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Broad and complex antifungal activity among environmental isolates of lactic acid bacteria

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Abstract

More than 1200 isolates of lactic acid bacteria isolated from different environments were screened for antifungal activity in a dual-culture agar plate assay. Approximately 10% of the isolates showed inhibitory activity and 4% showed strong activity against the indicator mould *Aspergillus fumigatus*. The antifungal spectra for 37 isolates with strong activity and five isolates with low or no activity were determined. Several of the strains showed strong inhibitory activity against the moulds *A. fumigatus*, *Aspergillus nidulans*, *Penicillium commune* and *Fusarium sporotrichioides*, and also against the yeast *Rhodotorula mucilaginosa*. *Penicillium roqueforti* and the yeasts *Pichia anomala* and *Kluyveromyces marxianus* were not inhibited. Several isolates showed reduced antifungal activity after storage and handling. The majority of the fungal inhibitory isolates were identified by 16S rDNA sequencing as *Lactobacillus coryniformis*. *Lactobacillus plantarum* and *Pediococcus pentosaceus* were also frequently identified among the active isolates. The degree of fungal inhibition was not only related to production of lactic or acetic acid. In addition, antifungal cyclic dipeptides were identified after HPLC separation and several other active fractions were found suggesting a highly complex nature of the antifungal activity.

Keywords: Phenyllactic acid; 16S rRNA; Antifungal activity; Lactic acid bacterium; Cyclic dipeptide; *Lactobacillus coryniformis*

1. Introduction

Food and feed spoiling moulds and yeasts cause great economic losses worldwide. Furthermore, the presence of moulds with the concomitant production of allergenic spores and possibly mycotoxins makes them serious potential health hazards [1]. The reduction of mould and yeast growth in food and feed production and storage is thus of primary importance and there is great interest in developing efficient and safe strategies for this purpose. In this context, the application of biopreservation, i.e. control of one organism by another, has received much attention in recent years. Lactic acid bacteria (LAB) are known to produce different antimicrobial compounds and are important in the biopreservation of food and feed [2–4]. LAB are of special interest as biopreservation organisms since they have a long history of use in food and are 'generally regarded as safe' organisms. Their preserving effect mainly relates to the production of organic acids, i.e. lactic and acetic acid, [5], but bacteriocins, produced by some strains, are also of importance [6].

The majority of the large numbers of reports on antimicrobial activity of LAB have focused on antibacterial effects [6], while reports on antifungal effects are few. Lavermicocca et al. [3] reported production of the antifungal compounds phenyllactic acid and 4-hydroxyphenyllactic acid by a sourdough *Lactobacillus plantarum* strain. In addition, bacteriocin-like substances and other low molecular mass compounds produced by LAB have been reported as antifungal [7,8]. Our group has recently discovered that *Lactobacillus coryniformis* strain Si3 can produce a proteinaceous antifungal compound [9]. We have also identified antifungal cyclic dipeptides from a silage *L. plantarum* strain [10].

In this study, the identity and fungal inhibitory spectra of 42 isolates of LAB were determined. The majority of

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the isolates were obtained from plant material but some isolates were collected from chicken intestines, honey and from soil. The isolates were screened against the five mould species, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Penicillium commune*, *Penicillium roqueforti* and *Fusarium sporotrichioides*, and the three yeasts species *Rhodotorula mucilaginosa*, *Pichia anomala* and *Kluyveromyces marxianus*. These fungi were selected to represent spoilage organisms of economic significance in the handling of food and feed [1].

A fractionation of the culture filtrate from five isolates was done to elucidate the nature of the compounds produced. The degree of fungal inhibition was not related to production of lactic or acetic acid only, but to a combination of different compounds. It can be concluded that LAB produce a wide spectrum of compounds that might act synergistically towards filamentous fungi and yeasts.

