

# Malt sprout extract medium for cultivation of *Lactobacillus plantarum* protective cultures

A. Laitila, M. Saarela, L. Kirk, M. Siika-aho, A. Haikara, T. Mattila-Sandholm and I. Virkajärvi

VTT Biotechnology, Espoo, Finland

2003/0950: received 21 October 2003, revised 18 February 2004 and accepted 2 June 2004

## ABSTRACT

A. LAITILA, M. SAARELA, L. KIRK, M. SIIKA-AHO, A. HAIKARA, T. MATTILA-SANDHOLM AND I. VIRKAJÄRVI. 2004.

**Aims:** The aim was to develop a cheap cereal-based alternative medium for the large-scale production of biopreservative *Lactobacillus plantarum* VTT E-79098. We examined the effect of growth medium and pH control on the cell yield of *Lact. plantarum* E-79098 and the antimicrobial activity of the cell-free extracts.

**Methods:** Fermentations using a novel Malt Sprout Extract Medium (MSE) were performed with different pH regimes. The antimicrobial activity of the cell-free extracts against *Pantoea agglomerans* VTT E-90396 and *Fusarium avenaceum* VTT D-80147 was assessed with automated turbidometry.

**Significance and Impact of the Study:** When compared with MRS, the MSE medium cultures produced equal growth yields of *Lact. plantarum* VTT E-79098 and enhanced antimicrobial potential against the Gram-negative bacterium *P. agglomerans* and a *Fusarium* fungus. The MSE medium can be used as a low-cost alternative to MRS for producing high cell yields and good antimicrobial activity of *Lact. plantarum*.

**Keywords:** antimicrobial, biocontrol, cultivation, growth medium, malt sprouts.

## INTRODUCTION

In recent years there has been growing interest in the use of lactic acid bacteria (LAB) and/or their products as natural biocontrol agents. Their success is due to the ability of LAB to acidify the environment, to out-compete other microbes for nutrients, and to produce a spectrum of antimicrobial compounds such as organic acids, hydrogen peroxide, bacteriocins and low molecular weight antimicrobial compounds (Holzapfel *et al.* 1995; Salminen and von Wright 1998). Natural biocontrol agents are attractive as they have a better public image than many currently available chemicals, and potentially they could be used in processes in which chemicals cannot be applied.

*Lactobacillus plantarum*, the organism of interest in this study, has been applied to improve the microbiological stability, quality, and safety of silage (Weinberg and Muck 1996), brewery spent grains (Suomalainen *et al.* 1995) and

barley used in malting (Haikara and Mattila-Sandholm 1994; Linko *et al.* 1998; Laitila *et al.* 2002). *Lact. plantarum* produces antimicrobial compounds which are effective against a range of microbes including other lactobacilli, lactococci, food-borne pathogens such as *Listeria monocytogenes*, *Bacillus cereus* and *Clostridium perfringens* (Enan *et al.* 1996; Suma *et al.* 1998), and fungal contaminants such as *Fusarium* fungi (Niku-Paavola *et al.* 1999; Lavermicocca *et al.* 2000; Laitila *et al.* 2002). The antimicrobial activity of *Lact. plantarum* is a complex phenomenon and probably the result of the synergistic action of several inter-related factors (Gourama and Bullerman 1995; Suma *et al.* 1998; Niku-Paavola *et al.* 1999; Lavermicocca *et al.* 2000; Laitila *et al.* 2002).

The production of antimicrobial compounds is greatly dependent on the composition of the growth medium. In *in vitro* studies, *Lact. plantarum* as well as other LAB are commonly cultured in MRS broth (de Man *et al.* 1960), but for large-scale commercial applications its cost is prohibitive. In addition MRS broth contains constituents not approved in food production such as components of bovine origin.

Correspondence to: Arja Laitila, VTT Biotechnology, PO Box 1500, FIN-02044 VTT, Finland (Tel.: +358 9 456 78606; e-mail: arja.laitila@vtt.fi).

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.

Our main aim was to develop an alternative media for the large-scale production of *Lact. plantarum*. For replacement of some inappropriate components and expensive peptone sources, we used a cereal-based by-product from the food industry: malt sprouts. We examined the effect of growth medium and different pH control regimes on the growth of *Lact. plantarum* E-79098, and the antimicrobial potential of cell-free extracts against a Gram-negative bacterium and a *Fusarium* fungus.

