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Antifungal Activity of *Pediococcus pentosaceus* Isolated from Whole Barley Sourdough

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Abstract

Background: Lactic acid bacteria (LAB) isolated originally from sourdough are probably the best candidates as biopreservatives, because they are well adapted to the non-aseptic conditions in sourdough fermentation. The aim of the present study was to study antifungal properties of a dominant endogenous LAB isolated from whole barley sourdough.

Methods: Whole barley flour from a mixture of barley varieties was purchased from a local market. The pure and single colonies obtained from streak plate of these bacteria, were subjected to species specific PCR. By sequencing of the PCR products, the dominant LAB isolate was identified. Antifungal activity of mentioned isolate and its cell-free culture filtrate (CCF) were evaluated against *Aspergillus flavus* and *A. niger* based on overlay method and fungal spore spot on mixture of medium-CCF, respectively. Statistical analyzing was performed by ANOVA and by post hoc Tukey using SAS-9.1 software.

Results: The dominant LAB isolate was identified as *Pediococcus pentosaceus* that had significant (p<0.05) antifungal effect compared to control group. Furthermore, among all CCF treatments, CCF obtained from stationary phase (CCF-S) had the highest antagonistic effect (p<0.05). The antifungal activity was also observed in neutral pH.

Conclusion: It was concluded that the secondary metabolites of *P. pentosaceus* were more effective than the primary metabolites in antifungal properties. Also, its antifungal activity in neutral pH indicated this isolate produced some antifungal compounds in both logarithmic and stationary phases. Therefore, *P. pentosaceus* with antifungal potential would be a proper candidate as biopreservative starter or adjunct culture in sourdough bread processing.

Introduction

Consumption of breads containing whole grain barley (*Hordeum vulgare*) reduces blood cholesterol, glycemic index, colorectal cancer, cardiovascular diseases; and promotes weight loss. The high content of dietary fiber, phenolics and β-glucan in barley, are so important in the

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health promoting effects of this grain. However, because of deteriorating impact on bread quality, barley has rarely been used in baked goods processing. The use of sourdough can be a proper strategy to improve the quality and characteristics of dough and bread containing barley (Mariotti et al., 2014; Rieder et al., 2012).

Sourdough as a very complex biological ecosystem is one of the oldest biotechnological processes. Sourdough consists of cereal and water fermentation with certain yeast and lactic acid bacteria (LAB) usually propagated by back-slopping (Golshan Tafti et al., 2014). Although, in the early days, spontaneous sourdough fermentation should have been used, but nowadays use of this ecosystem has already been more systematic, and emphasizes on development of specific cultures and control of fermentation process (Chavan and Chavan, 2011; Clarke and Arendt, 2005). Previously, impact of sourdough fermentation on different properties of baked goods such as product health (Katina et al., 2005), aroma and flavour (Paterson et al., 2006), texture (Arendt et al., 2007), shelf life (Sadeghi, 2008), nutritional value (Poutanen et al., 2009), and functional features (Gobbetti et al., 2014) has been investigated by several researchers.

Most of useful and valuable characteristics attributed to the sourdough fermentation are mainly related to its dominant microflora especially its LAB. These bacteria are important group of industrial starter cultures in food fermentation technologies and play a crucial role in the preservation and safety of these products (De Vuyst and Vancanneyt, 2007; Leroy and De Vuyst, 2004). Consumer demands for safe foods and decreased application of synthetic preservatives have stimulated study on LAB as antimicrobial biopreservatives (Schnurer and Magnusson, 2005).

LAB antibacterial activity is because of the production of some compounds with bacteriostatic or bactericidal mode of action. A number of these compounds e.g. cyclic dipeptides, organic acids, phenyl lactic acid as well as hydroxylated fatty acids have been also isolated from LAB as antifungal agents with synergistic effects (Dalie et al., 2010; Messens and De Vuyst, 2002). Studying specific interactions between LAB and food-borne pathogens is complicated by the general antimicrobial effects of the bacterial fermentation products that might act synergistically with more targeted antimicrobial compounds. It has been proven that antimicrobial compounds produced after LAB fermentation may induce an additional hurdle against spoilage microorganisms (Schnurer and Magnusson, 2005). Several investigations have explored the antimicrobial properties of sourdough LAB. For example, potential of LAB to inhibit rope producing strains of *Bacillus* spp. (Katina et al., 2002), sourdough LAB ability for production of bacteriocin-like inhibitory substances (Corsetti et al., 2004) and anti-mould potential of LAB (Fazeli et al., 2004) have been confirmed.