2. Materials and methods

2.1. Isolation of lactic acid bacteria

LAB isolates were collected from a variety of natural environments, but the majority of them were isolated from plant material (leaves, stems and flowers) after enrichment in mini-silos under anaerobic conditions. The remaining isolates came from soil, honey, chicken and pig intestines. *L. coryniformis* strain Si3, earlier isolated from grass silage [9], was also included in the study. Plant material was fermented at 10, 25 or 30°C for 10 days in 50-ml mini-silos supplied with a syringe needle to remove over-pressure from produced CO_2. After incubation, 10 g of plant material was suspended in 90 ml sterile peptone water (0.2% w/v) and treated for 2 min in a stomacher. For soil samples, portions of 10 g soil were suspended in 90 ml sterile peptone water (0.2% w/v). The mixture was shaken for 15 min on a rotary shaker at 120 rpm. Intestine from newly slaughtered animals was collected from the slaughterhouse. In the laboratory, the intestines were sectioned, cut open and rinsed with ice-cold phosphate-buffered saline (PBS) in order to remove loosely associated intestinal content. Mucosal material was then released by gently scraping the intestine with a spatula. The released material was collected in tubes with ice-cold PBS. For all above isolations, dilutions were made with sterile peptone water (0.2% w/v), surface spread on MRS agar (Oxoid) plates and incubated in anaerobic jars under CO_2+N_2 atmosphere (GasPak System, BBL) for either 7 days at 10°C, 3 days at 25°C, 2 days at 30°C or 2 days at 37°C (intestine isolates). After incubation, colonies were transferred to new MRS plates and incubated a second time. Working cultures were kept under anaerobic conditions on MRS agar plates at 5°C. Long-term storage of strains was done either at −70°C in a 15% glycerol salt solution (0.82 g K_2HPO_4, 0.18 g KH_2PO_4, 0.59 g Na-citrate, 0.25 g $MgSO_4 \cdot 7H_2O$ per litre) or lyophilised in skimmed milk powder.

2.2. Fungal inocula

The moulds *A. fumigatus* J9, *A. nidulans* J283 (FSGC A4 wt), *P. roqueforti* J268 (IBT 6754), *P. commune* J238 (IBT 12400), *F. sporotrichioides* J304 (ITEM168) and the yeasts *P. anomala* J121, *K. marxianus* J186 and *R. mucilaginosa* J350 (CFSQE 63) are kept in the culture collection of the Department of Microbiology, Swedish University of Agricultural Sciences. Moulds were grown on malt extract agar (MEA, Oxoid) slants at 25°C for 7 days, and stored at 4°C. Spore inocula were prepared by growing the moulds on MEA slants for 7 days (or until sporulation) and collecting spores after vigorously shaking slants with sterile peptone water (0.2% w/v). Yeast cell inocula were prepared from cultures, grown in malt extract broth (2%, Difco Laboratories) at 25 or 30°C for 24 h. Both mould spores and yeast cell concentration were determined using a haemocytometer, and adjusted to 10^5 spores/cells per ml of sterile peptone water (0.2% w/v).

2.3. Antifungal assays

LAB were screened for antifungal activity using a dual-culture overlay assay [9]. Bacteria were inoculated in two 2-cm lines on MRS agar plates and allowed to grow at 30°C for 48 h in anaerobic jars. The plates were then overlaid with 10 ml of malt extract soft agar (0.05% malt extract; Difco Laboratories, and 1% agar; Oxoid) containing 10^4 yeast cells or mould spores (conidia) per ml. After 48 h of aerobic incubation at 30°C, the zone of inhibition was measured. The inhibition was graded by relating the inhibited growth area per inoculation streak to the total area of the petri dish. The inhibition area was also related to the variation in length of the bacterial streak. The following scale was used: −, no visible inhibition; +, no fungal growth on 0.1–3% of plate area/bacterial streak; ++, no fungal growth on 3–8% of plate area/bacterial streak; +++, no fungal growth on >8% of plate area/bacterial streak. Inhibition tests were done in duplicate.

Determination of antifungal activity of the isolated compounds was performed in duplicate using a microtitre plate well method with *A. fumigatus* as the target organism [9]. In the microtitre plate well method, 50 μl MRS broth (Oxoid) containing 10^4 fungal spores per ml was added to each well. Air-dried fractions from the high performance liquid chromatography (HPLC) fractionation were dissolved in 50 μl 10 mM HAc, left at room temperature for 3 h, and then transferred to the corresponding wells in the microtitre plate assay. After 48 h incubation at 30°C the inhibition was detected by measuring optical density (OD) at 550 nm with a microplate autoreader. An inverted

microscope and measurement using the naked eye were also used for estimation of fungal growth in the wells.

2.4. Determination of antifungal spectra

More than 1200 isolates were screened against the indicator mould *A. fumigatus* J9, and among these, 42 isolates were selected for this study. The overlay method described above was used to determine the ability of the selected LAB isolates to inhibit growth of five mould and three yeasts species at 25 and 30°C. Inhibition tests were done in duplicate.

