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***ORIGINAL RESEARCH PAPER***

# SELECTION OF LACTIC ACID BACTERIA ABLE TO FERMENT INULIN HYDROLYSATES

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Eight homofermentative lactic acid bacteria isolates were tested for lactic acid production using chicory and Jerusalem artichoke hydrolysate as substrate. The pH, lactic acid yield and productivity were used to select the best homolactic bacteria for lactic acid production. The selected strains produced lactic acid at maximum yield after 24 hours of fermentation and the productivity was greater at 24 hours of fermentation. From all studied strains, Lb1 and Lb2 showed the best results regarding lactic acid yields and productivity. After 48 hours of chicory and Jerusalem artichhoke hydrolysates fermentation, from all the studied strains, Lb2 produced the highest lactic acid yield (0.97%). Lb2 produced after 48 hours of fermentation the lowest pH value of 3.45±0.01. Lb2 showed greater lactic acid productivity compared to the other studied lactic acid bacteria, the highest values, 0.13 $g \cdot L^{-1} \cdot h^{-1}$from Jerusalem artichoke hydrolysate and 0.11$g \cdot L^{-1} \cdot h^{-1}$ from chicory hydrolysate, being produced after 24 hours of fermentation.

**Keywords**: chicory, Jerusalem artichoke, lactic acid yield, productivity, fructose

## Introduction

Inulin is a reserve carbohydrate in plants, found in high amounts in Jerusalem artichoke, dahlia and chicory and in smaller amounts in garlic and onion (Chi*et al*., 2011; Singh*et al*., 2006), being a group of naturally-occurring polysaccharides. Inulin is a polydisperse fructan that ranges in its degree of polymerization from 2 to 60, or higher (De Leenheer *et al*., 1994). Inulin can be hydrolysed by enzymes or by chemical means to fructose. The acid hydrolysis of inulin has been investigated using sulphuric or hydrochloric acid (Barta, 1993; Duvnjak *et al*., 1987; Pekić *et al*., 1985). Inulin chemical stability decreases in an acidic environment at pH under 4 due to the heating time and temperature increase. Strong acidic environment (pH 1-3) caused intensive inulin hydrolysis and the amount of unbound reducing sugars is increasing with heating time and temperature (Glibowski *et al*., 2011).

Lactic acid (2-hydroxypropionic acid or 2-hydroxypropanoic acid) has many applications in food, pharmaceutical, textile, leather, and other chemical industries. Lactic acid is generally recognized as safe. In food industry, lactic acid is used as acidulant, flavoring or buffering agent or inhibitor of bacterial spoilage in a wide variety of processed foods, such as candy, breads and bakery products, soft drinks, soups, sherbets, dairy products, beer, jams and jellies, mayonnaise, and processed eggs, often in conjunction with other acidulants (John *et al*.,2007). Besides high product specificity, as it produces a desired optically pure L-(+)- or D-(−)-lactic acid, the biotechnological production of lactic acid offers several advantages compared to chemical synthesis like low cost of substrates, low production temperature, and low energy consumption (Hofvendahl *et al.*,1997).

Biotechnologically, from inulin, lactic acid can be produced by SHF - separate hydrolysis and fermentation (inulin is, firstly, transformed to reducing sugars by enzymatic or chemical means which then are fermented to lactic acid by microorganisms), by SSF – simultaneous saccharification and fermentation (inulin is hydrolysed and fermented directly to lactic acid by microorganisms) and by CHF – combined hydrolysis and fermentation (inulin is, firstly, enzimatic hydrolysed to a certain amount of fructose for enhancing the lactic acid conversion, and then inoculated with microorganisms).

Some of the lactic acid bacteria (LAB) such as: *Lactobacillus acidophilus, L. amylovorus, L. casei, L. fermentum, L. johnsonii, L. gasseri, L. paracasei, L. plantarum, L. rhamnosus, L. delbrueckii* can use fructose as carbon source (Makras *et al*., 2005; Patil *et al.*, 2006).

The present work reports on the selection and testing of eight homolactic acid production bacteria strains isolated from chicory roots for the best lactic acid production and acidic medium resistance.

## Materials and methods

### *Materials*

The inulin rich feedstock used in this study was chicory and Jerusalem artichoke flour. Chicory flour was produced in the laboratory by cleaning and cutting the roots followed by freeze-drying using Alpha 1-4 LD Plus lyophilizator (Martin Christ, Germany) and finally grinding with VC2011 grinder (Victronic, PRC).

Jerusalem artichoke (*Helianthus tuberosus* L.) flour was kindly delivered to us by the Romanian company S.C. Hofigal Export-Import S.A.

