Microbiological Research **159** (2004) 339—346

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Microbiological Research

www.elsevier.de/micres

Potential of selected lactic acid bacteria to produce food compatible antifungal metabolites

Cassandra De Muynck[a,*], Annelies I.J. Leroy[a], Sofie De Maeseneire[a], Filip Arnaut[b], Wim Soetaert[a], Erick J. Vandamme[a]

[a]*Laboratory of Industrial Microbiology and Biocatalysis, Department of Biochemical and Microbial Technology, Ghent University, Coupure links 653, B-9000 Gent, Belgium*
[b]*Puratos N.V., Industrialaan 25, B-1702 Groot-Bijgaarden, Belgium*

Accepted 28 July 2004

KEYWORDS
Lactic acid bacteria;
Antifungal metabolites;
Biopreservation;
Screening

Summary

The aim of this study was to assess the potential of lactic acid bacteria to inhibit the outgrowth of some common food-spoiling fungi. Culture supernatants of 17 lactic acid bacterial strains as well as of three commercial probiotic cultures were evaluated for antifungal activity using an agar-diffusion method. The method parameters were chosen in order to reveal compounds for potential use in food (bio)preservation. Thirteen strains showed antifungal activity of which five strains were very promising: *Lactobacillus acidophilus* LMG 9433, *L. amylovorus* DSM 20532, *L. brevis* LMG 6906, *L. coryniformis* subsp. *coryniformis* LMG 9196 and *L. plantarum* LMG 6907. Four of these five strains were further examined; it was found that the produced antifungal metabolites were pH-dependent. The exact chemical nature of these substances has not been revealed yet.

Introduction

Lactic acid bacteria are among the most powerful prokaryotes when it comes to antimicrobial potential. These bacteria not only produce several antimicrobials during carbon source metabolism, they also compete with other species by acidifying their environment and by rapidly depleting the nutrients. Besides these relatively simple antagonistic mechanisms, some lactic acid bacteria also produce potent antibiotic compounds via complex secondary metabolism pathways. Among these are bacteriocins (e.g. nisin), antibiotics (e.g. reutericyclin) and small antibiotic-like molecules such as reuterin. The current need for biopreservation has renewed the interest in the search for food compatible antimicrobials produced by microorganisms. Based on current literature, it can be concluded that

*Corresponding author.
E-mail address: Cassandra.DeMuynck@UGent.be (C. De Muynck).

0944-5013/$ - see front matter © 2004 Elsevier GmbH. All rights reserved.
doi:10.1016/j.micres.2004.07.002

antifungal compounds of lactic acid bacteria do exist and have potential for being effective in combating food borne yeasts and moulds. There have been several reports on antifungal properties of lactobacilli; e.g. *Lactobacillus acidophilus* (Batish et al., 1989, 1990b; Plocková et al., 1997a, b), *L. casei* (Suzuki et al., 1991; Gourama, 1997), *L. coryniformis* subsp. *coryniformis* (Magnusson and Schnürer, 2001), *L. pentosus* (Okkers et al., 1999), *L. plantarum* (Niku-Paavola et al., 1999; Lavermicocca et al., 2000; Laitila et al., 2002; Ström et al., 2002; Lavermicocca et al., 2003), *L. rhamnosus* (Suzuki et al., 1991; Stiles et al., 2002), *L. salivarius* (Stiles et al., 1999), *L. sanfrancisco* (Gobetti and Corsetti, 1997; Corsetti et al., 1998), *L. lactis* subsp. *lactis* (Roy et al., 1996; Roy et al., 2001) and *L. lactis* subsp. *lactis* var. *diacetylactis* (Batish et al., 1989, 1990a). The first investigations however have been conducted by El-Gendy and Marth (1980) on co-cultures of streptococci and lactobacilli.

Most of the antifungal capacity of the lactic acid bacteria described in these reports is due to the production of an antifungal protein or proteinaceous compound. Others, like *L. plantarum* and *L. sanfrancisco* produce special organic acids (3-phenyl-L-lactic acid and caproic acid, respectively) that have antifungal properties (Corsetti et al., 1998; Ström et al., 2002; Lavermicocca et al., 2003). The aim of the present research was to screen lactic acid bacteria for antifungal activity. This screening was focused on antifungal substances that can be used as a (bio)preservative in a complex and neutral (pH 5.0–6.0) food matrix, that usually undergoes a thermal treatment equivalent to pasteurization.