2.5. Identification of lactic acid bacteria

Cell morphology was observed by light microscopy. All strains were identified by sequence analysis of 16S rDNA. Bacterial DNA was isolated from bacteria grown in MRS broth using DNeasy™ Tissue Kit (Qiagen). 16S rDNA was amplified by PCR (94°C for 30 s, 54°C for 30 s and 72°C for 80 s, 30 cycles) using primers 16S.S (5′-AGAGTTTGATCCTGGCTC-3′) and 16S.R (5′-CGGGAACGTATTCACCG-3′). The resulting PCR product was purified using the Qiagen PCR purification kit. Both strands of the purified fragment were partially sequenced using the Thermo Sequenase Dye Terminator Cycle Sequencing Pre-mix (Amersham Biosciences) and the automated sequence analyser ABI Prism 377XL (Perkin Elmer). Primers used for sequencing were 16S.S and the additional internal primer 16S4 (5′-CCYACTGCTGCCTCCCGT-3′). The partial 16S rDNA sequences, approximately 450 bp encoding variable regions V1 and V2, were used for searching in public databases (GenBank®).

2.6. HPLC analysis of lactic and acetic acid in culture filtrate

The bacteria were grown in MRS broth to $OD_{540} = 2.6$ (± 0.2), centrifuged and sterile-filtered (0.45 μm, Millipore) to obtain a cell-free supernatant. The HPLC analysis was performed on a cation exclusion column (HC-75, 7.8×305 mm, Hamilton) at 60°C using 5 mM H_2SO_4 as the mobile phase. The flow rate was 0.6 ml min^{-1} and the eluate was monitored by refractive index (Agilent 1100 Series) at 40°C.

2.7. Preparation of cell-free supernatant

MiLAB strains 006, 016, 024 and 091, and strain Si3 were inoculated to 10^5 cells ml^{-1} in 200 ml of MRS broth and incubated as still cultures at 30°C for 48 h. Cell-free supernatant was prepared by centrifugation (7000 rpm for 15 min) and sterile filtration (0.45 μm, Millipore). The cell-free culture filtrate was used for further isolation of the antifungal compounds.

2.8. Solid phase extraction (SPE) and HPLC and structure determination of antifungal substances

The isolation of antifungal compounds was done according to Ström et al. [10]. The supernatants were fractionated on a C_{18} SPE column and the 95% aqueous acetonitrile fraction was further separated on a preparative HPLC C_{18} column. Fractions were collected in 2-ml 96-deep-well plates using a fraction collector. All fractions from the purification process were evaluated for antifungal activity after concentration under vacuum and/or by freeze-drying or evaporation under compressed air. The microtitre plate bioassay described above was used with *A. fumigatus* as the indicator fungus. Non-inoculated MRS broth, fractionated and evaluated in the bioassays using the same procedure as with the cell-free culture filtrate, was used as a negative control.

The structures of the antifungal compounds produced by strain Si3 were determined using nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) according to Ström et al. [10].

3. Results

3.1. Isolation of antifungal lactic acid bacteria

Out of 1200 LAB isolates from different sources screened against the indicator mould *A. fumigatus*, approximately 10% showed antifungal activity (+, ++ or +++ activity). About 4% of the total number of screened isolates had strong activity (+++). For this study, 37 isolates with strong (+++) or moderate (++) activity and five isolates with low or no activity (+ or −) were selected.

3.2. Inhibition of moulds and yeasts

Varying degrees of inhibition were detected against the moulds *P. commune*, *A. fumigatus*, *A. nidulans* and *F. sporotrichioides*. The latter, which was the most sensitive indicator strain, was inhibited (++ or +++) by all the bacterial strains. None of the isolates had activity against *P. roqueforti*. Among the yeasts, only *R. mucilaginosa* was inhibited by some of the isolates (Table 1). Notably, some isolates exhibited a lower inhibitory effect during determination of antifungal spectra than during the primary screening.