### *Hydrolysis*

3% (w/v) suspensions were prepared by mixing the chicory and Jerusalem artichoke flours with distilled water. The pH was measured using a pH meter S20 (Mettler Toledo, USA). For chemical hydrolysis the pH of the chicory and Jerusalem artichoke flours were subsequently adjusted to 2 using 98%, 1N and 0,1N sulphuric acid solutions, then heated at 100 ± 2°C for 30 minutes. For reducing sugars initial analysis and inoculation, the samples were cooled at 25°C and neutralized at pH = 6.0±0.2 using 33%, 1N and 0.1N NaOH solutions.

***Lactic acid bacteria isolation***

The lactic acid bacteria strains were isolated from rhizospheric soil of chicory roots. Soil samples were collected with sterile spoons, and saved into clean bags. 2 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 (2008). The MRS agar and MRS broth and calcium carbonate were purchased from Sigma-Aldrich, Germany.

Only 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 bacteria, according to Tofan *et al.* (2002). Their isolation was carried out in pure cultures in MRS broth, according to Tofan *et al.* (2002).

***Preservation of isolates***

All strains were stored in the laboratory freezer Platinum 500 (AS Biomedical, Germany), at -70°C in MRS broth supplemented with 15% (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 (2008). Eight lactic acid bacteria that showed homolactic fermentation were isolated from the rhizospheric soil of chicory roots, and were identified as Lb1 to Lb8.

As the fermentation medium was used the chemical hydrolysates prepared as described above. All the hydrolysates were pasteurized at 80°C for 30 minutes using Stericell 111 oven (MMM, Germany) and then cooled at 20°C. The hydrolysates were then inoculated with 1% from each lactic acid bacteria isolate and immediately incubated at 37°C using a BF 4000 incubator. The fermentations were conducted in 100 ml flat bottom flasks. Lactic fermented samples were taken every 24 hours and analyzed for pH, reducing sugars and acidity. The determinations were made in duplicate.

The conversion yield of substrate to product, expressed as g lactic acid /g reducing sugars, and the productivity were determined according to Pirt (1985).

***Physico-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 (2000). The concentration of fructose was estimated by 3,5-dinitrosalicylic acid method using 6505 UV-VIS spectrophotometer (Jenway, UK), according to Miller (1959). Fructose was used for the establishment of a standard curve. Inulin was determined using Fructan Assay Procedure for the measurement of Fructo-Oligosaccharides (FOS) and Fructan Polysaccharide (Megazyme International, Ireland), according to McCleary and Blakeney (1999). Romanian standard SR 90:2007 (2007) has used for pH determination. The lactic acid were determined at $\lambda$ = 470 nm with hydroquinone using the spectrophotometer, according to the method described in Banu *et al*. (1991). All the chemicals used were of analytical grade.

All the determinations were made in triplicate.

## Results and discussions

Eight lactic acid bacteria strains were isolated from rhizospheric soil of chicory roots. The colonies distinguished clearly because they had around them a clear halo, while the rest of the medium in the plate was opalescent. Table 1 presents the different characteristics of the eight isolates.

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

| Bacteria | Colony characteristics | Cell morphology |
|---|---|---|
| Lb1 | 2 mm white-cream colony, convex, glistening, opaque | Short rods, rounded ends, singly, pairs |
| Lb2 | 3 mm cream circular colony, convex, opaque, smooth | Long curved rods, rounded ends, single, pairs, short chains |
| Lb3 | 2-3 mm cream circular colony, convex, opaque, smooth | Long rods, rounded ends, single, pairs, in palisades |
| Lb4 | 2 mm white-cream colony, convex, glistening, opaque | Short rods, rounded ends, single, in palisade |
| Lb5 | 1-2 mm white-cream colony, convex, dull, opaque | Short rods, rounded ends, single, in palisade |
| Lb6 | 2 mm white-cream colony, convex, glistening, opaque | Short rods, rounded ends, single, short chains, in palisade |
| Lb7 | 1-2 mm white-cream colony, convex, dull, opaque | Short rods, rounded ends, single, in palisade |
| Lb8 | 2-3 mm cream circular colony, convex, opaque, smooth | Long curved rods, rounded ends, single, pairs, short chains |

The SHF technique was used for the lactic acid production, which consisted in 30 minutes of acid hydrolysis for fructose production, followed by 72 hours of lactic acid fermentation.

The composition of the Jerusalem artichoke and chicory flours and the yield of hydrolysis are presented in Table 2.

