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Texture promoting capacity and EPS formation by lactic acid bacteria in three different oat-based non-dairy media

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Abstract Four strains of lactic acid bacteria were used as pure or mixed cultures to investigate the texture-promoting capacity and exopolysaccharides (EPS) formation in three different oat-based, non-dairy milk products, Adavena G40, Adavena M40 (both with a dry matter content of 20%) and Mill Milk (with a dry matter content of 10%). Viscosity was measured at two different shear rates during 2 min of shear thinning. The highest viscosity was measured at a shear rate of 129 s^{-1} when a mixed culture consisting of *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 and *Lactobacillus brevis* DSM 1269 was grown at 30 °C in the medium containing mostly glucose as the carbohydrate source (Adavena G40). The mixed culture consisting *of Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 and *Streptococcus thermophilus* DSM 20259 gave the highest viscosity when using the medium with a dry matter content of 20% and maltose as the main carbohydrate source (Adavena M40). The EPS formation in the Adavena G40 medium was confirmed by isolating the soluble polymer fraction after fermentation. A higher yield of polymer dry mass was obtained from the samples with higher viscosity. The study shows that the co-operative growth that occurs when using mixed cultures also influences the EPS formation and final viscosity in the different oat-based media. This knowledge is of importance when strains are selected for the development of new kinds of fermented, oat-based, non-dairy products.

Keywords Adavena · Exopolysaccharides · *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 · Oats · Non-dairy

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Introduction

The consumer demand for non-dairy milk products with high acceptance and functionality has increased during recent years. Three commercial, non-dairy milk products, Adavena M40 (M40 medium), Adavena G40 (G40 medium) and Mill Milk (MM medium) are all products derived entirely from oats. These products are based on the same unique, patented, enzymatic process (USA patent No. 5686123) [1], which involves dry milling or wet milling of rolled oats or oat flour at 60 °C, followed by an enzymatic reaction. Maltose or glucose, together with β-limit dextrins, are thus the main carbohydrates in the final product formed from the oat starch. The final product can be modified further by the addition of nutrients, lipids and flavourings, and also processed further via a spray drying process to a fine powder [1]. Previous studies have shown that the beverage Mill Milk has high acceptability concerning taste and flavour and exhibits cholesterol and lipid-lowering effects [2, 3]. It has also been reported that this product is a suitable substrate for lactic acid bacteria (LAB) fermentation [4].

In general the choice of starter organisms in the production of fermented food products is important, in order to obtain the desired properties of the final product. Essential criteria in the selection of starter cultures include their ability to provide not only stability and texture, but also acidification, aroma and flavour to the final product [5]. Some cultures used in the dairy industry are generally described as promoters of "ropiness" or "mouth feel" because of their texture-enhancing properties. This function is due to the ability of these bacteria to secrete exopolysaccharides (EPS) synthesised mainly from mono- and disaccharides [6]. These polysaccharides are found outside the cell wall, either attached closely to the cell as a capsule or secreted into the environment as slime [7]. This EPS production is well studied in LAB in terms of structure and quantity [8]. However, very little has been investigated regarding the production of EPS and the texture-promoting capacity in mixed strain cultures. Some studies have observed a stimulation of EPS pro-

duction during growth of an EPS producing strain together with a non-EPS-producing strain in milk. This suggests that the production seems to be stimulated between certain bacterial stains [9, 10].

The aim of this study was to investigate whether any difference in terms of EPS yield, seen as increased viscosity, could be obtained by using pure or mixed cultures of LAB in three different oat-based, non-dairy media. Determination of the soluble polymer fraction after fermentation in one of the media used was also conducted.

Material and methods

Bacterial strains and growth conditions. *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 was obtained from the National Culture Collection of Food Bacteria, Aberdeen, Scotland. *Lactobacillus brevis* DSM 1269, *L. delbrueckii* subsp. *bulgaricus* DSM 20081, and *Streptococcus thermophilus* DSM 20259 were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany. *S. thermophilus* DSM 20259 was cultured in corynebacterium broth (DSM-medium 53) composed of 10 g l^{-1} casein peptone, tryptic digest (Difco, Detroit, Michigan, USA), 5 g l^{-1} yeast extract (Difco), 5 g l^{-1} sodium chloride, and 5 g l^{-1}glucose (Merck, Darmstadt, Germany). All other strains were cultured in MRS broth [11] (Merck). Stock cultures containing 20% (v/v) glycerol were kept at –80 °C. Before use the cultures were propagated twice in cultivation broth supplemented with cysteine-HCl (0.05%, v/v) (Fluka, Buchs, Switzerland) at 30 or 37 °C. The transfer inoculum was a 1% (v/v) culture grown for 24 h in a fresh medium under anaerobic conditions in anaerobic jars (85% N_2, 10% H_2, 5% CO_2). Solid medium was prepared by the addition of 15 g l^{-1} of granulated agar (Oxoid) to the broths.