First, some pre-screening tests were performed to identify possible undesirable side reactions of the screening method such as inhibition by the culture medium or by the low pH of the supernatants. Afterwards, seventeen lactic acid bacteria and three commercial probiotic cultures were screened for antifungal activity according to the agar-diffusion method. To be able to compare antifungal and antibacterial properties of the lactic acid bacteria, a bacterial indicator strain was included in the screening plan. For this purpose, a *Bacillus subtilis* strain was chosen. The antimicrobial potential of each culture supernatant was investigated against 14 microorganisms (13 fungi and 1 bacterium). In the present study, only the extracellular antifungal activity present in the supernatant of the selected lactic acid bacteria was checked. Finally some of the best performing strains were further investigated in order to identify the nature of the antifungal activity.

Materials and methods

Fungal test strains and *B. subtilis*

The fungal strains used in this investigation were mainly provided by the Mycothèque de l'Universite catholique de Louvain (MUCL, Louvain-la-Neuve, Belgium). They include *Aspergillus flavus* MUCL 19945, *Endomyces fibuliger* MUCL 11443, *Eurotium repens* MUCL 15977, *Penicillium paneum* MUCL 40611, *P. roqueforti* MUCL 40617 and *Rhizopus oryzae* MUCL 20145. Other strains were own spoiled food isolates and include *Cerinosterus* sp., *Cladosporium* sp. and 5 morphologically different *Penicillium* species, named A, B, C, F and G. The *B. subtilis* strain, which was used for comparison of antifungal and antibacterial activity, was isolated at our laboratory from ropy bread samples. All strains were maintained on Potato Dextrose Agar (PDA, Oxoid, Hampshire, UK) plates at 25 °C. Once good growth of the cultures was established, they were stored at 4 °C until further use and subcultured on a monthly basis. For longer-term conservation of the strains, spore (or cell) suspensions were kept in cryovials at −80 °C, with 90% glycerol added as cryoprotectant.

Lactic acid bacteria cultures

All the lactic acid strains used were obtained from international culture collections. According to their specific needs of temperature and aeration level as shown in Table 1, they were cultured on deMan, Rogosa and Sharpe (MRS, Oxoid) agar (12 g l^{-1}) plates. Anaerobic strains were kept in an anaerobic jar (Anaerogen, Oxoid). Again fully grown colonies were stored on plates at 4 °C until further use and subcultured on a monthly basis. Cell suspensions were stored at −80 °C in the presence of 90% glycerol as cryoprotectant, for long-term conservation.

Probiotic cultures

Three commercial probiotic dairy products, Actimel® (Danone, France), Yakult® (Yakult, UK) and Beneflora® (Ortis, Belgium) were screened for strains with antifungal properties. The lactic acid bacteria present in these dairy products, according to their manufacturer, are *L. casei Immunitas, L. bulgaricus* and *Streptococcus thermophilus* for Actimel®, *L. casei Shirota* for Yakult® and *Bifidobacterium* sp., *Bifidobacterium longum, L. acidophilus, L. bulgaricus, L. casei* and *S. thermophilus* for Beneflora®. Beneflora® was suspended (2%) in

Table 1. Origin and incubation conditions of the selected lactic acid bacteria

Strain	Growth temperature (°C)	O_2 need
L. acidophilus LMG[a] 9433	37	Anaerobic
L. amylovorus DSM[b] 20532	37	Anaerobic
L. amylovorus LMG 9496	30	Anaerobic
L. brevis LMG 6906	30	Aerobic
L. casei subsp. *casei* LMG 6904	30	Aerobic
L. coryniformis subsp. *coryniformis* LMG 9196	30	Aerobic
L. delbrueckii subsp. *delbrueckii* ATCC[c] 9649	37	Aerobic
L. fermentum LMG 6902	30	Aerobic
L. hilgardii LMG 6895	30	Aerobic
L. plantarum LMG 6907	30	Aerobic
L. rhamnosus ATCC 7469	37	Aerobic
L. sanfranciscensis DSM 20451	30	Aerobic
L. lactis subsp. *lactis* LMG 7930	30	Aerobic
L. lactis subsp. *lactis* LMG 9441	30	Aerobic
L. mesenteroides NRRL[d] B-512F	30	Aerobic
L. mesenteroides subsp. *cremoris* LMG 6909	30	Aerobic
L. pseudomesenteroides ATCC 12291	25	Aerobic