Chicory flour fructans (inulin and oligosaccharides) amount is greater than the fructans amount of Jerusalem artichoke flour. Also, the fructose amount of chicory flour is greater than the amount of the Jerusalem artichoke flour.

After acid hydrolysis the fructose amount of Jerusalem artichoke increased from 0.4 % to 35.55 %, with a hydrolysis yield of 55.24 %. Razmovski *et al.* (2011) stated that 30 minutes and pH = 2.0 were needed for 52 % hydrolysis of Jerusalem artichoke inulin. The difference in yield value can be due to the differences in hydrolysis conditions and artichoke flours composition.

**Table 2.** Chemical composition and fructose amount after hydrolysis of the analysed flours (values presented as mean ± standard deviation)

| Flour type | Dry matter (%) | Composition of flour | | Flour hydrolysates | Fructans hydrolysis yield (%) |
|---|---|---|---|---|---|
| | | Fructose (% dry weight) | Fructans (% dry weight) | Fructose (% dry weight) | |
| Jerusalem artichoke | 95.25±0.3 | 0.4±0.16 | 63.64±1.27 | 35.55±0.86 | 55.24 |
| Chicory | 95.04±0.5 | 3.9±0.14 | 66.50±2.01 | 39.81±1.22 | 54.12 |

Chicory flour had an initial amount of fructose of 3.9 % and after hydrolysis it incresed to 39.81 %, with an acid hydrolysis yield of 54.12 %.

Chicory root flour had an amount of fructans (inulin and oligosaccharides) of 66.50 %, a value close to the one reported by Beirão-da-Costa *et al.* (2005) of 65.6 %.

Lactic acid yields from chicory and Jerusalem artichoke hydrolysates are presented in Figures 1 and 2.

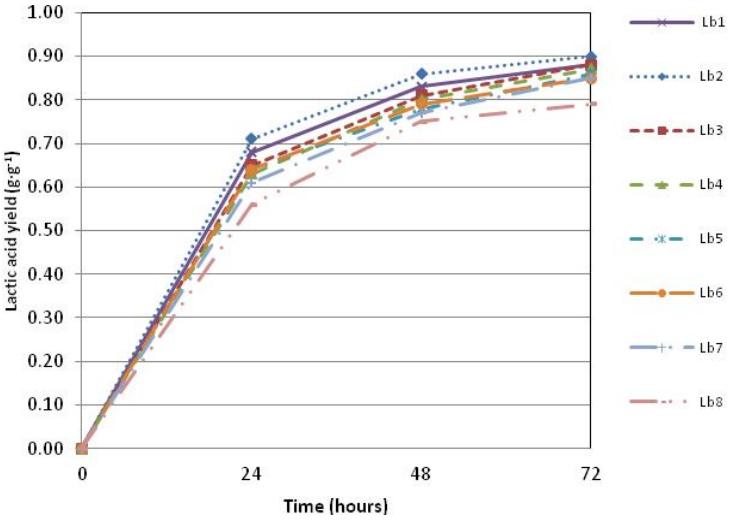


**Figure 1.** Lactic acid yield variation for chicory hydrolysate

After 24 hours of chicory hydrolysate homolactic acid fermentation, Lb2 had a yield of 0.71 g lactic acid/g fructose, and Lb1 a yield of 0.68 $g \cdot g^{-1}$. The other lactic

acid bacteria had yields below 0.65 $g \cdot g^{-1}$. It was an increase in yield values until 48 hours of fermentation, where Lb2 had a value of 0.86 $g \cdot g^{-1}$ and Lb1 of 0.83 $g \cdot g^{-1}$.