## MATERIALS AND METHODS

### Micro-organisms

The following micro-organisms were obtained from the VTT Culture Collection: *Lactobacillus plantarum* VTT E-79098 (E98 = ATCC 14917), *Pantoea agglomerans* VTT E-90396 (E396) and *Fusarium avenaceum* (teleomorph *Gibberella avenacea*) VTT D-80147 (D147).

### Preparation of the Malt Sprout Extract Medium (MSE) and fermentation experiments

Malt sprouts, a by-product from the malting industry, were used to prepare the cereal-based culture medium. Dried malt sprouts obtained from Viking Malt (Lahti, Finland) were coarsely sieved in order to remove the bulky grain husks from the nitrogen-rich root sprouts. The sprouts were soaked for 2 h in distilled water (1 l of water per 100 g of dry material) and then autoclaved at 110°C for 30 min. After autoclaving, the MSE was kept at room temperature overnight and the liquid extract was separated from the coarse solids by centrifugation and filtration through a Whatman No. 3 filter paper with a Büchner funnel. The extract was used instead of water to make the media. The MSE medium was tested in flask cultivation with different levels of yeast extract. MSE was supplemented with 20 g l<sup>-1</sup> glucose and yeast extract at 0 g l<sup>-1</sup> (MSE\_0), 4 g l<sup>-1</sup> (MSE\_4) or 8 g l<sup>-1</sup> (MSE\_8). In addition, we examined the effects of inclusion of extra glucose, Mg<sup>2+</sup>, Mn<sup>2+</sup>, the vitamins niacin and pantothenic acid, and improved buffering capacity (phosphates). The MSE\_extra medium contained the following components dissolved in the MSE prepared as above: 40 g l<sup>-1</sup> glucose, 1 g l<sup>-1</sup> Tween-80, 5 g l<sup>-1</sup> each of KH<sub>2</sub>PO<sub>4</sub> and K<sub>2</sub>HPO<sub>4</sub>, 5 g l<sup>-1</sup> CH<sub>3</sub>COONa·3H<sub>2</sub>O, 2 g l<sup>-1</sup> each of diammonium citrate and MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 g l<sup>-1</sup> MnSO<sub>4</sub>·1H<sub>2</sub>O, and 1 mg l<sup>-1</sup> each of niacin and pantothenic acid. All ingredients (excluding niacin and

pantothenic acid) were sterilized at 121°C for 20 min. The vitamins were filter sterilized with a 0.45 µm pore-size filter (Millex-HA, Millipore, Bedford, MA, USA) and added to the medium after sterilization. MRS broth (Oxoid Ltd, Basingstoke, UK) was used as a reference medium.

The MSE\_extra medium was used in bioreactor cultivations. All ingredients (excluding niacin and pantothenic acid) were sterilized *in situ* in fermenters at 121°C for 30 min. Two drops of Struktol antifoam (Schill & Seilacer, Hamburg, Germany) were added to minimize foaming during sterilization. The vitamins were filter sterilized with a 0.45 µm pore-size filter (Millex-HA, Millipore) into each fermenter immediately prior to pH adjustment and inoculation.

*Lactobacillus plantarum* E98 was grown at 30°C in MRS. Overnight culture was diluted 10-fold in fresh medium and incubated for 16 h at 30°C. Cells were harvested by centrifugation and washed once with sterile saline solution. Cell suspension (1 ml) was inoculated into 100 ml of MRS or MSE. The inoculum size for bioreactor cultivations was 1% (v/v). Bioreactor fermentations were performed in Chemap CMF fermenters (Chemap AG, Volketswill, Switzerland) with a working volume of 1 l. The medium was agitated at 50 rev min<sup>-1</sup> and maintained at 30°C. Prior to inoculation, the pH was adjusted to 7.0 with 5 M NaOH. After inoculation, the pH of fermentation #1 was allowed to decline without adjustment, whereas the pH of fermentations #2 and #3 was controlled (using 5 M NaOH) at pH 4.5 and 5.0, respectively. Samples for viability and antimicrobial activity assessments were taken after 12, 24 and 48 h cultivation.

The viable cell number of *Lact. plantarum* E98 was determined by plate counting on MRS agar in duplicate or triplicate plates. Samples were incubated in anaerobic conditions at 30°C for 24–30 h.