The three oat-based products, Mill Milk (MM medium) (aseptically packed) and the two powders, Adavena M40 (M40 medium) and Adavena G40 (G40 medium) were provided by Ceba (Lund, Sweden), and analyzed for protein, fat, various carbohydrates, dietary fibres, various vitamins and minerals by an authorised laboratory (AnalyCen Nordic, Kristianstad, Sweden). The results are shown in Table 1.

Preparation of the media. The two Adavena powders (G40 and M40 media) were reconstituted in water to a final concentration of 20% (w/v) during continuous stirring at 350 rpm, followed by heat treatment at 90 °C for 5 min in a water bath, and then cooled to incubation temperature before inoculation. The MM medium (10% dry matter) was transferred to sterile E-flasks and cooled to incubation temperature 30 min before inoculation.

Fermentation with pure and mixed cultures. The fermentations were carried out in sterile and sealed bottles containing 200 ml of G40 medium inoculated with a 24 h-fresh culture at an inoculum concentration of 5% (v/v) and incubated at four different temperatures, namely, 25, 30, 37 or 43 °C for 24 h. Three different combinations of pure cultures were investigated in the G40 medium. The *L. delbrueckii* subsp. *bulgaricus* 2772 strain was mixed with *L. delbrueckii* subsp. *bulgaricus* 20081, *S. thermophilus* 20259 and *L. brevis* 1269 at 25, 30, 37 and 43 °C at a ratio of 1:1. Samples of the three media, G40, M40 and MM medium were inoculated with a pure culture of *L. delbrueckii* subsp. *bulgaricus* 2772 and with the three different combinations of mixed cultures, and incubated at 30 °C for 24 h to compare the viscosity in the three media after incubation.

Viscosity measurements. After incubation, samples were collected and cooled down to 25 or 9 °C for viscosity measurements using a Bohlin Visco 88 (Bohlin Reologi, Lund, Sweden) with the spindle C30 mm and shear rates of 395 s^{-1} or 129 s^{-1} for 120 s. The viscosity was expressed as milliPascals.

Table 1 Chemical composition (g/100 g) of three different oat-based media, M40, G40 and MM medium. *ND* Not determined

Component	M40	G40	MM
Protein g	1.1	1.1	1.1
Fat g	0.8	0.8	1.5
Glucose	ND	4.4	ND
Maltose g	4.2	0.3	4.2
Maltodextrin g	2.7	2.7	2.7
Total fibre g	0.8	0.8	0.8
β-glucan g	0.4	0.4	0.4
α-tocopherol mg	0.1	0.1	0.1
Thiamin mg	0.04	0.04	0.04
Riboflavin µg	9.6	9.6	9.6
Niacin mg	0.1	0.1	0.1
Folic acid µg	3.3	3.3	3.3
Pyridoxine mg	0.01	0.01	0.01
Iron mg	0.1	0.1	0.1
Magnesium mg	4.7	4.7	4.7
Manganese mg	0.1	0.1	0.1
Phosphorus mg	27	27	27
Sodium mg	11	11	11
Zinc mg	0.1	0.1	0.1

Quantitative determinations of the soluble polymer fraction. After 24 h of incubation, 25 g of fermented sample was weighed into a centrifuge tube (50 ml). The pH of the samples was adjusted to 6.2. Proteins were hydrolysed by a combination of Alcalase and Esperase (Novo Nordisk, Bagsværd, Denmark) at a ratio of 1:1 with a concentration of 1% (v/v) and incubated for 16 h at 30 °C. Before incubation, sodium azide (Sigma, St Louis, USA) was added (0.01%, w/v). After incubation the samples were centrifuged using a Heraeus Sepatech centrifuge for 30 min at 2,325×*g*. The supernatant was collected and washed through a filter (200 µm) (Spectra Mesh, Houston, USA). The filtrate was collected and precipitated with 4 volumes of cold 95% ethanol, followed by storage overnight at 4 °C. The precipitate was recovered by centrifugation at 5,230×*g* for 20 min at 4 °C, dissolved in distilled water and dialysed against distilled water for 3 days at 4 °C and then lyophilised. The amount of soluble polymer was expressed as milligrams of polymer dry mass (PDM) per litre.