3.3. Identification of the isolates

Approximately 450 bp of the 16S rDNA sequence were determined for the 42 selected isolates included in this study. The sequences derived from the isolates were used to search databases for highest similarity rank to determine the species identity of the isolates (Table 1). The majority of the 37 isolates with high or moderate activity,

Table 1
Source of isolation, species identity and fungal inhibition spectra of LAB strains

Strain	Species	Initial activity	J9	J283	J304	J238	J268	J121	J186	J350	Source
MiLAB 006	*Lactobacillus plantarum*	+++	++	++	+++	++	–	–	–	–	Lilac flowers
MiLAB 014	*Lactobacillus plantarum*	+++	+++	+	+++	++	–	–	–	+	Lilac flowers
MiLAB 016	*Pediococcus pentosaceus*	+++	++	+	+++	++	–	–	–	–	Chestnut flowers
MiLAB 018	*Pediococcus pentosaceus*	+++	++	++	+++	++	–	–	–	++	Chestnut flowers
MiLAB 022	*Pediococcus pentosaceus*	+++	++	++	+++	+	–	–	–	–	Clover
MiLAB 024	*Pediococcus pentosaceus*	++	+++	++	+++	++	–	–	–	–	Clover
Si3	*Lactobacillus coryniformis*	+++	+++	++	+++	++	–	–	(+)	++	Grass
MiLAB 029	*Pediococcus pentosaceus*	+++	++	++	+++	+	–	–	–	+	Clover
MiLAB 031	*Pediococcus pentosaceus*	+++	++	++	+++	++	–	–	–	++	Clover
MiLAB 037	*Lactobacillus plantarum*	+++	++	+	+++	++	–	–	–	–	Dandelion flower
MiLAB 039	*Lactobacillus plantarum*	+++	++	++	+++	++	–	–	–	+++	Dandelion flower
MiLAB 047	*Pediococcus pentosaceus*	+++	++	+	+++	+	–	–	–	++	Clover
MiLAB 049	*Lactobacillus acidophilus*	+++	–	–	+++	–	–	–	–	–	Chicken intestine
MiLAB 051	*Lactobacillus salivarius*	+++	+	++	+++	++	–	–	–	++	Chicken intestine
MiLAB 052	*Lactobacillus salivarius*	+++	+	++	+++	++	–	–	–	–	Chicken intestine
MiLAB 091	*Lactobacillus sakei*	+++	++	+	+++	–	–	–	–	++	Dandelion
MiLAB 099	*Pediococcus parvulus*	+++	++	+	+++	+	–	–	–	–	Dandelion
MiLAB 101	*Pediococcus pentosaceus*	+++	+	+	+++	+++	–	–	–	–	Dandelion
MiLAB 120	*Lactobacillus coryniformis*	+++	++	+	+++	++	–	–	–	++	Dandelion
MiLAB 123	*Lactobacillus coryniformis*	+++	+	+	+++	+	–	–	–	++	Dandelion
MiLAB 125	*Lactobacillus coryniformis*	+++	+	++	+++	++	–	–	–	++	Dandelion
MiLAB 148	*Lactobacillus sakei*	++	++	+	+++	+	–	–	–	+	Dandelion leaves
MiLAB 166	*Pediococcus pentosaceus*	+++	++	+	+++	+	–	–	–	++	Grass
MiLAB 170	*Pediococcus pentosaceus*	++	++	++	+++	++	–	–	–	++	Grass
MiLAB 248	*Lactobacillus plantarum*	++	+++	+	+++	++	–	–	–	+++	Chestnut flower
MiLAB 262	*Lactobacillus plantarum*	++	+++	+	+++	++	–	–	–	++	Chestnut flower
MiLAB 274	*Lactobacillus coryniformis*	+++	+++	++	+++	++	–	–	–	+++	Coltsfoot flower
MiLAB 275	*Lactobacillus coryniformis*	+++	+++	++	+++	++	–	–	–	++	Coltsfoot flower
MiLAB 282	*Lactobacillus coryniformis*	+++	+++	++	+++	++	–	–	–	+++	Hepatica flower
MiLAB 283	*Lactobacillus coryniformis*	+++	+++	++	+++	+	–	–	–	–	Hepatica flower
MiLAB 290	*Lactobacillus coryniformis*	+++	+++	+	+++	++	–	–	–	++	Rowan leaves
MiLAB 291	*Lactobacillus coryniformis*	+++	+++	++	+++	++	–	–	–	++	Rowan leaves
MiLAB 303	*Lactobacillus coryniformis*	++	+++	++	+++	+	–	–	–	++	Coltsfoot flower
MiLAB 311	*Lactobacillus coryniformis*	++	+++	++	+++	++	–	–	–	++	Dandelion flower
MiLAB 355	*Lactobacillus coryniformis*	+++	+++	++	+++	++	–	–	–	+	Dandelion flower
MiLAB 357	*Lactobacillus coryniformis*	+++	+++	++	+++	++	–	–	–	++	Dandelion flower
MiLAB 359	*Lactobacillus coryniformis*	++	+++	++	+++	++	–	–	–	++	Dandelion stalk
MiLAB 199	*Lactobacillus sakei*	+	+	–	++	+	–	–	–	–	Grass
MiLAB 026	*Enterococcus hirae*	–	+	–	++	–	–	–	–	–	Dandelion flower
MiLAB 062	*Lactobacillus salivarius*	–	+	+	+++	++	–	–	–	–	Chicken intestine
MiLAB 268	*Weissella soli*	+	+	–	++	–	–	–	–	+	Soil
MiLAB 069	*Enterococcus durans*	–	+	–	++	–	–	–	–	–	Honey