The total lactate concentrations in cell-free extracts were determined with an L-lactic acid/D-lactic acid spectrophotometric assay kit (Cat. # 1112821, Boehringer Mannheim, Mannheim, Germany). The amount of undissociated lactate was calculated using the Henderson-Hasselbach equation, the measured concentrations of total lactate, and the corresponding pH values (lactate pK<sub>a</sub> = 3.86 at 30°C; Perrin and Dempsey 1974).

Glucose was measured with the 'GLU' MPR 3 (God-Period method) spectrophotometric assay kit (Cat. # 184047 Boehringer Mannheim).

### Detection of antimicrobial activity with automated turbidometry (Bioscreen assay)

The method used for the assessment of antimicrobial activity of *Lact. plantarum* E98 culture filtrates was similar to that described by Niku-Paavola *et al.* (1999). To prepare

the cell-free extracts *Lact. plantarum* E98 cultures were centrifuged at 3000 *g* for 10 min and then filtered through a 0.45 µm pore-size filter (Millex-HA, Millipore). The indicator bacterium, *P. agglomerans* E396, was grown in Nutrient Broth, NB (Difco, Detroit, MI, USA) with shaking at 30°C for 24 h. Prior to the Bioscreen assay, the culture was diluted 100-fold in NB. The indicator fungus *F. avenaceum* D147 was induced to sporulate in 1% (w/v) CMC broth (10 g l<sup>-1</sup> carboxymethylcellulose, 1 g l<sup>-1</sup> NH<sub>4</sub>NO<sub>3</sub>, 1 g l<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 0.5 g l<sup>-1</sup> MgSO<sub>4</sub>·7H<sub>2</sub>O, 1 g l<sup>-1</sup> yeast extract), with shaking at 25°C for 7 days. The fungal culture was diluted 10-fold with 0.05% (v/v) Tween-80 and then mixed carefully with sterile glass beads (10–15 per test tube) to break the mycelium. The mycelial debris was removed by filtration through sterile glass wool. The number of conidia was counted microscopically using a counting chamber (Thoma, Knittel Gläser, Germany). Prior to the Bioscreen assay the spore suspension was diluted and adjusted to 1000 spores per microtitre plate well.

The antimicrobial assays were conducted using a Bioscreen<sup>®</sup> automated turbidometer (Thermolabsystems, Vantaa, Finland). For the *P. agglomerans* E396 assay, 10 µl of *Lact. plantarum* E98 cell-free extract and 30 µl of the diluted *P. agglomerans* E396 culture were mixed with 260 µl of NB in each microtitre plate well. For the *F. avenaceum* D147 assay, 30 µl of cell-free extract and 30 µl of the diluted spore suspension were mixed with 240 µl of potato dextrose broth (Difco). Control samples without indicator organisms were included to form the baseline absorbance profile. Maximum growth of both indicator organisms was ascertained in samples without inhibitory compounds. The test samples were performed in triplicate, and five replicates were used for each control. *P. agglomerans* E396 was incubated in the Bioscreen<sup>®</sup> with shaking at 30°C for 24 h, and *F. avenaceum* D147 with shaking at 25°C for 72 h. The optical measurements were made at 620 nm, and readings were taken every 20 min. The area under the curve

calculated by the Bioscreen<sup>®</sup> software was used as a measure of microbial growth. Area-reduction percentage values were used to describe the inhibitory effects of the cell-free extracts.

## RESULTS

Our results indicate that MRS medium could be replaced by the MSE medium without affecting the cell count or the antimicrobial potential of *Lact. plantarum* E98 (Table 1). The MSE medium required addition of yeast extract in order to support the cell growth and antimicrobial activity. All media formulations containing yeast extract performed similarly or even better than the MRS control. Increasing the concentration of yeast extract from 4 to 8 g l<sup>-1</sup> did not improve the cell growth, but the antimicrobial activity was more pronounced with extra nutrients (Table 1). Antimicrobial activity of protective cultures is usually strongly dependent on the cultivation medium. It is clear that the addition of certain nutrients (e.g. glucose, buffering agents, vitamins) can improve the performance of complex by-product derived media.

MSE\_extra medium, with a maximum amount of nutrients, was selected for the fermenter cultivations. Table 2 shows the results for cell count, antimicrobial activity and lactate concentration in MSE\_extra bioreactor cultivations. Regardless of the medium or pH control regime used, all cultures reached the stationary phase in 12 h, when almost all the glucose was depleted from the growth medium. Lactate production continued for a number of hours thereafter (data not shown). In both the *P. agglomerans* and *F. avenaceum* growth inhibition assays, the cell-free extract of pH-controlled cultures displayed reduced antimicrobial activities compared with the control cultures without pH control (Table 2). The activity of the extract from the non-pH-controlled culture compared well with that of MSE\_extra in bottle cultivations.

