GROWTH PARAMETERS OF PROBIOTIC STRAIN LACTOBACILLUS PLANTARUM, ISOLATED FROM TRADITIONAL WHITE CHEESE

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ABSTRACT

Different aspects, including safety, functional and technological characteristics, have to be taken into consideration in the selection of each probiotic microorganism. The aim of the present work was to determine the kinetic growth parameters of L. plantarum strain after cultivation in media with different carbon sources. The strain was isolated from traditional Bulgarian white cheese and previously characterized as putative probiotic, based on commonly accepted in vitro criteria. For further biotechnological implementation was necessary to select a suitable and economically relevant growth media. Thus, reconstituted permeate (6% w/v) and the following modification of de Man, Rogose Sharpe media (MRS): (i) MRS-glucose; (ii) MRS-lactose; (iii) MRS-galactooligosaccharide; (iv) MRS-fructooligosaccharide were used. The strain growth, lactic acid production and carbon source utilization were monitored by pH and cell number determination, and HPLC analysis at different time points of the cultivation process. The highest cell growth and carbohydrate conversation were detected in the presence of glucose and lactose. The main product of the fermentation was lactate with detectable level of acetate. The permeate and MRS-galactooligosaccharide also support good growth and lactic acid production, which indicate a great potential for industrial applications of studied L. plantarum strain into the food system.

Keywords: Growth kinetics, L. plantarum, lactic acid production

Introduction

Lactic acid bacteria (LAB) have been widely used as starters or probiotic cultures in various food fermentation processes, contributing to the organoleptic properties, flavour and texture. Due to the production of lactic acid or other antimicrobial compounds, they also assist in the safety of the final product. However, before the successful application of each LAB strain in corresponding food processing, several factors have to be estimated. The conditions of fermentation, such as temperature, pH, the type of growth media, oxygen, and addition of some neutralizers have a large effect on the growth activity of lactobacilli (3). Among these, the type of growth media plays an important role in the growth activity and production of storage-stable concentrated cultures. Various media for LAB, such as de Man Rogosa, Sharp (MRS) broth, M-17, Eliker's broth, skim milk and whey permeate have been widely used (10). In order to choose appropriate growth medium, different aspects have to be considered: cost, the ability to reach a high number of cells, and harvesting methods. Therefore, changes in medium composition or searching of cheap raw materials have been proposed as way for feasible economic production (4, 13).

In our previous study, one L. plantarum strain was selected as promising strain with good probiotic properties (2). Its additional biotechnological characterization is of great importance for future application in probiotic functional foods. The aim of the present work was to study the efficiency of different growth media, biomass and lactic acid production of L. plantarum strain. In addition, the kinetic growth parameters were evaluated after cultivation in whey permeate recommended as economically relevant and cheap growth media for the production of LAB cultures.

Materials and methods

Bacterial strain and growth conditions
One L. plantarum strain isolated from traditional white
cheese and characterized as strain with good probiotic properties were included in the present study (2). Before the assay the strain was subcultured twice in MRS broth at 37°C, under anaerobic condition (BBL® Gas Pak Anaerobic System Envelopes, Becton Dickinson).

**Media and culture conditions**

A batch fermentation process, with final volume 250 ml, was carried out in static conditions at 37°C. As growth media were used (i) modified MRS broth (MRS without beef extract and Tween 80) supplemented with different carbon sources: glucose (20 g/l)- MRS-Glu; lactose (20 g/l)-MRS-Lac; galactooligosaccharide (10 g/l, Yacult, Japan)- MRS-GOS; fructooligosaccharide (10 g/l, Orafti, Belgium)- MRS-FOS and in (ii) whey permeate powder (6%, w/v) supplemented with (0.5%, w/v) yeast extract (WP/YE). The WP/YE medium was prepared as described by Schepers et al. (13). The different media were inoculated with overnight culture of *L. plantarum* (1%, v/v) washed twice with saline solution and resuspended to the initial volume. Gram staining was used to check the purity and the rate of growth.