Recently, the use of sourdough to improve the baking and sensory properties of barley bread and also the microbial composition of barley sourdough, as well as its organic acid profiles and leavening ability, were investigated (Rieder et al., 2012; Zannini et al., 2009). The objective of the present study was to molecular identification of a dominant LAB isolated from whole barley sourdough with emphasis on its antifungal ability for using as biopreservative.

Materials and methods

Whole barley sourdough fermentation for obtaining dominant LAB

Whole barley flour from a mixture of barley varieties was purchased from a local market and its components (protein: 11.6%, carbohydrate: 61.7%, ash content: 1.9%) were determined based on standard methods (AACC International, 2010). Spontaneous sourdough fermentation was carried out by mixing 600 g of whole barley flour and 2100 ml of sterile distilled water (dough yield: 450) for 24 h at 36 °C. Dominant LAB of this sourdough was obtained by back sloping (addition 20% of previous sourdough on next day sourdough formulation). The sourdough total titratable acidity (TTA) values were also measured in 24 h interval during this period. Usually, when TTA does not change after two continuous back sloping, dominant sourdough LAB will be isolated. For determination of TTA, an aliquot of 10 g fermented barley sourdough was blended with 90 ml of distilled water. Then, this suspension was titrated with 0.1 M NaOH (Merck, Germany) to a final pH of 8.5, and TTA was expressed as the amount of NaOH used (Katina et al., 2002).

Isolation and molecular identification of dominant LAB

Ten g sourdough was suspended in 90 ml sterile 0.1% peptone (Merck, Germany) solution and homogenized with a stomacher apparatus (Seward BA7020, USA) at 260 rpm for 3 min. For microbial counting, aliquot in serial 10-fold dilutions from homogenate was spread on MRS agar (Merck, Germany). The LAB colonies were counted after 48 h incubation at 36 °C in anaerobic jar. The most abundant bacterial colonies were picked up from the countable plates and streaked to purity. The microscopic cell morphology and the Gram and catalase reactions of the LAB isolate were also checked (Zannini et al., 2009).

For molecular identification of dominant LAB isolate, after DNA extraction (Bioneer, AccuPrep K-3032 DNA

extraction kit, South Korea) from its pure and single colony (according to manufacture protocol), DNA was subjected to species specific PCR according to Ferchichi et al. (2007) and also Heuer et al. (1997) with the specific primers F: 5'-GAACGCGAAGAACCTTAC-3' and also R: 5'-GCGTGTGTACAAGACCC-3'. The PCR test was performed on a Corbett N15128 thermocycler (Australia). Briefly, optimized PCR reaction (initial denaturation at 94 °C for 4 min, amplification for 35 cycles at 94 °C for 45 s, 60 °C for 60 s, 72 °C for 45 s and final extension for 5 min at 72 °C) was carried out in 25 µl final volume including 1 µl *Taq* DNA polymerase with 2.5 units activity, 2 µl buffer 10x, 2 µl $MgCl_2$ at concentration of 25 mM, 1 µl of dNTPs mixture with 10 mM concentration, 2 µl of each primer at concentration of 0.5 mM and 2 µl DNA with concentration of 100 ng/µl. Then, PCR products were electrophoresed on 1.5% (w/v) agarose gel in TBE buffer with pH 8, stained by SYBR Safe DNA gel stain (Invitrogen, USA) and observed under UV light. Based on the LAB target gene sequence, the amplified PCR products should be 500 bp. Finally, the PCR product was sent for sequencing to MWG Co. (Germany) and sequencing result was evaluated by BLASTn (http://www.blast.ncbi.nlm.nih.gov/Blast) procedure available in NCBI (Altschul et al., 1997).

Determination of logarithmic and stationary phases of LAB isolate

Optical density (OD) values of the isolate were measured by spectrophotometer (PG Instruments LTD T80, England) at absorbance of 600 nm at each 1 h intervals, until receiving the growth curve to stationary phase (Gulahmadov et al., 2009).

