pH = 3.3±0.06).

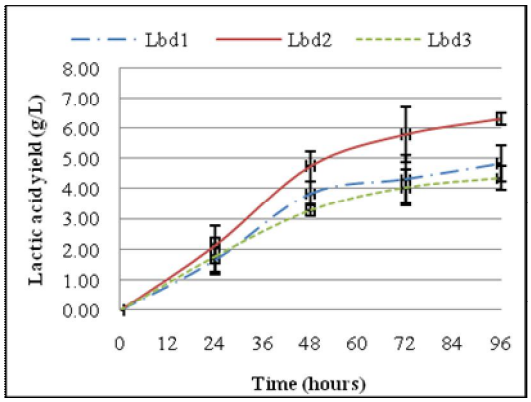



**Figure 2.Lactic acid yield variation for dahlia hydrolysate**

The reduced value of pH is proportionally with the increasing value of the lactic acid in the fermentation media because of the lactic acid accumulation. In figure 2 we

can observe that the maximum lactic acid yield is produced by Lbd2, increasing from 2.16 ± 0.6 g/L after 24 hours of fermentation to 6.30 ± 0.2 g/L after 96 hours. Lbd2 presented the best lactic acid yield of the homolactic acid bacteria studied. Lbd3 had the lowest lactic acid yield of all the homolactic acid bacteria studied.

For the lactic acid production we used batch fermentation and separate hydrolysis and fermentation techniques. It is known the fact that in batch cultures lactic acid concentrations can be higher than in continuous cultures [16]. Comparing the lactic acid yield produced by Lbd2 with ones produced by batch fermentation [16] we can say that our values are very low because the researchers used fermentation media enriched with nutrients or they used lactic acid bacteria selected for lactic acid production or another substrate for fermentation than dahlia hydrolysate.

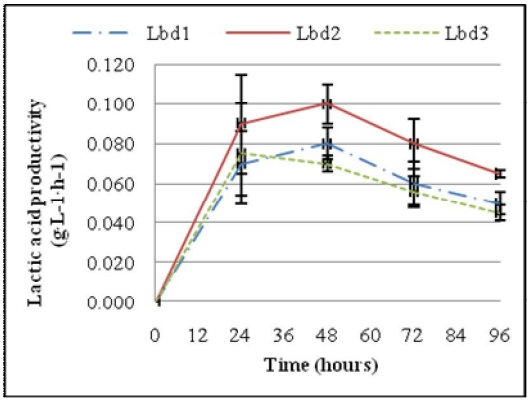



**Figure 3. Lactic acid productivity from dahlia hydrolysate**

The productivity of dahlia hydrolysate, as shown in figure 3, increased for Lbd1 and Lbd2 after the first 48 hours of fermentation and decreased until the end of lactic acid fermentation. This is due to the increasing in the period of time between 48 and 96 hours of fermentation simultaneously with the slow accumulation of lactic acid in the fermentation media. Lbd2 showed the best lactic acid productivity after 48 hours of fermentation with a value of 0.10 ± 0.01 $g \cdot L^{-1} \cdot h^{-1}$. Comparing the productivity of the homolactic bacteria isolated, the lowest values were registered by Lbd3.

## 4.Conclusions

We isolated three strains of homolactic acid bacteria from rhizospheric soil of dahlia tubers. Lbd2 showed the best biotechnological production of lactic acid using batch fermentation and separate hydrolysis and fermentation technique, with no other nutrients added to the fermentation medium. Also, it has high toleration to acid environment. It can be used to produce lactic acid from chicory hydrolysate. Further studies needs to be made to improve the lactic acid production by enrichment of the fermentation medium with nutrients.

## 5.Acknowledgements

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## 6. References

[1] CHI Z.M., ZHANG T., CAO T.S., LIU X.Y., CUI W., ZHAO C.H., Biotechnological potential of inulin for bioprocesses, *Bioresource Technol.*, 102. 4295-4303, (2011)

[2] ANAN´INA N.A., ANDREEVA O.A., MYCOTS L.P., OGANESYAN E.T., Standardization of inulin extracted from *Dahlia*

*single* tubers and some physicochemical properties of inulin, *Pharmaceutical Chemistry Journal*, 43. 157-159, (2009)

[3] JOHN R.P., NAMPOOTHIRI K.M., PANDEY A., Fermentative production of lactic acid from biomass: an overview on process developments and future perspectives, *Appl. Microbiol. Biotechnol*., 74. 524–534, (2007)

[4] HOFVENDAHL K., HAHN-HÄGERDAL B., L-lactic acid production from whole-wheat starch hydrolysate using strains of Lactobacilli and Lactococci, *Enzyme Microb. Tech*., 20. 301–307, (1997)

[5] MAKRAS L., VAN ACKER G., DE VUYST, L., *Lactobacillus paracasei* subsp. *paracasei* 8700:2 degrades inulin-type fructans exhibiting different degrees of polymerization, *Appl. Env. Micro*., 71. 6531-6537, (2005)

[6] PATIL S.S., KADAM S.R., BASTAWDE K.B., KHIRE J.M., GOKHALE D.V., Production of lactic acid and fructose from media with cane sugar using mutant of *Lactobacillus delbrueckii* NCIM 2365, *Lett. Appl. Microbiol*., 43. 53–57, (2006)

[7] BARBU V., Phenotypical characterization of several lactic acid bacteria strains isolated from wheat's epiphyte microbiota, *Rom. Biotechnol. Lett*., 13. 4074-4085, (2008)

[8] TOFAN C., BAHRIM G., NICOLAU A., ZARA M., Food Microbiology. Techniques and Laboratory Analysis, AGIR, Bucharest, 34-61 p, 192-195 p, (2002)

[9] PIRT S.J., Principles of microbe and cell cultivation, Blackwell Scientific Publications, Oxford, 4-14 p, (1985)

[10] AOAC, Official methods of analysis of AOAC International, Gaithersburg, MD, USA, (2000)

[11] MILLER G.L., Use of dinitrosalicylic acid reagent for determination of reducing sugar, *Anal. Chem*., 31. 426-428, (1959)

[12] MCCLEARY B.V., BLAKENEY, A.B., Measurement of inulin and oligofructan, *Cereal Foods World*, 44. 398–406, (1999)

[13] SR 90:2007, Wheat. Methods of analysis, Romanian standard, (2007)

**[14]** BANU C., IONESCU A., BERZA, M., Control Methods and Techniques for Fish and Fish products, Dunărea de Jos University of Galati, Galati, 131 p, (1991)

[15] RAZMOVSKI R.N., ŠĆIBAN M.B., VUČUROVIĆ V.M., Bioethanol production from Jerusalem artichoke by acid hydrolysis, *Rom. Biotechnol. Lett.*, 16. 6497-6503, (2011)

[16] WEE Y.J., KIM J.N., RYU H.W. Biotechnological production of lactic acid and its recent applications, *Food Technol. Biotech.,* 44. 163–172, (2006)