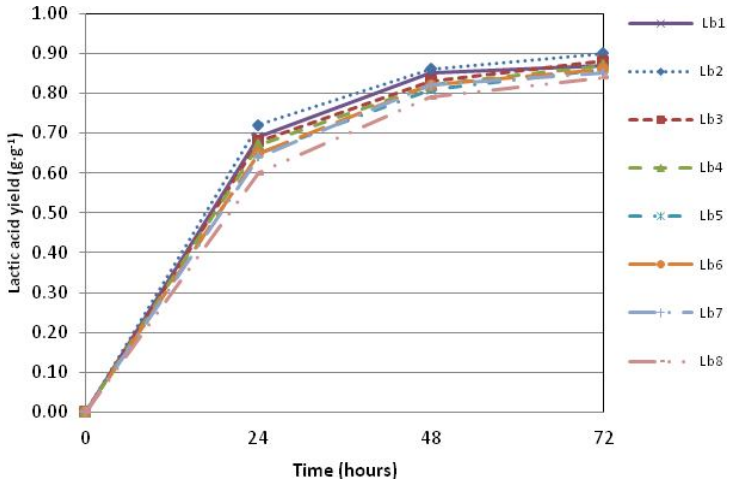


**Figure 2.** Lactic acid yield variation for Jerusalem artichoke hydrolysate

Within 24 hours of lactic acid fermentation of the Jerusalem artichoke hydrolysate, the yield for all the bacteria studied increased between 0.60 and 0.71 $g \cdot g^{-1}$. Lb2 had the highest yield value of 0.71 $g \cdot g^{-1}$, being followed by Lb1 with 0.69 $g \cdot g^{-1}$. After 48 hours of fermentation, the Lb2 showed the highest yield value (0.86 $g \cdot g^{-1}$). Comparing with scientific literature data, Ge *et al.* (2010), using *Lactobacillus casei* G-02 by SSF technique, obtained 93.6% lactic acid yield, and produced 52.4 g lactic acid/100 g Jerusalem artichoke flour. Choi *et al.* (2012) by fermenting for 72 hours with *L. paracasei* KCTC13169 directly, without any hydrolysis of the inulin from Jerusalem artichoke tubers, obtained 98% of the theoretical yield of lactic acid. Also, using a mixed culture of *A. niger* and *Lactobacillus* sp. G-02, Ge *et al.* (2009) produced by fed-batch fermentation for 36 hours directly from Jerusalem artichoke tubers, a yield of 94.5% lactic acid. The difference between literature values and our lactic acid yields is due to the fact that we used submerged fermentation, batch method. It is well known that batch and fed-batch method of fermentation produces the highest lactic acid yield (Hofvendahl *et al.*, 2000).

From the data obtained in Figures 1 and 2, it can be observed that Lb2 strain produced the highest yield for all studied homolactic acid bacteria studied.

The pH variation along 72 hours of lactic acid fermented chicory and Jerusalem artichoke hydrolysates is presented in Figures 3 and 4.

In the case of fermented chicory hydrolysate, from the initial pH of 6.0, Lb2 produced a fast pH decrease in the first 24 hours until 3.6 and a slower decrease after another 24 hours until 3.45. For the Lb2 strain, the pH variation was the

lowest of all the lactic acid bacteria analysed. As pH values decreased, after 24 and respectively 48 hours of fermentation, the Lb2 was followed by Lb1 bacteria.

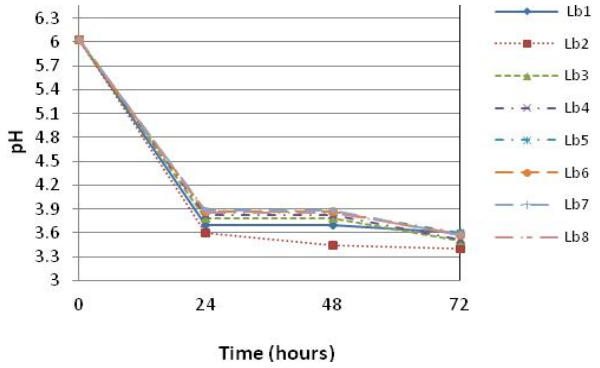


**Figure 3.** pH variation of lactic acid fermented chicory hydrolysate

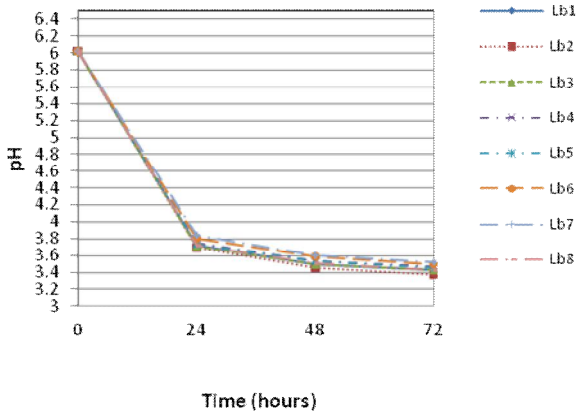


**Figure 4**. pH variation of lactic acid fermented Jerusalem artichoke hydrolysate

The initial pH of Jerusalem artichoke hydrolysate had a fast decrease along the first 24 hours of fermentation and a much slower decrease after 48 hours. Lb2 presented the lowest pH decrease from 6.0 to 3.7 after 24 hours of fermentation and to 3.46 after 48 hours of fermentation.

The Lb2 strain is tolerant to low acidity, because after 72 hours of lactic acid production, the medium pH decreased below 3.40.

In Figures 3 and 4 it can be seen that a fast decrease of pH took place in the first 24 hours of fermentation. For the next 24 hours of fermentation, the pH had a slow decrease. This is due to lactic acid production and accumulation in the fermented medium of the lactic acid bacteria.