Indicator microorganisms

Lactobacillus sp. (PTCC 1332), *Aspergillus flavus* (PTCC 5006) and *A. niger* (PTCC 5012) were purchased from Persian culture collection (Iran) as lyophilized and after activation on MRS or nutrient broth (Merck, Germany) at 30-36 °C for 24-48 h, were cultured again on mentioned conditions and then stored at -80 °C in nutrient supplemented with 25% (v/v) glycerol (Magnusson et al., 2003).

Preparation of cell-free culture filtrate (CCF) from LAB isolate

LAB was inoculated in MRS broth at 36 °C (0.5% v/v). The fermentation culture was collected at 8 and 12 h (logarithmic and stationary phases, respectively) and subsequently centrifuged (Hermle Z-323K, Germany) at 4 °C, 4000 g for 10 min. Then, for preparation of CCF without any treatment, the supernatant was filtered using a 0.22 mm sterile filter (Biofil, China) and for pH adjusted CCF, the supernatant was neutralized to pH 6.0 by 1 M NaOH and filtered again such as above (Yang et al., 2012). Abbreviated titles for mentioned CCF used in this article are including CCF-L (logarithmic phase, without any treatment), CCF-LN (logarithmic phase, neutralized pH), CCF-S (stationary phase, without any treatment) and CCF-SN (stationary phase, neutralized pH).

Antifungal activity of LAB isolate

Antifungal activity assay was based on the method previously described by Magnusson and Schnurer (2001) with slight modifications. MRS agar (15 ml) was added to a petri dish, and then LAB were inoculated as two 3 cm long lines and incubated at 36 °C for 72 h. The plates were overlaid with 15 ml of PDA agar (Merck, Germany) containing 10^4 fungal spores/ml and incubated at 30 °C. Fungal growth was examined at an interval of 24 h. The control samples were prepared using the same procedure as the experimental group, but LAB was not inoculated in the control culture.

Antifungal activities of CCF

Antifungal activity of CCF was evaluated according to the method of Wang et al. (2012) with some modifications. Media containing 50% (v/v) CCF were inoculated in agar discs, in which the test fungi (6 mm) were placed at the center. The control plates containing the media were mixed with MRS (50%, v/v) instead CCF. Antifungal activity of CCF against indicator fungi was monitored in 24 h intervals, until fungal growth in the control plates was almost complete. During this period, mycelial growths (cm) in both treated and control petri dishes were determined.

Statistical analysis

All experiments were performed in triplicate. Results of these assays are presented as mean values±standard deviation. Data were compared by the one way analysis of variance (ANOVA) and by post hoc Tukey. Statistical significance (p<0.05) was assessed with the SAS-9.1 software.

Results

After 4 days back slopping, sourdough TTA was fixed and then, dominant LAB was isolated. Dominant LAB isolate, was Gram positive, catalase negative coccus with a tetrad cell organization. Also, its growth was optimal at 30 °C, slow at 16 °C and no growth was seen at 45 °C. Logarithmic phase of the LAB isolate was also started 9 h after incubation.

Fig. 1 shows the PCR results for specific detection of dominant LAB isolate. Extracted DNA from pure and single colonies as described in materials and methods were examined as template. Specificity of primers and absence of nonspecific products were tested by analyzing PCR products on 1.5% agarose gel stained with SYBR safe in present of positive and negative controls. Only the expected 500 bp amplicon could be observed after electrophoresis and no other bands were visible. To confirm the identity of the amplicon and further characterization of the mentioned gene sequence, the PCR product was sequenced and the result was verified by BLASTn software. Based on the results, dominant LAB isolate was identified as *Pediococcus pentosaceus* (99% identity).

Based on the results, *P. pentosaceus* had significant (p<0.05) antifungal effect on *A. niger* and *A. flavus* in comparison to control samples (Fig. 2). Furthermore, this antagonistic effect against *A. niger* was more than the effect on *A. flavus*. Antifungal activities of *P. pentosaceus* isolate CCF-L, CCF-S, CCF-LN and CCF-SN against *A. niger* and *A. flavus* were confirmed by fungal spore spot on mixture of medium-CCF (Fig. 3).