**Table 1** Viable cell count (log CFU ml<sup>-1</sup>) and antimicrobial activity of *Lactobacillus plantarum* E98 cultivated in Malt Sprout Extract (MSE) medium in flask cultures. Values are averages with ±S.D.

		Viable cell count (log CFU ml <sup>-1</sup> )			Growth inhibition (%)				
		<i>L. plantarum</i> E98			<i>P. agglomerans</i> E396		<i>F. avenaceum</i> D147		
Medium*	<i>n</i>	0	24 h	48 h	24 h	48 h	24 h	48 h	pH 48 h
MRS	6	7.5 ± 0.1	9.6 ± 0.1	9.5 ± 0.1	81 ± 4	91 ± 5	24 ± 8	37 ± 13	3.7
MSE_0	2	7.5 ± 0.1	9.1 ± 0.1	8.9 ± 0.1	40 ± 11	68 ± 1	17 ± 4	37 ± 9	3.8
MSE_4	2	7.5 ± 0.1	9.6 ± 0.1	9.5 ± 0.1	83 ± 2	95 ± 6	42 ± 4	56 ± 16	3.6
MSE_8	6	7.5 ± 0.1	9.6 ± 0.1	9.4 ± 0.1	86 ± 11	99 ± 1	41 ± 11	59 ± 11	3.6
MSE_extra	2	7.5 ± 0.1	9.7 ± 0.1	9.7 ± 0.1	81 ± 1	99 ± 1	39 ± 13	62 ± 4	3.8

\*MSE was supplemented with 20 g l<sup>-1</sup> glucose and yeast extract at 0 g l<sup>-1</sup> (MSE\_0), 4 g l<sup>-1</sup> (MSE\_4) or 8 g l<sup>-1</sup> (MSE\_8).

**Table 2** Maximum viable count of *Lactobacillus plantarum* E 98 cultures, growth inhibition (%) of *Pantoea agglomerans* E396 and *Fusarium avenaceum* D147 by cell-free extracts of the cultures as measured by an automated turbidometer (Bioscreen®) and total/undissociated lactate in the cultures. Culture filtrates of *Lact. plantarum* E 98 were collected after 12, 24 or 48 h fermentation in MSE\_extra

		Growth inhibition (%) at three fermentation times						Total/undissociated lactate* (g l <sup>-1</sup> ) 48 h
		<i>P. agglomerans</i> E396			<i>F. avenaceum</i> D 147			
		12 h	24 h	48 h	12 h	24 h	48 h	
No pH control	9.7	38	95	99	21	41	57	23/14
pH 4.5	9.8	23	62	84	22	26	39	38/7
pH 5.0	9.9	13	38	52	8	20	21	36/1

\*Measured sum of undissociated and dissociated lactate, calculated using the Henderson-Hasselbach equation.

## DISCUSSION

The antimicrobial potential of *Lact. plantarum* cultures has been applied in a large variety of plant-based bioprocesses such as sourdough-baked products, silage and malting (Fleming *et al.* 1985; Haikara and Mattila-Sandholm 1994; Holzapfel 1997; Lavermicocca *et al.* 2000; Laitila *et al.* 2002). It is well known that the growth medium plays an important role in production of antimicrobial metabolites. Various growth media such as MRS broth, M-17, Elliker's broth, skimmed milk and whey permeate have been widely used for LAB cultivation (Mäyrä-Mäkinen and Bigret 1998). In the present study the expensive MRS broth was replaced with an MSE medium. Malt sprouts are a by-product from the malting industry and are normally used as animal feed due to their rich vitamin and mineral composition. As in the MSE medium the expensive peptone sources were replaced with an extract derived from the protein-rich barley malt sprouts (Pomeranz and Robbins 1971; Hujanen and Linko 1996), the medium can be considered to be a low-cost alternative to MRS. The medium cost was estimated to be approximately 20% of that of MRS. Lactobacilli are known to be fastidious organisms. In order to support the maximum growth and antimicrobial activity, the MSE required supplementation with yeast extract, which provided extra proteins, B vitamins and minerals for *Lact. plantarum*.