[a]Belgian Coordinated Collections of Microorganisms, Belgium.
[b]Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany.
[c]American Type Culture Collection, USA.
[d]Agricultural Research Service Culture Collection, USA.

physiological solution and after 24 h inoculated in liquid MRS medium. The cultures were incubated both aerobically and anaerobically at 37 °C, since they contained both aerobic and anaerobic species. Actimel® and Yakult® were inoculated directly into liquid MRS medium and incubated aerobically at 37 °C. These (mixed) cultures were further treated in the same way as the lactic acid bacteria monocultures.

Agar-diffusion method

The agar-diffusion method consists in placing sterile filter disks (∅ 5 mm), perforated from Whatmann No. 1 filter paper, on a Petri dish inoculated with 100 µl of a fungal spore solution. A potential antifungal substance is then applied on these filter disks: three filter disks per plate were used for each antifungal substance. The volume spotted was 10 µl and the agar medium used was a modified Wheat Flour Hydrolysate (WFH) medium described by Gobetti et al. (1994). For each fungal strain, a blank without filter disks was made. The test plates were incubated aerobically at 25 °c and examined for inhibition zones around the filter disks during 10 days. The radius of the observed inhibition zones was measured as an average of three.

Pre-screening tests

As a preliminary experiment, 10 times concentrated and pasteurized MRS medium was evaluated for antifungal activity with the agar-diffusion test. Furthermore, this assay was repeated with MRS media at different pH values ranging from 1.0 to 6.0. The pH was adjusted with HCl after concentration and before pasteurization.

Production of potential antifungal substances

The lactic acid bacteria were cultured in liquid medium according to their specific needs of temperature and aeration. Aerobic strains were shaken in Erlenmeyer flasks, whereas anaerobic ones were grown as static culture. All strains, except the lactococci, were grown in MRS medium. *Lactococcus lactis* sp. were grown in Elliker's broth, according to Batish et al. (1989). For the isolation of the potentially produced antifungal substances, 10 ml of each culture was centrifuged (2600*g*, 20 min, 4 °C) in order to remove the cells. This was done after specific time intervals (48, 72, 96 and 120 h) to give an idea of the time dependency of the production. The resulting supernatant was lyophilized to dryness (Alpha 1–4 freeze dryer,

Martin Christ Gefriertrocknungsanlagen, Osterode, Germany) and resuspended in 1 ml distilled water, resulting in a tenfold concentration compared to the original supernatant. Subsequently the concentrates were pasteurized (70 °C, 20 min) and stored at 4 °C for further antifungal activity investigation based on the agar-diffusion method. Where indicated, pH adjustments were made with HCl or NaOH after concentration and before pasteurization.

Results

Pre-screening tests

Since most supernatants of the tested lactic acid bacteria contained MRS medium, a test was performed to determine antifungal properties originating from components of this medium. No inhibition zones were observed with concentrated and pasteurized MRS medium spotted on the filter disks, indicating that MRS medium did not contain any antifungal substances.

In another pre-screening test, the acid tolerance of the fungal test strains used was tested. The observed results of the agar-diffusion method with concentrated MRS at different pH-values, ranging from 1.0 to 6.0, are shown in Fig. 1. From these data, it can be concluded that none of the fungi are inhibited by MRS medium, as long as the pH-value is above 3.0. Below this pH value the growth of *R. oryzae* MUCL 20145 and *Penicillium* species B, F and G is slightly inhibited. As expected, the *B. subtilis* strain was more sensitive towards acid pH values. From these results it can be concluded that observed antifungal effects cannot be attributed to supernatant acidity, as long as the pH-value is above 3.0.

