Lactic acid bacteria fermentations in oat-based suspensions

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Comparison of growth characteristics and exopolysaccharide formation of two lactic acid bacteria strains, *Pediococcus damnosus* 2.6 and *Lactobacillus brevis* G-77, in an oat-based, nondairy medium

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Abstract

The fermentation characteristics of two strains of lactic acid bacteria (LAB), *Lactobacillus brevis* G-77 and *Pediococcus damnosus* 2.6 were compared in an oat-based, nondairy milk medium (Adavena® G40). Viscosity and ropiness were the main growth parameters studied. Both strains are reported to produce an exopolysaccharide (EPS) with a β-glucan structure; in addition, the *L. brevis* strain produces also an EPS with an α-glucan structure. Both strains were able to ferment and produce EPS in the oat-based, nondairy medium to the extent that an obvious change was observed in terms of viscosity and ropiness during the fermentation period. These results show the potential of both LAB strains as possible starter cultures in new kinds of fermented, nondairy milk products.

Keywords: Lactic acid bacteria; Exopolysaccharides; β-glucan; Fermented oat; Adavena®

1. Introduction

Lactic acid bacteria (LAB) producing exopolysaccharides (EPS) play an important role in the food industry by improving the viscosity and the texture of fermented products (Cerning, 1990; Ricciardi & Clementi, 2000). It has previously been reported that various physiological conditions (composition of the medium, physio-chemical and kinetic parameters) have a major influence on the production of EPS by LAB (Cerning & Sutherland, 1985, Dupont, Roy, & Lapointe, 2000). Most of the polysaccharides produced by LAB have been described as having a combination of both α- and β-linkages with variations in monosaccharide composition (Bouzar, Cerning, & Desmazeaud, 1997; De Vuyst, Vanderveken, Van de Ven, & Deegest, 1998). However, few studies have been conducted on EPS-producing strains of *Lactobacillus brevis* and *Pediococccus damnosus* known to produce β-glucans (Dueñas-Chasco et al., 1997; Dueñas-Chasco et al., 1998) and their potential as starter cultures in food. Nevertheless, strains from these species are two of the few reported LAB that produce EPS with a β-glucan structure (Fig. 1).

There is general agreement that β-glucans from oats have a beneficial physiological effect in terms of their ability to reduce blood cholesterol and that these polysaccharides could be defined as dietary fiber (Wood, 1997; Behall, Scholfield, & Hallfrisch, 1997). The revised definition of dietary fibers not only includes nondigestible plant polysaccharides, but also their analogues, such as polysaccharides from microorganisms (Chung, 2000). Polysaccharides produced by microorganisms may therefore be related within this definition of dietary fibers if they can be shown to have similar physiological effects. Hence, this can make these bacterial strains important as starter cultures in fermented products with the aim to increase the amount of dietary fibers. A diet generally rich in dietary fibers is accepted to be beneficial in terms of low blood cholesterol levels (Andersson & Bridges, 1986). In this study, the growth of two strains of LAB, *L. brevis* G-77 and *P. damnosus* 2.6 were

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(a) →3)-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→
2
↑
1
β-D-Glc*p*

(b) →6)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→
2
↑
1
α-D-Glc*p*

Fig. 1. Structure of exopolysaccharide (EPS) produced by *Pediococcus damnosus* 2.6 (a) (Dueñas et al., 1997) and *Lactobacillus brevis* G-77 (a,b) (Dueñas et al., 1998).

studied in an oat-based, nondairy medium, Adavena® G40 (G40 medium) (Ceba Foods AB, Lund, Sweden). This product is derived entirely from oats and produced by an enzymatic process (Lindahl, Ahldén, Öste, & Sjöholm, 1997). One application of this oat base is as a milk substitute produced commercially (Ceba Foods AB, Lund, Sweden) that has been shown to have a cholesterol-lowering effect (Önning et al., 1999) and also to be a good substrate for various kinds of LAB (Mårtensson, Öste, & Holst, 2000, 2002; Mårtensson, Andersson, Andersson, Öste, & Holst, 2001).

The objective of the present study was to investigate the fermentation characteristics in an oat-based, nondairy medium and the structural effect of the EPS formation of these bacterial strains, measured as changes in viscosity and ropiness. This study is the first step in the evaluation of the technological performances of these strains with the aim to investigate their potential as cultures in new kinds of fermented, oat-based, nondairy products.