When *Lact. plantarum* E98 was grown in flasks on MRS or MSE, the maximum viable count levels detected were similar ( $4 \times 10^9$  CFU ml<sup>-1</sup>). However, in both the *P. agglomerans* E396 and *F. avenaceum* D147 growth inhibition assays, the MSE flask cultures displayed better antimicrobial activities compared with the MRS cultures. The implementation of pH control (at 4.5 or 5.0) increased the viable cell yield. The maximum cell count of  $8 \times 10^9$  CFU ml<sup>-1</sup> was obtained with MSE\_extra medium in bioreactor fermentation when the pH was controlled at 5.0. The increases in cell density in pH-controlled cultivations were obtained at the cost of decreased antimicrobial activity: the lower the pH the higher the antimicrobial activity.

Whereas the cultivations with pH control produced more total lactate than that without pH-control, the concentrations of the undissociated form of lactate were considerably lower due to the elevated pH. Gätje and Gottschalk (1991) concluded that the undissociated form of lactate was probably the major factor responsible for growth inhibition of *Lact. helveticus* DSM 20075 at pH  $\leq 5$ . The non-pH-controlled MSE\_extra fermentation (final pH 3.9), which resulted in the lowest viable *Lact. plantarum* E98 cell yields, contained two- and 14-fold levels of undissociated lactate compared with pH 4.5- and 5.0-controlled MSE fermentations, respectively. Although lactate accumulation limits the growth of the producer organism, production of large quantities of lactate is often considered to be necessary for maximal antimicrobial action of LAB. Organic acids such as lactic, acetic and volatile fatty acids produced by LAB are powerful antimicrobial compounds, especially when acting in synergy with other metabolites (Ouwehand 1998). Furthermore, media components with strong antimicrobial properties, such as sodium acetate (one component of MRS), may contribute to the antimicrobial action (Stiles *et al.* 2002). Several studies have reported that low pH and lactic acid alone could not explain the inhibitory action of lactobacilli against fungi. Rather it is the combined effect of several antimicrobial factors acting in synergy (Corsetti *et al.* 1998; Niku-Paavola *et al.* 1999; Lavermicocca *et al.* 2000). As the nature of the antimicrobial substances was not studied in the present investigation it is unclear to what extent different compounds contributed to the antimicrobial properties of the *Lact. plantarum* E98 MSE culture filtrates.

An alternative growth medium to MRS, named MSE, can be applied in the production of *Lact. plantarum* and probably for other LAB cultures used as biocontrol agents in plant-based bioprocesses such as in malting and in silage applications. The optimization of sprout extract processing and the need for extra ingredients are subjects for further research. The results of the present study demonstrated that the MSE medium supported the growth of *Lact. plantarum* E98 at the same level as the commercial MRS. In addition, it

enhanced the antimicrobial activity against *P. agglomerans* and *F. avenaceum*.

## ACKNOWLEDGEMENTS

The authors are grateful to Viking Malt Oy for providing the malt sprouts. They thank Michael Bailey for critical reading of the manuscript. This study was partly supported by the Commission of the European Communities, specific RTD programme 'Quality of Life and Management of Living Resources', QLK1-2000-30042, 'Nutritional enhancement of probiotics and prebiotics: Technology aspects on microbial viability, stability, functionality and on prebiotic function'. It does not necessarily reflect its views and in no way anticipates the Commission's future policy in this area.