Screening of lactic acid bacteria monocultures

The antifungal spectrum of 17 lactic acid bacterial strains was evaluated. Antifungal activity of some of the examined lactic acid bacteria had previously been reported (El-Gendy and Marth, 1980; Batish et al., 1989; Suzuki et al., 1991). Initially, the concentrated and pasteurized supernatant of the cultures was tested with the filter disk method. A summary of the results of this first screening is given in Table 2. In this table, all data obtained are "condensed" into one inhibition value. This value is an average of the inhibition zone radii obtained for all 13 tested fungi. Thirteen out of the 18 tested lactic acid bacteria showed antifungal activity, which usually varied with the growth stadium of the culture. The remaining five lactic strains displayed no inhibition towards the fungi in the screening test. Almost all lactic acid bacteria supernatants tested showed antibacterial activity at a certain age, probably due to the acidifying of the supernatants (as indicated by the acid tolerance test in Fig. 1, *B. subtilis* can only grow at pH-values higher than 5.0). The antifungal activity of the 13 "active" lactic acid bacteria was most powerful for *L. acidophilus* LMG 9433, *L. amylovorus* DSM 20532, *L. brevis* LMG 6906, *L. coryniformis* subsp. *coryniformis* LMG 9196 and *L. plantarum* LMG 6907.

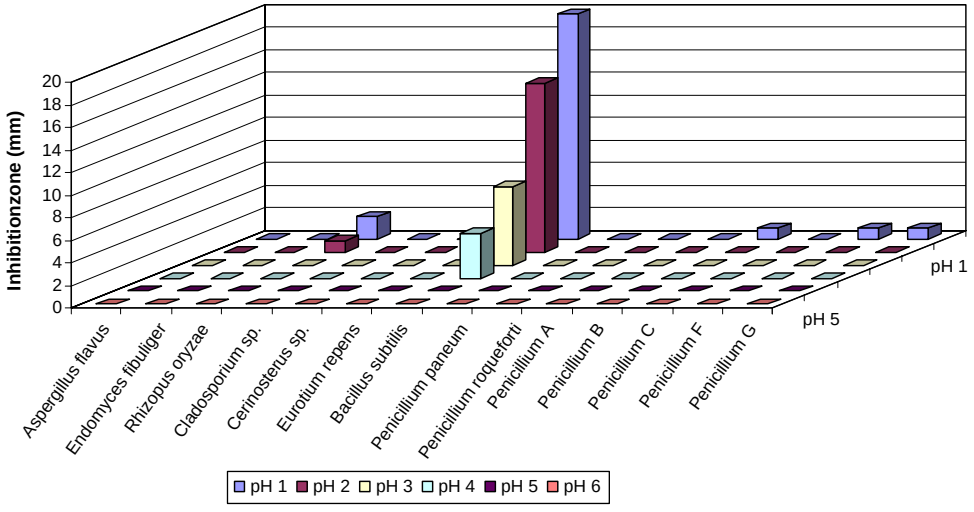

Figure 1. Effect of different medium acidities on the growth of selected fungi; the inhibition of growth is expressed as the radius of the inhibition zone around the filter disk.

Table 2. Antimicrobial activity of selected lactic acid strains: the antifungal activity is expressed as an average inhibition radius around filter disks (averaged over all fungi tested) and the antibacterial activity as the culture age (h) where antibacterial activity was first observed

Strain	Average antifungal activity (mm)				Antibacterial activity (h)
Sample time of the supernatants, hours after inoculation	48	72	96	120	
L. acidophilus LMG 9433	0.1	0.9	1.1	1.6[a]	72
L. amylovorus LMG 9496	0.4	0.3	0.3	0.2	48
L. amylovorus DSM 20532	0.7	1.0	0.7	0.5	48
L. brevis LMG 6906	0	1.1	1.6	1.3[a]	72
L. casei subsp. *casei* LMG 6904	0.3	0.5	0.3	0.2	48
L. coryniformis subsp. *coryniformis* LMG 9196	0	0.5	0.6	1.1	72
L. delbrueckii subsp. *delbrueckii* ATCC 9649	0.3	0.4	0.4	0.3	48
L. fermentum LMG 6902	0	0.5	0.4	0.7[a]	72
L. hilgardii LMG 6859	0	0.4	0.7	0.6[a]	72
L. plantarum LMG 6907	0.3	0.8	0.8	1.3[a]	72
L. rhamnosus ATCC 7469	0.3	0.3	0.4	0.2	48
L. sanfransiscensis DSM 20451	0	0	0	0	No inhibition
L. lactis subsp. *lactis* LMG 7930	0	0	0	0	48
L. lactis subsp. *lactis* LMG 9441	0	0	0	0	48
L. mesenteroides NRRL B-512F	0.1	0.3	0	0.2	48
L. mesenteroides subsp. *cremoris* LMG 6909	0.1	0.1	0	0	48
L. pseudomesenteroides ATCC 12291	0	0	0	0	48

[a]This value is actually the average antifungal activity 144 h after inoculation.