2. Materials and methods

2.1. Bacterial strains and media

L. brevis G-77 and *P. damnosus* 2.6 were obtained from the UPV culture collection, Universidad de Pais Vasco, San Sebastian, Spain. The strains were stored at −80°C in de Man Rogosa Sharpe (MRS) broth (De Man, Rogosa, & Sharpe, 1960) plus 25 mL glycerol 100 mL. Before use the cultures were propagated twice in MRS (Merck, Darmstadt, Germany) at 30°C. The oat-based, nondairy medium (Adavena® G40 medium) was obtained as a powder from Ceba Foods AB, Lund, Sweden and is made from rolled oats and water by an enzymatic method described previously (Lindahl, Ahldén, Öste, & Sjöholm, 1997). It was analysed for protein, fat, different carbohydrates, dietary fiber, various vitamins and minerals by an authorized laboratory (AnalyCen Nordic AB, Lidköping, Sweden) (Table 1).

Table 1
Chemical composition of the nondairy oat base (G40 medium)

Components	G40 medium g/100 g
Dry matter	20
Protein	2.2
Fat	1.6
Glucose	8.8
Maltose	0.6
Maltodextrin	5.4
Total fiber	1.6
β-glucan	0.8
	mg/100 g
α-Tocopherol	0.2
Thiamin	0.08
Riboflavin	0.002
Niacin	0.2
Folic acid	0.006
Pyridoxine	0.01
Iron	0.2
Magnesium	9.4
Manganese	0.1
Phosphorus	54
Sodium	22
Zinc	0.2

2.2. Fermentation experiments

Batches of 3L were cultivated in a small-scale fermentor (ChemoFerm, Stockholm, Sweden). The fermentor was sterilized at 121°C for 30 min. The inoculum (150 mL) of an exponentially growing culture was introduced into the fermentor, which was operated at 28°C for 24 h. For the preparation of the medium, oat-based powder (G40 medium) was dissolved to produce a mixture containing 20 g dry matter/100 mL and was heat treated at 90°C for 5 min with continuous stirring and then transferred to the sterile fermentor.

2.3. Sampling

Over a time period of 24 h, samples were withdrawn from the fermentation vessel to determine viscosity, ropiness, lactic acid (LA) concentration and residual glucose (Glc). Samples were immediately cooled on ice. Cell numbers (cfu/mL) were estimated by plate counts on MRS agar (Merck) incubated for 72 h at 30°C under anaerobic conditions using anaerobic jars (Anaerocult® A system) (Merck).

2.4. Measurement of viscosity and ropiness during growth

The viscosity was measured with a Brookfield DV-I viscometer (Brookfield Viscometers LTD, Harlow, UK) and the S85 spindle. The viscosity was measured at fermentation temperature for 2 min at 0.3 rpm and expressed in mPa. Ropiness was measured using an Instron 4442 instrument (Instron LTD, Buckinghamshire, UK). The probe (3.8 cm diameter) was placed on the surface of the sample and lifted at a speed of 100 cm/min. The ropiness value was shown as a linear unit (equal to the length of the "rope") and expressed in centimeter.

2.5. Determination of glucose and LA concentration

Residual glucose (Glc) content was analysed by high pH anion exchange chromatography (HPHEC) using a Carbopac PA 10 column (Dionex, Jouy-en-Josas, France) with 0.2 M NaOH as the mobile phase, at a flow rate of 1.4 mL/min. LA concentration was determined using an enzymatic kit (Boehringer, Manheim, Germany).

3. Results and discussion

In this study, the fermentation characteristics of two strains of LAB, *L. brevis* G-77 and *P. damnosus* 2.6, in the nondairy, oat-based medium (G40) were compared. Other studies have used viscosity as a verification of EPS production during growth (Dupont, Roy, & Lapointe, 2000, Thorne, Mikolajczak, Armentrout, & Pollock, 2000). In this study, we used both viscosity and ropiness as indications of EPS production during the fermentation period. A previous study has shown that no change of viscosity is found when the pH is lowered in the oat-based medium by the addition of glucono-δ-lactone (Mårtensson et al., 2000). Thus, methods to measure the structural characteristics such as viscosity and ropiness are appropriate for the detection of EPS formation in this kind of nondairy, oat-based medium. Basic fermentation characteristics of the two strains are listed in Table 2. The G40 medium supported growth and the formation of EPS of the bacterial strains to such an extent that there was an obvious change in viscosity and ropiness during the fermentation period, indicating that EPS production had occurred.