## REFERENCES

- Corsetti, A., Gobbetti, M., Rossi, J. and Damiani, P. (1998) Antimould activity of sourdough lactic acid bacteria: identification of a mixture of organic acids produced by *Lactobacillus sanfrancisco* CB1. *Applied Microbiology and Biotechnology* **50**, 253–256.
- Enan, G., El-Essawy, A.A., Uyttendaele, M. and Debevere, J. (1996) Antibacterial activity of *Lactobacillus plantarum* UG1 isolated from dry sausage: characterization, production and bactericidal action of plantaricin UG1. *International Journal of Food Microbiology* **30**, 189–215.
- Fleming, H.P., McFeeters, R.F. and Daeschel, M.A. (1985) The lactobacilli, pediococci, and leuconostocs: vegetable products. In *Bacterial Starter Cultures for Foods* ed. Gilliland, S.E. pp. 97–118. Boca Raton, FL: CRC Press.
- Gätje, G. and Gottschalk, G. (1991) Limitation of growth and lactic acid production in batch and continuous cultures of *Lactobacillus helveticus*. *Applied Microbiology and Biotechnology* **34**, 446–449.
- Gourama, H. and Bullerman, L.B. (1995) Antimycotic and antiaflatoxinigenic effect of lactic acid bacteria: a review. *Journal of Food Protection* **57**, 1275–1280.
- Haikara, A. and Mattila-Sandholm, T. (1994) *Procedure for Treatment of Seed Material to be Germinated*. International Patent Cooperation Treaty (PCT) WO 9416053. Panimolaboratorio Oy, PO Box 15, Espoo, Finland.
- Holzappel, W. (1997) Use of starter cultures in fermentation on a household scale. *Food Control* **8**, 241–258.
- Holzappel, W.H., Geisen, R. and Schillinger, U. (1995) Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *International Journal of Food Microbiology* **24**, 343–362.
- Hujanen, M. and Linko, Y.-Y. (1996) Effect of temperature and various nitrogen sources on L(+)-lactic acid production by *Lactobacillus casei*. *Applied Microbiology and Biotechnology* **45**, 307–313.
- Laitila, A., Alakomi, H.-L., Raaska, L., Mattila-Sandholm, T. and Haikara, A. (2002) Antifungal activities of two *Lactobacillus plantarum* strains against *Fusarium* moulds in vitro and in malting of barley. *Journal of Applied Microbiology* **93**, 566–576.
- Lavermicocca, P., Valerio, F., Evidente, A., Lazzaroni, S., Corsetti, A. and Gobbetti, M. (2000) Purification and characterization of novel antifungal compounds from sourdough *Lactobacillus plantarum* strain 21B. *Applied and Environmental Microbiology* **66**, 4084–4090.
- Linko, M., Haikara, A., Ritala, A. and Penttilä, M. (1998) Recent advances in the malting and brewing industry. *Journal of Biotechnology* **65**, 85–98.
- de Man, J.C., Rogosa, M. and Sharpe, M.E. (1960) A medium for the cultivation of lactobacilli. *Journal of Applied Bacteriology* **23**, 130–135.
- Mäyrä-Mäkinen, A. and Bigret, M. (1998) Industrial use and production of lactic acid bacteria. In *Lactic Acid Bacteria. Microbiology and Functional Aspects* ed. Salminen, S. and von Wright, A. pp. 73–102. New York: Marcel Dekker.
- Niku-Paavola, M.L., Laitila, A., Mattila-Sandholm, T. and Haikara, A. (1999) New types of antimicrobial compounds produced by *Lactobacillus plantarum*. *Journal of Applied Microbiology* **86**, 29–35.
- Ouweland, A.C. (1998) Antimicrobial components from lactic acid bacteria. In *Lactic Acid Bacteria. Microbiology and Functional Aspects* ed. Salminen, S. and von Wright, A. pp. 139–159. New York: Marcel Dekker.
- Perrin, D.D. and Dempsey, B. (1974) *Buffers for pH and Metal Ion Control*. p. 158. London: Chapman and Hall.
- Pomeranz, Y. and Robbins, G.S. (1971) Malt sprouts; their composition and use. *The Brewer's Digest* **46**, 58–64.
- Salminen, S. and von Wright, A. (1998) *Lactic Acid Bacteria. Microbiology and Functional Aspects*. 617 p. New York: Marcel Dekker.
- Stiles, J., Penkar, S., Plockova, M., Chumchalova, J. and Bullerman, L.B. (2002) Antifungal activity of sodium acetate and *Lactobacillus rhamnosus*. *Journal of Food Protection* **65**, 1188–1191.
- Suma, K., Misra, M.C. and Varadaraj, M.C. (1998) Plantaricin LP84, a broad spectrum heat-stable bacteriocin of *Lactobacillus plantarum* NCIM 2084 produced in a simple glucose broth medium. *International Journal of Food Microbiology* **40**, 17–25.
- Suomalainen, T., Storgårds, E., Mäyrä-Mäkinen, A. and Haikara, A. (1995) Lactic acid bacteria starter cultures in the preservation of spent grains used as animal feed. In *Proceedings of the 25th Congress of the European Brewer's Convention, Brussels*. pp. 733–739. Oxford: IRL Press.
- Weinberg, Z.G. and Muck, R.E. (1996) New trends and opportunities in the development and use of inoculants for silage. *FEMS Microbiology Reviews* **19**, 53–68.