Statistical analysis. All values are the mean of three replicates. Values were expressed as means and standard deviation. Mean values of treatments were compared by Student's *t* test. Differences were considered significant at $P<0.05$.

Results and discussion

Viscosity in three different oat-based media after incubation with one pure culture and three mixed cultures

Viscosity was measured after incubation with one pure and three mixed cultures in three different oat-based media, G40, M40 and MM. The viscosity was measured after 24 h of incubation at 30 °C (Table 2). It was found that there was a higher increase in viscosity after incubation in the G40 medium than in other media. The highest final viscosity was measured in the sample incubated with the mixed culture consisting of *L. delbrueckii* subsp. *bulgaricus* 2772+*L. brevis* 1269.

There was no increase in viscosity when pure cultures of the non-EPS producing strains were used. Two of

Table 2 Viscosity was measured (mPas) using a shear rate of 129 s^{-1} after 24 h of incubation at 30 °C with pure or mixed cultures (*L. delbrueckii* subsp. *bulgaricus* 2772, *L. delbrueckii* subsp. *bulgaricus* 20081, *L. brevis* 1269 and *S. thermophilus* 20259) in three different oat-based media, M40, G40 and MM. Values are mean±standard deviation

Pure and mixed cultures	M40		G40		MM	
	Time s					
	0	120	0	120	0	120
	21±2[ae]	21±2[ae]	21±2[ae]	21±2[ae]	<20±0[af]	<20±0[af]
L. bulgaricus 2772	243±45[b]	176±21[b]	1047±139[b]	523±49[b]	187±5[b]	120±2[b]
L. bulgaricus 2772+*L. bulgaricus* 20081	420±25[c]	242±11[c]	953±12[b]	494±51[b]	262±25[c]	152±15[c]
L. bulgaricus 2772+*L. brevis* 1269	552±36[d]	275±9[d]	1148±31[b]	585±28[b]	281±20[c]	165±13[c]
L. bulgaricus 2772+*S. thermophilus* 20259	603±67[d]	296±25[d]	847±64[b]	526±27[b]	163±15[d]	111±10[d]

[a, b, c, d] Means marked with the same letter did not differ at $P<0.05$; [e] Unfermented M40 or G40 media; [f] Unfermented MM medium

Table 3 Viscosity in the G40 medium after incubation with four different bacterial strains (*L. delbrueckii* subsp. *bulgaricus* 2772, *L. delbrueckii* subsp. *bulgaricus* 20081, *L. brevis* 1269 and *S. thermophilus* 20259) at four different temperatures: 25, 30, 37 and 43 °C. The viscosity in the unfermented G40 medium (control) was 22 mPas. The viscosity values were measured after 120 s of shear thinning using a shear rate of 395 s^{-1}. Values are mean±standard deviation

Bacterial strains	Incubation temperatures °C			
	25	30	37	43
L. delbrueckii subsp. *bulgaricus* 2772	174±4.9[a]	153±7.1[a]	135±0.7[a]	96±12.7[a]
L. delbrueckii subsp. *bulgaricus* 20081	23±1.4[b]	22±1.4[b]	21±0.0[b]	23±2.1[b]
L. brevis 1269	21±0.0[b]	21±0.0[b]	21±0.0[b]	21±0.0[b]
S. thermophilus 20259	30±1.4[c]	21±0.0[b]	21±0.0[b]	22±1.4[b]

[a, b, c] Means marked with the same letter did not differ at $P<0.05$

these non-EPS producing strains, *L. delbrueckii* subsp. *bulgaricus* 20081 and *S. thermophilus* 20259 have been observed in a previous study to increase the viscosity after fermentation in a milk-based, semi-defined medium [4]. The contribution of the ropy bacterial strains to textural properties, such as increased viscosity in different milk-based media, has been suggested to be the ability of the bacterial EPS to form strands that may be connected to the casein micelle [12, 13]. As our different oat-based media do not contain this micelle structure and have an overall lower content of proteins, these interactions can be excluded and the change in viscosity after fermentation can be attributed to the formation of EPS only.