**Growth parameters and lactic acid production**

Samples were taken at regular intervals during the growth to determine the cell number (cfu/ml), turbidity at 600 nm and pH (SensoDirect pH110, Lovibond®, Germany) at different time point up to 48 h. A calibration curves was completed to relate the absorbance value to the cell dry weight. One gram per liter of dry cell weight corresponded to 2.7 OD<sub>600</sub>. Lactic acid production and carbon source utilization were monitored by HPLC analysis (HPLC system Perkin Elmer LC-25 with a refractive-index detector) performed at 24 h. The HPLC column (Aminex HPX-87H, Bio-Rad) was maintained at the ambient temperature. The mobile phase was 5 mM sulfuric acid at a flow rate of 0.6 ml/min. Twenty μl of sample by subsequent treatments with centrifugation (8000×/15 min), filtering (0.22 μm, Millipore) and the addition of H<sub>2</sub>SO<sub>4</sub> was injected into the HPLC column.

Specific growth rate (μ<sub>n</sub>) was calculated as μ<sub>n</sub> = lnN<sub>f</sub>/N<sub>0</sub>/t<sub>f</sub>-t<sub>0</sub>/ln2. The maximum specific growth rate (μ<sub>nmax</sub>) was determined from curve of μ<sub>n</sub> vs time. Yield coefficients were estimated as followed:

Y<sub>x/s</sub>- yield coefficient biomass/substrate

\[
Y_{x/s} = \frac{(OD_{1} - OD_{0})}{(t_{1} - t_{0})} \cdot \frac{0.37}{(S_{0} - S_{1})}
\]

Y<sub>p/s</sub>- yield coefficient product (LA)/substrate

Y<sub>p/s</sub> = \frac{(LA_{1} - LA_{0})}{(S_{0} - S_{1})}

Productivity (φ) is evaluated as the biomass or the lactic acid production per unit time (h) per unit culture volume (l).

\[
\phi_{biomass} = \frac{(OD_{1} - OD_{0})}{(t_{1} - t_{0})}
\]

\[
\phi_{lactic acid} = \frac{(LA_{1} - LA_{0})}{(t_{1} - t_{0})}
\]

**Results and Discussion**

Characterization of the growth parameters in different commonly accepted media is the first and important step toward further commercial application of one probiotic strain. We examined the growth of previously selected *L. plantarum* in modified MRS broth supplemented with glucose, lactose and two prebiotic ingredients GOS and FOS. In the assay a cheap growth media commonly used for *Lactobacillus* cultivation were also included. The rate of growth was evaluated up to 48 h of incubation at optimal temperature for mesophilic lactobacilli. The obtained result indicated good growth (Fig. 1) and the highest productivity (φ) of the strain in both culture media supplemented with glucose and lactose. During the fermentation process high μ<sub>nmax</sub> values were reached at 6 and 8 h respectively. The *L. plantarum* growth, in the presence of glucose and lactose, was characterized with short lag phase, steep exponential phase up to 12 h, then a growth stage having a much slower speed for 2 h followed by the stationary stage (Fig.1).

The addition of prebiotics, as sole carbon source, resulted with high cell number and substrate utilization only in MRS-GOS. In the presence of FOS almost no growth was detected (Fig. 1). The yield coefficients corresponding to biomass production (Y<sub>x/s</sub>) and substrate conversion into product (Y<sub>p/s</sub>) were the highest in the presence of glucose and GOS (Table 1). Concerning the lactose containing media, WP/YE was less efficient for the strain’s growth. Although the high specific growth rate demonstrated in permeate, the conversion ratio (Y<sub>p/s</sub>) of the substrate into product was low with only 32 % carbohydrate utilisation.

The major product of the carbohydrates conversion was lactic acid, accomplished with detectable level of acetic acid. The highest amount of lactic acid was produced in the presence of glucose and lactose (about 13.6 g/l), while only 0.41 g/l in the presence of FOS (Fig. 2). The production of lactic acid may continue in time, taking into consideration the remaining amount of glucose and lactose in the media.
Fig. 1. Growth of *L. plantarum* strain in modified MRS broth with different carbon sources.