The fermentation profiles of *P. damnosus* 2.6 and *L. brevis* G-77, in terms of the effect on pH, viscosity, ropiness and the production of lactic acid and the consumption of glucose during growth in the G40 medium are shown in Fig. 2a–e. The pH decreased and lactic acid was produced during the whole fermentation period (Fig. 2a,b). Between 30% and 40% of the glucose was consumed during the fermentation period (Fig. 2c). The increase in viscosity was almost equal for both strains, although less glucose was consumed by the *P. damnosus* strain than by the *L. brevis* strain. The viscosity increased earlier in the fermentation period than the ropiness (Fig. 2d,e). Even if both of these parameters are related to EPS production this shows that the two parameters measure different kinds of structural behavior which are both linked to in situ production of EPS. For both strains the viscosity decreased during the last phase of the fermentation period. However, the medium still had visually a thicker appearance after 24 h of fermentation than prior to fermentation, thus this decrease is most likely due to an inhomogenicity in the medium due to the absence of stirring during the fermentation period. Another reason for this decrease in viscosity may also be originated from a partial hydrolysis of the EPS. This kind of phenomenon has been reported earlier for other EPS-producing LAB strains (De Vuyst & Deegest, 1999). The *P. damnosus* 2.6 strain showed both higher viscosity and ropiness in comparison with the *L. brevis* G-77 strain when the G40 medium was used (Fig. 2d,e). An earlier study in milk has shown that produced EPS from LAB can interact with proteins in milk (Hess, Roberts, & Ziegler, 1997). That these kinds of interaction also may

Table 2
Fermentation characteristics for *Lactobacillus brevis* G-77 and *Pediococcus damnosus* 2.6 in the G40 medium

Strain	Final pH[a]	Viable count[b] (cfu/ml)	Viscosity[c] (mPa)	Ropiness[d] (cm)	Residual Glc[e] (g/L)	Lactic acid (LA)[f] (g/L)
L. brevis G-77	4.0	4×10^8	1211	7.6	57	4.2
P. damnosus 2.6	4.2	7×10^8	1423	13.2	62	3.7

[a] Measured after 24 h, initial pH values were 7.2.
[b] Highest obtained value during the fermentation period. Initial value was 2×10^7 cfu/mL.
[c] Highest obtained value during the fermentation period. The viscosity was measured after 2 min of shear thinning at 0.3 rpm. Initial value was 95 mPa.
[d] Highest obtained value. The ropiness was measured with a speed of 100 cm/min. Initial value was 1.5 cm.
[e] Residual amount of glucose (Glc) was determined after 24 h of fermentation.
[f] Amount of lactic acid (LA) produced after 24 h of fermentation.