Viscosity in the G40 medium fermented with pure and mixed cultures at different temperatures

An increase in viscosity after 24 h of incubation using the G40 medium was observed only for the *L. delbrueckii* subsp. *bulgaricus* 2772 strain at all the temperatures tested (Table 3). It was seen that the highest increase in viscosity after fermentation was in samples incubated at 30 °C. The smallest change in viscosity was observed in samples incubated at 43 °C.

The increase in viscosity after incubation with a pure culture, *L. delbrueckii* subsp. *bulgaricus* 2772, and three different mixed cultures, all containing the 2772 strain, were measured after incubation at four different temperatures (Fig. 1). Two of the mixed cultures tested, *L. delbrueckii* subsp. *bulgaricus* 2772+*L. brevis* 1269 and *L. delbrueckii* subsp. *bulgaricus* 2772+*L. delbrueckii* subsp. *bulgaricus* 20081, gave a higher viscosity both after incubation and after 120 s of shear thinning than the sample fermented with *L. delbrueckii* subsp. *bulgaricus* 2772 only. At the incubation temperature of 25 °C, the *L. delbrueckii* subsp. *bulgaricus* 2772 strain gave higher viscosity after incubation than the mixed cultures tested. At higher incubation temperatures the biggest change in viscosity was found in the mixed cultures.

When growing the EPS producing strain *L. delbrueckii* subsp. *bulgaricus* 2772, an increase in viscosity was observed in all media when compared to the unfermented samples. This indicates not only the suitability of the oat-based, non-dairy medium as a non-dairy growth medium for LAB, but also that it is a good medium when LAB is selected for its function of producing EPS. The highest increase in viscosity was observed at an incubation temperature of 30 °C when using the G40 medium at different incubation temperatures. This is in line with findings in [14] where the highest EPS productions were found at a non-optimal growth temperature. The highest increase in viscosity was obtained in the G40 medium at a shear rate of 129 s^{-1}. The highest viscosity was measured when the ropy strain *L. delbrueckii* subsp. *bulgaricus* 2772 was included in two mixed cultures consisting of the non-ropy strains, *L. brevis* 1269 and *L. delbrueckii* subsp. *bulgaricus* 20081 and incubated at 30 °C. When

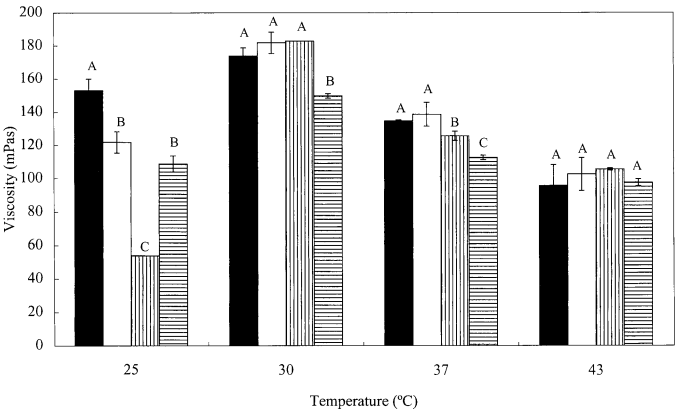

Fig. 1 Viscosity in the G40 medium after incubation at four different temperatures (25, 30, 37 and 43 °C) using an exopolysaccharides (EPS)-producing strain (*L. delbrueckii* subsp. *bulgaricus* 2772) and three different mixed cultures consisting of *L. delbrueckii* subsp. *bulgaricus* 2772 and a non-EPS producing strain. The viscosity values were measured after 120 s of shear thinning using a shear rate of 395 s^{-1}. Black bars *Lactobacillus delbrueckii* subsp. *bulgaricus* 2772, white bars *L. delbrueckii* susp. *bulgaricus* 2772+*L. delbrueckii* subsp. *bulgaricus* 20081, vertically striped bars *L. delbrueckii* susp. *bulgaricus* 2772+*L. brevis* 1269, horizontally striped bars *L. delbrueckii* subsp. *bulgaricus* 2772+*S. thermophilus* 20259. The values are the means±standard deviation. Bars marked with the same letter did not differ at $P<0.05$

the cultures were incubated at a lower temperature (25 °C), the highest viscosity was obtained in the samples fermented with a pure culture of *L. delbrueckii* subsp. *bulgaricus* 2772. This suggests that both interactions and co-operative growth between bacterial strains in a mixed culture may occur. This has been reported for other mixed cultures in different milk substrates [15, 16, 17, 18, 19].