Table 3. Antimicrobial activity of selected probiotics: the antifungal activity is expressed as an average inhibition radius around filter disks (averaged over all fungi tested) and the antibacterial activity as the culture age (h) where antibacterial activity was first observed

Probioticum	Average antifungal activity (mm)					Antibacterial activity (h)
Sample time of the supernatants, hours after inoculation	48	72	96	120	144	
Actimel®	0.2	0.2	0.2	0.3	0.4	48
Yakult®	0.1	0.1	0.1	0.4	0.2	48
Beneflora®, aerobic	0.1	1.1	1.1	1.1	1.0	48
Beneflora®, anaerobic	0	0.2	0.2	0.2	0.2	48

Screening of commercial probiotic cultures

Since most probiotic cultures are mainly selected on the basis of their antimicrobial potential (Casas and Dobrogosz, 2000), a screening for antifungal activity of three commercial preparations was performed. A summary of the inhibition zone values obtained is given in Table 3. Upon testing the supernatant of the Actimel® mixed aerobic culture (*L. casei Immunitas*, *L. bulgaricus* and *S. thermophilus*), only *Cerinosterus* sp. and *E. fibuliger* MUCL 11443 seemed to experience slight inhibition (data not shown). Yakult®, containing only one strain, *L. casei Shirota*, displayed in the filter disk method a similar result: only few of the fungal strains were slightly inhibited. Beneflora®, a dehydrated mix of six lactic cultures, was tested both under aerobic and anaerobic conditions. The aerobic strains, *L. casei* and *L. bulgaricus*, showed a fairly high antifungal effect as is shown in Table 3, while the

anaerobic fraction (*L. acidophilus*, *Bifidobacterium* sp., *B. longum* and *S. thermophilus*) did not inhibit the fungi at all. On the other hand, they all could inhibit the *B. subtilis* strain to a great extent.

Antifungal potential of *L. acidophilus* LMG 9433

With *L. acidophilus* LMG 9433, inhibition zones were observed against several fungi. Especially the *Penicillium* strains were very sensitive to the supernatant of this strain (data not shown). These results are similar to those reported by Batish et al. (1989, 1990b) and by Plocková et al. (1997a, b). *L. acidophilus* LMG 9433 did not lower the acidity of the supernatant below pH 3.0 (Fig. 2). The antifungal activity could therefore not be attributed to a simple pH-effect, as inhibition of fungal growth was only observed below pH 3.0 in the prescreening test. To examine the pH-dependency of the antifungal effect, the supernatant was neutralized to pH 5.0, 5.5 and 6.0 and tested again with the agar-diffusion method. None of the fungi were inhibited in this test, indicating that either organic acids or other pH-dependent antifungal compounds were responsible for the antifungal effect.

Antifungal potential of *L. amylovorus* DSM 20532

The supernatant of *L. amylovorus* DSM 20532 was fairly inhibiting for *Cerinosterus* sp., *Cladosporium* sp., *E. fibuliger* MUCL 11443, *Penicillium* A, F and G and *R. oryzae* MUCL 20145. Again the course of the pH-value was followed before and after concentrating; this showed that the pH-value did not drop below 3.5 and that it was not altered by concentration (data not shown). Moreover, upon neutralization of the supernatant, all antifungal activity disappeared, which indicated the presence of an antifungal compound with pH-dependent activity.

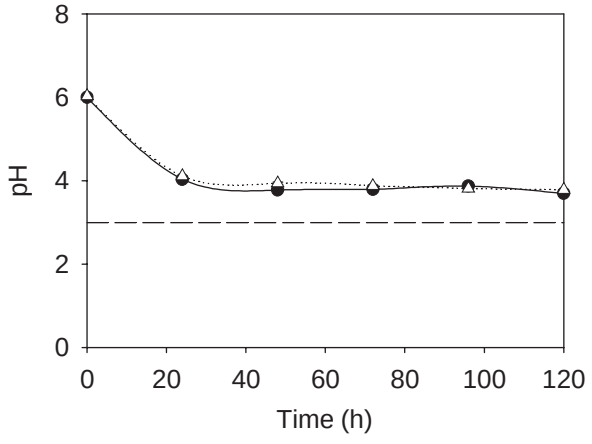

Figure 2. Course of the pH value during cultivation of *L. acidophilus* LMG 9433 before (●) and after (△) the concentration of the supernatant; (—) upper limit for fungal inhibition.