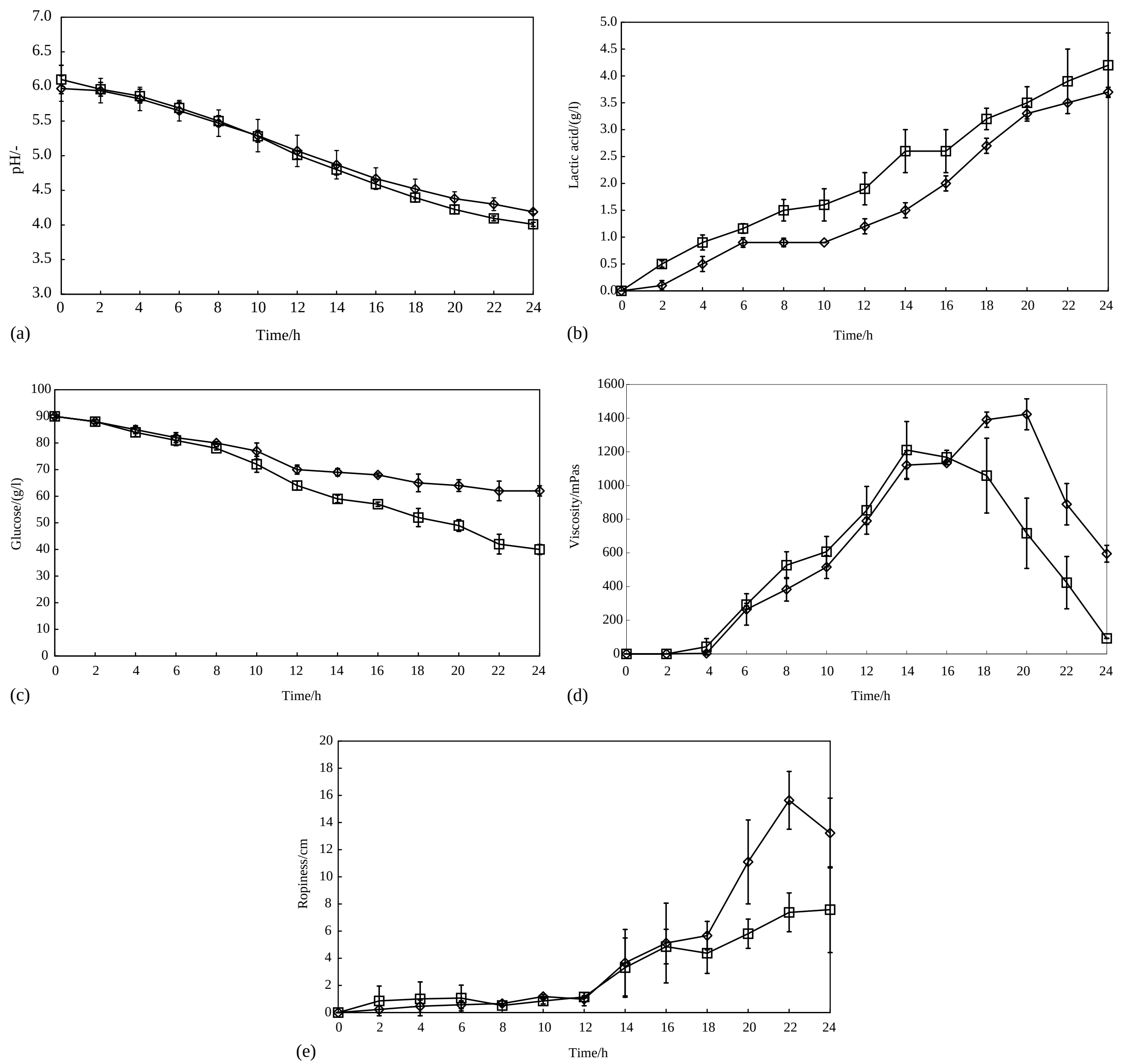

Fig. 2. pH (a), lactic acid concentration (b), glucose concentration (c), viscosity (d) and ropiness (e) during growth of *Pediococcus damnosus* 2.6 (◇) and *Lactobacillus brevis* G-77 (□) in G40 medium at 28°C for 24 h. The values are the mean of two fermentation experiments. All parameters were measured in triplicate at each fermentation experiment. Bar = standard deviation.

be able to occur between in situ produced EPS and the soluble fibers (β-glucans) in our oat-base medium cannot be neglected but has, however, to be further investigated.

After a fermentation period of 24 h the final pH in the G40 medium was below 4.3 for both strains, which is an indication of good growth conditions (Mårtensson et al., 2001). The highest viscosity was obtained after 20 h of fermentation for the *P. damnosus* 2.6 strain and after 13 h for the *L. brevis* G-77 strain. The highest ropiness occurred somewhat later during the fermentation period for both strains.

4. Conclusions

In this study, we show the fermentation characteristics and the EPS production of the two strains, *P. damnosus* 2.6 and *L. brevis* G-77, in an oat-based, nondairy medium. These initial characterisations have revealed a potential for these bacterial strains from a technological point of view as starter cultures and contributors for viscosity for use in new fermented, oat-based, nondairy products. Future work should both deepen the knowledge available concerning the parameters that influence EPS production and also involve investigations of the physiological effects of these fermented, viscous and ropy, oat-based products.

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