All isolates were initially screened against the indicator mould *A. fumigatus* J9, with degree of inhibition given under Initial activity. The table further shows activity, after long-term storage and handling, against moulds *A. fumigatus* J9, *A. nidulans* J283 (FSGC A4 wt), *F. sporotrichioides* J304 (ITEM168), *P. commune* J238 (IBT 12400) and *P. roqueforti* J268 (IBT 6754), and the yeasts *P. anomala* J121, *K. marxianus* J186 and *R. mucilaginosa* J350 (CFSQE 63).

i.e. 15 of the isolates, were identified as *L. coryniformis*. Among the remaining, 10 isolates were identified as *Pediococcus pentosaceus*, six as *L. plantarum*, two as *Lactobacillus sakei*, two as *Lactobacillus salivarius*, one as *Pediococcus parvulus* and one as *Lactobacillus acidophilus*. None of the five isolates with low or no activity belonged to the three major antifungal species found in this study.

3.4. HPLC analysis of lactic acid

To investigate if the differences in mould inhibition were due to variations in organic acid production, HPLC analysis of lactic and acetic acid in culture supernatant was performed on isolates of the same species with high or low activity. Two *P. pentosaceus* strains, MiLAB 024 (+++, inhibition of *A. fumigatus* J9) and MiLAB 101 (+), were compared, and three strains of *L. coryniformis*, MiLAB 123 (+), MiLAB 283 (+++) and MiLAB 311 (+++), were compared (Fig. 1). MiLAB 101 and MiLAB 123 had initially shown strong activity (+++), but lost most of this activity during storage or handling. The concentration of acetic acid in the samples, 29–31 mM, corresponds to the amount present in MRS broth. The highest concentrations of lactic acid, 90 and 95 mM, were observed with

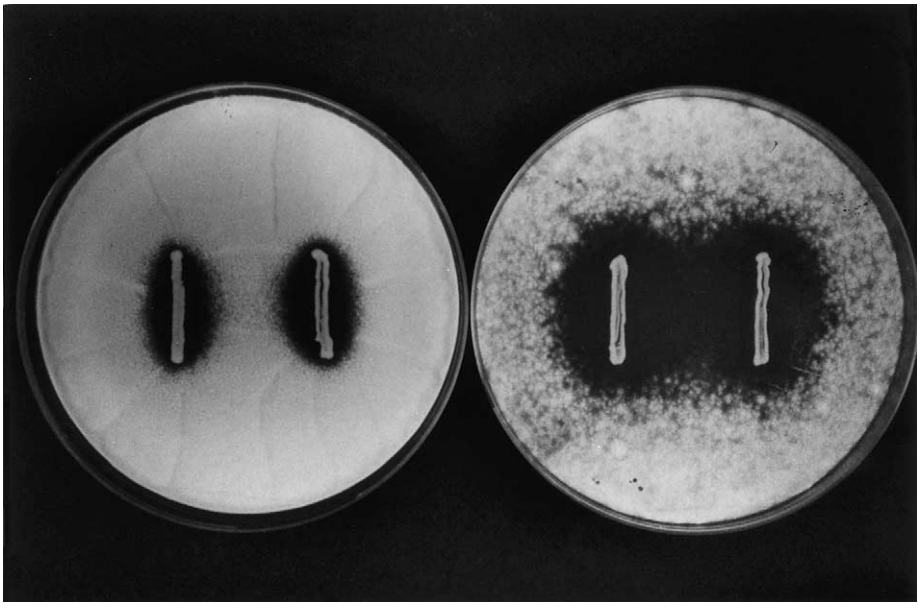

Fig. 1. Activity of two different *L. coryniformis* isolates, MiLAB 123 (left, +) and MiLAB 283 (right, +++) after long-term storage at −70°C, against the indicator mould *A. fumigatus*. Both isolates had initially shown strong (+++) activity against *A. fumigatus*.

the two isolates with the lowest antifungal activity. The three isolates with high antifungal activity produced low amounts of lactic acid (60, 62 and 62 mM).