Antifungal potential of *L. brevis* LMG 6906

The inhibition zone radii obtained with *L. brevis* LMG 6906 supernatant showed that this strain is capable of inhibiting the growth of almost all tested microorganisms. The pH of the supernatant was never below 3.5 throughout the experiment. After neutralization of the supernatant to pH 5.0, 5.5 and 6.0, all antifungal activity disappeared (data not shown).

Antifungal potential of *L. coryniformis* subsp. *coryniformis* LMG 9196

With *L. coryniformis* subsp. *coryniformis* LMG 9196, clear inhibition zones were detected against *Cerinosterus* sp., *Cladosporium* sp., *Penicillium* species F and G and *R. oryzae* MUCL 20145. Also *A. flavus* MUCL 19945, *E. fibuliger* MUCL 11443 and *Penicillium* A and B showed slight inhibition. The supernatant never had a pH-value lower than 3.5 and all antifungal activity disappeared upon neutralization. This again suggests that the antifungal properties of the *L. coryniformis* supernatant were due to a pH-dependent antifungal substance.

Discussion

The above screening has proven the potential of lactic acid bacteria to inhibit growth of several common food-spoiling fungi in their growth. This opens up new perspectives for the biopreservation of food products. Almost 75% (13 out of 18) of all tested lactic acid bacterial strains showed some (or clear) antifungal activity. For some species, like *L. amylovorus, L. brevis, L. delbrueckii, L. fermentum* and *L. hilgardii* this is, to our knowledge, the first report of observed antifungal activity. Further examination of four strains, *L. acidophilus* LMG 9433, *L. amylovorus* DSM 20532, *L. brevis* LMG 6906 and *L. coryniformis* subsp. *coryniformis* LMG 9196 indicated that the antifungal effect of these lactic acid bacteria could not simply be assigned to the low pH, but most probably to the formation and secretion of antifungal organic metabolites. Since lactic acid bacteria have long been known to produce organic acids into their production medium (De Vuyst and Vandamme, 1994), these metabolites

could be acids like acetic, lactic or special acids. Organic acids can only penetrate the microbial cell wall in their undissociated form, thus ideally at a pH beneath their pK_a. The pK_a value of the most common acids produced by lactic acid bacteria are below 5.0. The pK_a of lactic, acetic, 3-phenyl-L-lactic and caproic acid is 3.8, 4.7, 3.5 and 4.9, respectively. Thus, adjusting the pH values of the supernatants above 5.0, should exclude antifungal activity of organic acids. Neutralization of the supernatant to pH values of 5.0, 5.5 and 6.0 indeed removed the antifungal activity with all four of the lactic acid bacteria. This finding strongly suggests that organic acids are responsible for the antifungal activity. However, the presence of antifungal proteinaceous compounds was not eliminated by this test. Since lactic acid bacteria produce organic acids, these might also activate other antifungal compounds (like peptides) by lowering the pH. These compounds are therefore also eliminated by neutralization. For example, the antifungal substance described by Magnusson and Schnürer (2001) is such a low pH-activated compound. They observed a weak inhibition of *P. paneum* caused by a peptide produced by their *L. coryniformis* subsp. *coryniformis* Si3 strain. The activity of this peptide was stable at pH-values between 3.0 and 4.5 but rapidly decreased between 4.5 and 6.0. No inhibitory activity was detected at a pH above 6.0. The antifungal activity of *L. acidophilus* R described by Batish et al. (1989, 1990b) was also caused by a proteinaceous compound, but there were no results on the pH-dependency of this peptide available. Furthermore, it has been suggested that some lactic acid bacteria produce a wide spectrum of compounds that might act synergistically towards filamentous fungi and yeasts (Magnusson et al., 2003).

As to the antifungal potential of three commercial probiotic lactic cultures, Actimel®, Yakult® and Beneflora®, their supernatants displayed mainly antibacterial properties, which seems logical given their claimed probiotic activity. However, the concentrated supernatant of the aerobically cultured Beneflora® strains did inhibit the growth of several moulds.