In our study this co-operative growth was also observed to be strain-dependent. When using a lower shear rate (129 s^{-1}), the decrease in viscosity after 2 min of shear thinning was lower in the G40 and the MM media after fermentation with the mixed culture consisting of *L. delbrueckii* subsp. *bulgaricus* 2772 and *S. thermophilus* 20259 in comparison to the other viscosity-promoting, mixed cultures. This shows the importance of having both a sufficient concentration and type of carbon source in the medium together with a suitable mixed culture to obtain an increase in viscosity after fermentation with these LAB strains.

When comparing pure and mixed cultures in the different media, it was observed that when using a medium containing mainly maltose as the carbohydrate source (M40 or MM) the mixed cultures gave a higher final viscosity than when a pure culture was used. This suggests that when there is a greater limitation of fermentable carbohydrates, the co-operative growth between bacterial strains is more crucial.

Isolation of the soluble fibre fraction in the G40 medium

The EPS production in the G40 medium was examined by isolating the soluble fibre fraction after fermentation with three pure cultures and three mixed cultures (Table 4). The mixed cultures *L. delbrueckii* subsp. *bulgaricus* 2772+*L. delbrueckii* subsp. *bulgaricus* 20081 and *L. delbrueckii* subsp. *bulgaricus* 2772+*L. brevis* 1269 gave the highest amounts (4.8 g and 4.6 g PDM l^{-1}, respectively) after incubation. These mixed cultures also gave the highest final viscosity (>180 mPas) after fermentation when the viscosity was measured with a higher shear rate (395 s^{-1}).

The highest yield of the PDM was obtained when the mixed cultures of *L. delbrueckii* subsp. *bulgaricus* 2772+*L. delbrueckii* subsp. *bulgaricus* 20081 and *L. delbrueckii* subsp. *bulgaricus* 20081+*L. brevis* 1269 were used. These cultures also gave the highest viscosity after fermentation at 30 °C when using a higher shear rate (395 s^{-1}). However, our analytical results for samples fermented with the non-ropy bacterial strains, *L. delbrueckii* subsp. *bulgaricus* 20081, *L. brevis* 1269 and *S. thermophilus* 20259, gave a lower value for the soluble fibre fraction than for the unfermented, control sample, although there was no significant ($P<0.05$) difference in viscosity between these samples. This shows the complexity of the fermentation medium and indicates that there might be interactions between the isolation procedure and the medium, which may be explained by chemical changes in the medium caused by the fermentation process.

In this study we have compared three different oat-based media, which differ in carbohydrate composition and dry matter. All media were found to be suitable for lactic acid fermentation. The biggest change in viscosity after incubation was observed in the G40 medium. This was also confirmed when the soluble fibre fraction was isolated after fermentation. This study shows new possibilities of using different oat-based media, Adavena G40, Adavena M40 and Mill Milk, as substrates for LAB fermentation and the effect on the final viscosity when using pure and mixed cultures of ropy and non-

Table 4 Soluble fibre fraction expressed as polymer dry mass (PDM) per litre in the fermented G40 medium after incubation for 24 h at 30 °C using pure and mixed cultures. Values are mean±standard deviation

[a, b, c, d] Means marked with the same letter did not differ at $P<0.05$

Culture	Soluble fibre fraction (g PDM l^{-1})
L. delbrueckii subsp. *bulgaricus* 2772	4.4±0.20[ad]
L. delbrueckii subsp. *bulgaricus* 20081	3.5±0.10[b]
L. brevis 1269	2.5±0.20[c]
S. thermophilus 20259	3.3±0.20[b]
L. delbrueckii subsp. *bulgaricus* 2772+*L. delbrueckii* susp. *bulgaricus* 20081	4.8±0.30[a]
L. delbrueckii subsp. *bulgaricus* 2772+ *L. brevis* 1269	4.6±0.30[a]
L. delbrueckii subsp. *bulgaricus* 2772+ *S. thermophilus* 20259	4.3±0.20[ad]
Unfermented G40 medium	4.0±0.20[d]

ropy strains. This knowledge is important in the selection of strains for different oat-based, non-dairy applications. Further work should focus on using different kinds of EPS-producing strains in these oat-based milk substitutes and on optimising and characterising the EPS formation in order to better evaluate its potential in different kinds of non-dairy applications.

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