### TABLE 1

<table>
<thead>
<tr>
<th>Medium</th>
<th>Kinetic parameters*</th>
<th>Substrat utilization (%)</th>
<th>Lactate production [g/l]</th>
<th>Y_X/S</th>
<th>Y_P/S</th>
<th>ϕ_biomas [g/l/h]</th>
<th>ϕ_lacticacid [g/l/h]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRS-Glu</td>
<td>ln N: 21.1, μ_max: 0.627</td>
<td>74.6</td>
<td>13.68</td>
<td>0.143</td>
<td>0.917</td>
<td>0.240</td>
<td>0.57</td>
</tr>
<tr>
<td>MRS-Lac</td>
<td>ln N: 21.0, μ_max: 0.508</td>
<td>84.9</td>
<td>13.64</td>
<td>0.117</td>
<td>0.803</td>
<td>0.224</td>
<td>0.568</td>
</tr>
<tr>
<td>MRS-GOS</td>
<td>ln N: 20.6, μ_max: 0.619</td>
<td>86.3</td>
<td>7.33</td>
<td>0.152</td>
<td>0.849</td>
<td>0.148</td>
<td>0.305</td>
</tr>
<tr>
<td>WP/YE</td>
<td>ln N: 19.8, μ_max: 0.640</td>
<td>41.1</td>
<td>6.61</td>
<td>0.029</td>
<td>0.321</td>
<td>0.067</td>
<td>0.275</td>
</tr>
</tbody>
</table>

*All parameters (except μ\_max) are calculated for 24 h of cultivation.

Fig. 2. Chromatographic analysis of substrate utilization and lactic acid production and visualization of the microbial growth of *L. plantarum* strain in different media: A: MRS-Glu; B: MRS-Lac; C: MRS-Fos/ MRS-Gos; D: WP/YE.
Conclusions

The choice of a suitable medium plays a central role in the attempt at improving biomass and related product yield in a fermentation process. LAB have limited biosynthetic abilities (5) and are perfectly adapted to environments abundant in nutrients and energy sources. Such rich media, well balanced and satisfying the growth requirements of the lactobacilli is MRS. For the tested *L. plantarum* good growth rate was achieved in MRS media supplemented with glucose, lactose and GOS as carbon source. Similar results were reported for *L. plantarum* strains, isolated from koumiss and cultivated in MRS broth with glucose (1). The growth in MRS-GOS was very similar to those in MRS-glucose. Despite of the presence of free glucose residues specified in the commercial product, the kinetic parameters and especially production of 7 g/l lactic acid proved the capacity of *L. plantarum* to utilize this prebiotic. Such probiotic characteristic is strain-dependent and express additional potential for dietary and functional foods implementation. Insufficient growth in media with FOS proved inability of tested *L. plantarum* to ferment this oligosaccharide and its potential use in symbiotic formulas. In a study of the probiotic potential of lactobacilli, Pennacchia et al. reported the ability of *L. plantarum* strains to ferment FOS, GOS and lactulose but not inuline (11). Screening investigation of the ability of commercial probiotic strains to utilize FOS, proved species-specific behavior of its fermentation. The amount of lactic acid produced is of great importance with respect to technological properties of tested *L. plantarum* strain. We observed that the process of lactic acid fermentation in the presence of glucose was faster in comparison with other dairy lactobacilli including *L. plantarum* strains (1, 7).

In different *in vitro* studies, *L. plantarum* as well as other LAB are commonly cultured in MRS broth, but for large-scale commercial applications its cost is prohibitive. Both the success of MRS as a growth medium and its high cost are due to the complex extracts it contains (i.e. peptone, meat extract, and yeast extract), which accommodate the fastidious growth requirements (e.g. vitamins, amino acids, etc.) of many LAB (8). So the possibility of obtaining high biomass and lactic acid yield from a low cost medium is very challenging. As such media we choose whey permeate. Due to its high lactose content (83.1%), it may be used as a base-culture medium for production of starters or metabolites, such as lactic acid (9). *L. plantarum* showed relatively good growth and lactic acid production in permeate. Good growth of other *Lactobacillus* spp. in permeate was reported after periodic batch cultivation in membrane reactor (2, 13). Thus, the whey permeate is a promising medium for industrially cultivation of lactobacilli, after optimization process.

In conclusion, the present study demonstrated good growth of probiotic *L. plantarum* strain in the presence of prebiotic galactooligosaccharide, in addition of the standard media with glucose and lactose. These results are a promising base for future development of new symbiotic formulas. The whey permeate, as alternative and economically relevant growth medium, can be applied in the production of *L. plantarum*. Additional process of optimization will give a possibility for more efficient biomass and lactate production and it will be subject for further research.

Acknowledgment

The contributors express their gratitude for the funding by NATO grant SEP 982164 and research grant ДОО2-187/2008 of National Science Fund, Bulgaria

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