Finally, a remark has to be made concerning the size of the inhibition zones observed. Indeed for all lactic acid bacteria tested, inhibition zones never exceeded a radius of 3–4 mm. However, other reports mention strong antifungal effects of similar strains to the ones used in our study (Batish et al., 1990a, b; Corsetti et al., 1998; Magnusson and Schnürer, 2001; Stiles et al., 2002; Lavermicocca et al., 2003). This effect should not only be explained by variable factors such as culture medium and environmental conditions; but merely by the very stringent conditions of our screening method. First, the supernatants were pasteurized, a treatment which can eliminate several active compounds. Secondly, the screening method is based on a solid, complex medium, which only allows small, diffusable and non-complexing molecules to migrate through the agar. Another very important factor is chemical dissociation of organic acids. Organic acids (i.e. lactic and acetic acid) can only perform their antimicrobial effect, when they occur in a non-dissociated form. This is directly related to the pH, since these acids show little dissociation when the pH is lower than the pK_a of the acid. Due to this effect and to the fact that the agar-diffusion medium pH is around 6.0, most organic acids will not have optimal conditions to display their antifungal activity. However, since these acids can diffuse through the medium, they could locally lower the pH by dissociating and thus creating a suitable environment for the remaining undissociated molecules to inhibit the fungi. This locally acidifying effect could be the reason for the observed small size of the inhibition zones, when indeed organic acids are at the basis of the antifungal activity. When higher molecular weight or more hydrophobic molecules are causing the activity, the diffusion effect seems a more plausible explanation. These limitations are, however, by no means negative aspects of our screening method, since it was the aim to find (an) antifungal substance(s) that can be used in a complex, solid, neutral pH food product, which furthermore undergoes thermal treatment.

Conclusion

The aim of this study was to evaluate the antifungal capacity of lactic acid bacteria. Four strains, *L. acidophilus* LMG 9433, *L. amylovorus* DSM 20532, *L. brevis* LMG 6906 and *L. coryniformis* subsp. *coryniformis* LMG 9196, showed to be excellent producers of antifungal metabolites. Furthermore, these metabolites were heat stable, as they remained active after a pasteurization process. They were capable of migrating through a complex medium, causing clear inhibition zones, and their activity was shown to be pH-dependent. Given this pH-dependency and the results of previous reports (Magnusson and Schnürer, 2001), we conclude that they are either (organic) acids, proteinaceous compounds with low pH optima for activity or other pH-dependent organic metabolites. No further investigation of their chemical nature has been performed yet.

References

Batish, V.K., Grover, S., Lal, R., 1989. Screening lactic starter cultures for antifungal activity. Cultured Dairy Prod. J. 24, 21–25.

Batish, V.K., Lal, R., Chander, H., 1990a. Effect of nutritional factors on the production of antifungal substance by *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*. Aust. J. Dairy Technol. 45, 74–76.

Batish, V.K., Lal, R., Grover, S., 1990b. Studies of environmental and nutritional factors on production of antifungal substance by *Lactobacillus acidophilus* R. Food Microbiol. 7, 199–206.

Casas, I.A., Dobrogosz, W.J., 2000. Validation of the probiotic concept: *Lactobacillus reuteri* confers broad-spectrum protection against disease in humans and animals. Microb. Ecol. Health Dis. 12, 247–285.

Corsetti, A., Gobetti, M., Rossi, J., Damiani, P., 1998. Antimould activity of sourdough lactic acid bacteria: identification of a mixture of organic acids produced by *Lactobacillus sanfrancisco* CB1. Appl. Microbiol. Biotechnol. 50, 253–256.

De Vuyst, L., Vandamme, E.J., 1994. Antimicrobial potential of lactic acid bacteria. In: De Vuyst, L., Vandamme, E.J. (Eds.), Bacteriocins of Lactic Acid Bacteria: Microbiology, Genetics and Applications. Blackie Academic & Professional, London, pp. 91–142.

El-Gendy, S.M., Marth, E.H., 1980. Growth of toxigenic and non-toxigenic aspergilli and penicillia at different temperatures and in the presence of lactic acid bacteria. Arch. Lebensm. Hyg. 31, 189–220.

Gobetti, M., Corsetti, A., 1997. *Lactobacillus sanfrancisco,* a key sourdough lactic acid bacterium: a review. Food Microbiol. 14, 175–187.