3.5. Isolation of antifungal compounds

Antifungal activity of the cell-free supernatants resulting from growth of strains MiLAB 006, 016, 024 and 091, and strain Si3 in MRS broth was recovered both in the hydrophilic and in the 95% acetonitrile (hydrophobic) phase after SPE on a C_{18} column, indicating a complex nature of the antifungal compound or compounds. Further fractionation of the hydrophobic phase by HPLC using a C_{18} column and concomitant activity assay in microtitre wells revealed different antifungal inhibition patterns for the five isolates against *A. fumigatus* (Fig. 2). No activity was observed from the corresponding fractions from non-inoculated MRS broth.

The structures of the active compounds produced by strain Si3, corresponding to the fractions from the HPLC purification step, were elucidated. After NMR and MS it was determined that the active compounds were cyclo(Phe-Pro), eluted in wells C10–C11, and cyclo(Phe-4-OH-Pro) and phenyllactic acid, both compounds eluted in well D10.

4. Discussion

Yeasts and moulds are common spoilage organisms in different food and feed systems. Yeasts included in this study, such as *R. mucilaginosa* and *K. marxianus*, are important spoilage organisms in dairy products such as yoghurt, cream and cheese [1]. Moulds such as *P. roqueforti* and *P. commune* commonly spoil hard cheese, and different *Fusarium* species are associated with wheat and rye grain and can produce mycotoxins in cereal grains [11]. Thus, there is a definite need for safe and efficient ways to prevent fungal growth in raw material and food products. LAB have a long history as a preservative agent in food and feed systems and are regarded as safe organisms to use. Antimicrobial substances from LAB have in some cases been well studied, especially regarding antibacterial effect in several forms. However, there are relatively few reports on antifungal activity of LAB. We have recently described *L. coryniformis* subsp. *coryniformis* strain Si3 with a broad inhibitory spectrum against moulds and yeasts [9]. In the present study, we identified 36 new isolates with strong or moderate antifungal activity to species level. Among these, 14 belonged to the same species as strain Si3, *L. coryniformis*. The majority of the 15 *L. coryniformis* isolates, including Si3, were inhibitory against a broad range of fungi. Besides our previous publication, we have not found any literature reports on the antimicrobial activity of *L. coryniformis*. Thus, there is a striking bias in the selection of bacterial species that were identified as antifungal in our screening. Whether this is a result of the screening procedure itself or could be explained by the fact that *L. coryniformis*, *L. plantarum* and *P. pentosaceus* are more commonly antagonistic against fungi could not be concluded from our data. It is notable though that from the chicken intestinal isolates we could identify two *L. salivarius* strains with strong activity.

In this study, isolates of LAB were primarily selected for their ability to inhibit the mould *A. fumigatus*. Among these 37 isolates with strong inhibitory activity and five isolates with no or low activity were chosen for further investigation. However, during the determination of fun-