Lactic acid productivity for the isolated homolactic bacteria is shown in Figures 5 and 6. It can be seen that the productivity of lactic acid is increased for the first 24

hours of fermentation and then decreased slowly. This is due to the producing of lactic acid faster by the lactic acid bacteria in the first 24 hours than for 48 hours of fermentation. After the first 24 hours of fermentation, the lactic acid accumulation and the pH low values of the fermentation medium have led to inhibition of large quantities of lactic acid production by the selected bacteria.

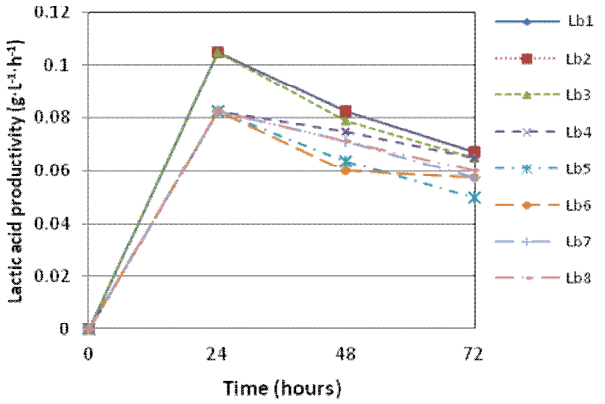


**Figure 5.** Lactic acid productivity from chicory hydrolysate

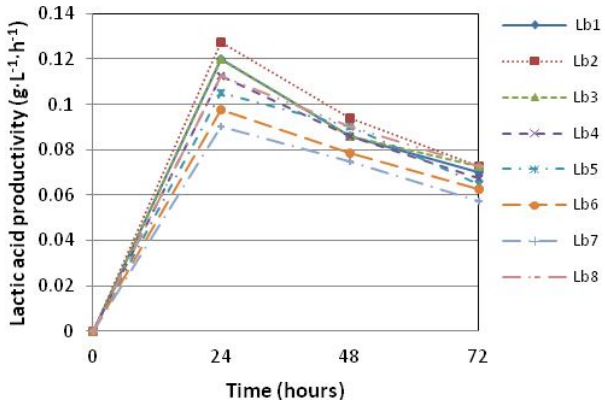


**Figure 6.** Lactic acid productivity from Jerusalem artichoke hydrolysate

Fermenting for 24 hours the chicory hydrolysate Lb1 and Lb2 showed the highest productivities from all the studied bacteria. For Lb1 and Lb2, the productivity increased after 24 hours at 0.11 $g \cdot L^{-1} \cdot h^{-1}$, and after 48 hours it slowly decreased to 0.08 $g \cdot L^{-1} \cdot h^{-1}$.

In Figure 6, the Lb2 productivity after 24 and 48 hours of fermentation is higher compared to the other lactic acid bacteria. The productivity of Lb2 increased to 0.13$g \cdot L^{-1} \cdot h^{-1}$ after 24 hours of fermentation and then slowly decreased to 0.09 $g \cdot L^{-1} \cdot h^{-1}$ after 48 hours of fermentation. The Lb3 strain showed a good productivity after Lb1, for 24 hours of fermentation. Ge *et al*. (2010) fermenting for 30 h using

SSF technique in fed-batch culture of *Lactobacillus casei* G-02 obtained a volumetric productivity of 4.7 $g \cdot L^{-1} \cdot h^{-1}$. The difference in the productivity is due to the fact that we used batch fermentation without any nutrients added.

## Conclusions

The lactic acid bacterium with the highest yield on chicory and Jerusalem artichoke hydrolysates is Lb2. After 48 hours of fermentation, the Lb2 strain produced lactic acid yield of 0.86 $g \cdot g^{-1}$ on both fermentation mediums, and after 72 hours of fermentation the lactic acid yield was 0.90 $g \cdot g^{-1}$.

The Lb2 strain showed the best tolerance to acidic medium. Below 3.40 it is still producing lactic acid.

The productivity after 24 hours of fermentation for Lb2 (0.11 $g \cdot L^{-1} \cdot h^{-1}$ on chicory hydrolysate and 0.13 $g \cdot L^{-1} \cdot h^{-1}$ on Jerusalem artichoke) is higher than the other homolactic bacteria. Its performance, in the case of chicory hydrolysate, is equalized only by Lb1 and Lb3.

Lb2 can be used in further studies for lactic acid production. For productivity increasing it can be made a nutritive improving of the chicory or Jerusalem artichoke hydrolysates.

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