Gobetti, M., Corsetti, A., Rossi, J., 1994. The sourdough microflora: interactions between lactic acid bacteria and yeasts: metabolism of carbohydrates. Appl. Microbiol. Biotechnol. 41, 456–460.

Gourama, H., 1997. Inhibition of growth and mycotoxin production of *Penicillium* by *Lactobacillus* species. Lebensm.-Wiss. Technol. 30, 279–1280.

Laitila, A., Alakomi, H.L., Raaska, L., Matilla-Sandholm, T., Haikara, A., 2002. Antifungal activities of two *Lactobacillus plantarum* strains against *Fusarium* molds in vitro and in malting of barley. J. Appl. Microbiol. 93, 566–576.

Lavermicocca, P., Valerio, F., Evidente, A., Lazzaroni, S., Corsetti, A., Gobetti, M., 2000. Purification and characterization of novel antifungal compounds from the sourdough *Lactobacillus plantarum* strain 21B. Appl. Environ. Microbiol. 66, 4084–4090.

Lavermicocca, P., Valerio, F., Visconti, A., 2003. Antifungal activity of phenyllactic acid against molds isolated from bakery products. Appl. Environ. Microbiol. 69, 634–640.

Magnusson, J., Schnürer, J., 2001. *Lactobacillus coryniformis* subsp. *coryniformis* strain Si3 produces a broad-spectrum proteinaceous antifungal compound. Appl. Environ. Microbiol. 67, 1–5.

Magnusson, J., Ström, K., Roos, S., Sjörgen, J., Schnürer, J., 2003. Broad and complex antifungal activity among environmental isolates of lactic acid bacteria. FEMS Microbiol. Lett. 219, 129–135.

Niku-Paavola, M.L., Laitila, A., Matilla-Sandholm, T., Haikara, A., 1999. New types of antimicrobial compounds produced by *Lactobacillus plantarum*. J. Appl. Microbiol. 86, 29–35.

Okkers, D.J., Dicks, L.M.T., Silvester, M., Joubert, J.J., Odendaal, H.J., 1999. Characterization of pentocin TV35b, a bacteriocin-like peptide isolated from *Lactobacillus pentosus* with a fungistatic effect on *Candida albicans*. J. Appl. Microbiol. 87, 726–734.

Plocková, M., Chumchalová, J., Tomanová, J., 1997a. Antifungal activity of *Lactobacillus acodophilus* CH5 metabolites. Food Sci. 15, 39–48.

Plocková, M., Tomanová, J., Chumchalová, J., 1997b. Inhibition of mould growth and spore production by *Lactobacillus acidophilus* CH5 metabolites. Bull. Food Res. 36, 237–247.

Roy, U., Batish, V.K., Grover, S., Neelakantan, S., 1996. Production of antifungal substance by *Lactococcus lactis* subsp. *lactis* CHD-28.3. Int. J. Food Microbiol. 32, 27–34.

Roy, U., Grover, S., Neelakantan, S., Batish, V.K., 2001. Mode of inhibition of *Aspergillus flavus* IARI antifungal substance produced by *Lactococcus lactis* subsp. *lactis* CHD-28.3. J. Food Sci. Technol. 38, 489–492.

Stiles, J., Penkar, S., Plocková, M., Chumchalová, J., Bullerman, L.B., 2002. Antifungal activity of sodium acetate and *Lactobacillus rhamnosus*. J. Food Prot. 65, 1188–1191.

Stiles, J., Plocková, M., Toth, V., Chumchalová, J., 1999. Inhibition of *Fusarium* sp. DMF 0101 by *Lactobacillus* strains grown in MRS and Elliker broth. Adv. Food Sci. 21, 117–121.

Ström, K., Sjörgen, J., Broberg, A., Schnürer, J., 2002. *Lactobacillus plantarum* MiLAB 393 produces the antifungal cyclic dipeptides cyclo(L-Phe-L-Pro) and cyclo(L-Phe-*trans*-4-OH-L-Pro) and 3 phenyllactic acid. Appl. Environ. Microbiol. 68, 4322–4327.

Suzuki, H.W., Nomura, M., Morichi, T., 1991. Isolation of lactic acid bacteria which suppress mold growth and show antifungal action. Milchwiss. 46, 635–639.