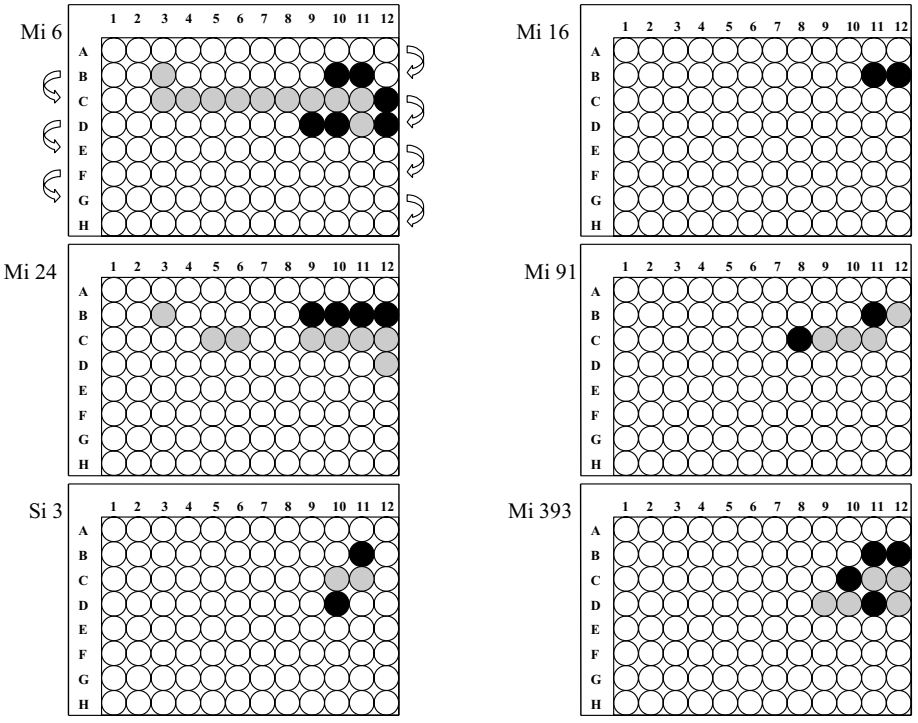

Fig. 2. The distribution of antifungal effect of MiLAB strains 006, 016, 024, 091, 393 and strain Si3 after partial purification. The antifungal effect is recorded as total inhibition (black circles), partial antifungal effect (grey circles) or no inhibition (white circles). When fractions from strains Si3 and MiLAB 393 are characterised the cyclic dipeptide cyclo(Phe-4-OH-Pro) is found in fractions C10–C12, cyclo(Phe-Pro) and phenyllactic acid in fractions D9–D12 and lactic acid in fractions B10–B12. The remaining wells with antifungal activity contain unknown substances. Arrows indicates movement of fraction collector.

gal inhibition spectra, only 29 of the 37 isolates retained their initial strong activity. In addition, all isolates originating from chicken intestines included in this study lost their activity during storage and handling. Several of the isolates that lost their activity belong to the same species as isolates with stable strong inhibitory activity. The reason for the loss of activity is not known. However, in our previous work we encountered similar problems regarding production and stability of the active antifungal compounds [9].

Cabo et al. [12] have recently suggested that antifungal activity of LAB is due to a synergistic effect of lactic acid produced by the bacteria and acetic acid from the MRS growth medium. To exclude the possibility that the observed differences in antifungal activity in our study originated from varying degrees of organic acid production, we performed an HPLC analysis of the bacterial supernatants. The production of lactic acid was the same or even higher in the tested negative isolates. The concentration of acetic acids corresponded to the amount detected in MRS broth. Thus, the HPLC analysis of lactic and acetic acid in the culture supernatant from these strains gave no explanation for the varying degrees of inhibition of fungi in dual-culture agar assay. This activity is then likely to be caused by the production of other antifungal compounds.

Ström et al. [10] found that *L. plantarum* MiLAB 393 produced three antifungal substances: cyclo($_L$-Phe-$_L$-Pro), cyclo($_L$-Phe-*trans*-4-OH-$_L$-Pro) and phenyllactic acid. In this study, cyclo(Phe-Pro), cyclo(Phe-4-OH-Pro) and phenyllactic acid were identified from the supernatant of *L. coryniformis* strain Si3. Although the complete stereochemistry has not been elucidated the compounds from *L. coryniformis* Si3 appears to be very similar to those of *L. plantarum* MiLAB 393. The HPLC isolation method used was reproducible, i.e. lactic acid, phenyllactic acid and the two cyclic dipeptides always appeared in specific fractions. It is thus likely that these substances, previously only reported from *L. plantarum* strains [3,10], are also produced by *P. pentosaceus* (MiLAB 024), *L. sakei* (MiLAB 091) and yet another *L. plantarum* (MiLAB 006).

This indicates that some antifungal compounds are widely distributed among different species of LAB. Beside the activity found in fractions mentioned above there are several other fractions with antifungal activity. These fractions containing possibly unknown antifungal substances are presently under investigation.

The current study shows that LAB from different environments and from different genera and species can exhibit antifungal activity against a number of common spoilage moulds and yeasts. The inhibitory activity is caused by several different compounds. Further investigations of the nature of the inhibiting compounds and their mechanism of action, together with development of suitable applications, could have a great potential for the control of spoilage fungi.

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