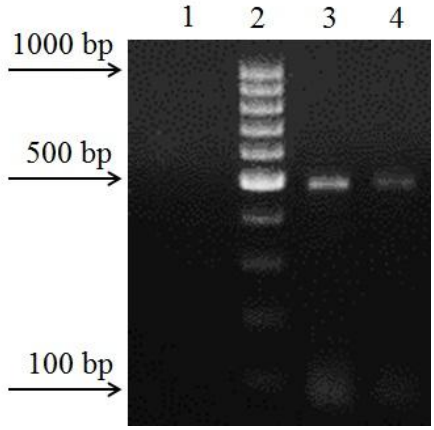

Fig. 1: Agarose gel electrophoresis of PCR products obtained under optimized conditions for detection of dominant LAB isolate (500 bp). Extracted DNA from single and pure colonies of dominant LAB isolate (lane 4), extracted DNA from cultured cells of *Lactobacillus* spp. in MRS broth as positive control (lane 3), 100 bp DNA marker (lane 2) and non DNA as negative control (lane 1)

Results of antifungal activity of CCF against indicator fungi showed that in all time intervals (24-120 h), diameter of *A. flavus* growth zone under treatment of *P. pentosaceus* isolate CCF, were significantly (p<0.05) lower than control samples. According to this result, antifungal activity of CCF-L and CCF-S were significantly (p<0.05) higher than CCF-LN and CCF-LS. Furthermore, in both cases (with neutralized pH or without any treatment), CCF-S was more effective than CCF-L.

Fig. 5 shows the effects of *P. pentosaceus* isolate CCF on *A. niger* growth. As it becomes obvious, in all time intervals (24-120 h), CCF-S treatments were significantly (p<0.05) more effective than the others. CCF-SN and CCF-L had the highest antagonistic activity after 24-96 h and 120 h incubation period, respectively. The lowest antagonistic effects were also observed in CCF-LN treatments.

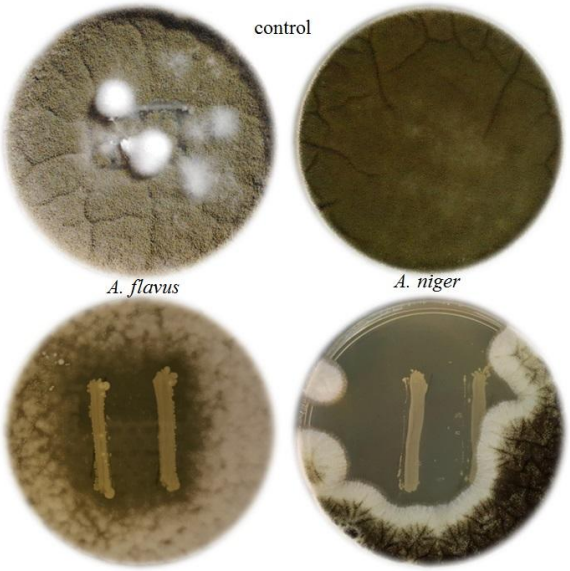

Fig. 2: Activity of *P. pentosaceus* isolate, against indicator fungi in comparison to control samples based on overlay assay

Discussion

The fungal spoilage in cereal products as sources of mycotoxins, involving public health problems, and also as the main cause of substantial economic losses is very important. So, there is a definite need for safe and efficient ways to prevent fungal growth in these products. For prevention of fungal spoilage, LAB may be considered as an alternative for conventional preservation methods. Antimicrobial characteristics of LAB have been well investigated, especially in case of antibacterial effect. However, there are few published data in related to antifungal activity of these bacteria (Dalie et al., 2010; Rouse et al., 2008).

As a functional LAB, anti-pathogen activity is one of the most important properties. LAB originally isolated from sourdough are probably the best candidates for biopreservatives, because they are well adapted to the non-aseptic conditions in sourdough fermentation and should therefore be more competitive and harbor stronger antimicrobial potentials than LAB from other fermented foods (Messens and De Vuyst, 2002; Simsek et al., 2006). The anti-mould potential of sourdough LAB has been elucidated. Some studies have described the isolation and characterization of antifungal activity of LAB isolates and also applications of the antifungal strains in

baking (Dal Bello et al., 2007; Lavermicocca et al., 2003). In the present study, after molecular identification of *P. pentosaceus* as dominant LAB isolated from whole barley sourdough, antifungal activities of this isolate and its CCF obtained from logarithmic and stationary phases were studied against *A. niger* and *A. flavus*. Results showed that among all CCF treatments, CCF-S had the highest antagonistic effect on indicator fungi showing that secondary metabolites are more effective than the primary metabolites in antifungal characteristics of the *P. pentosaceus* isolate against *A. niger* as well as *A. flavus*.

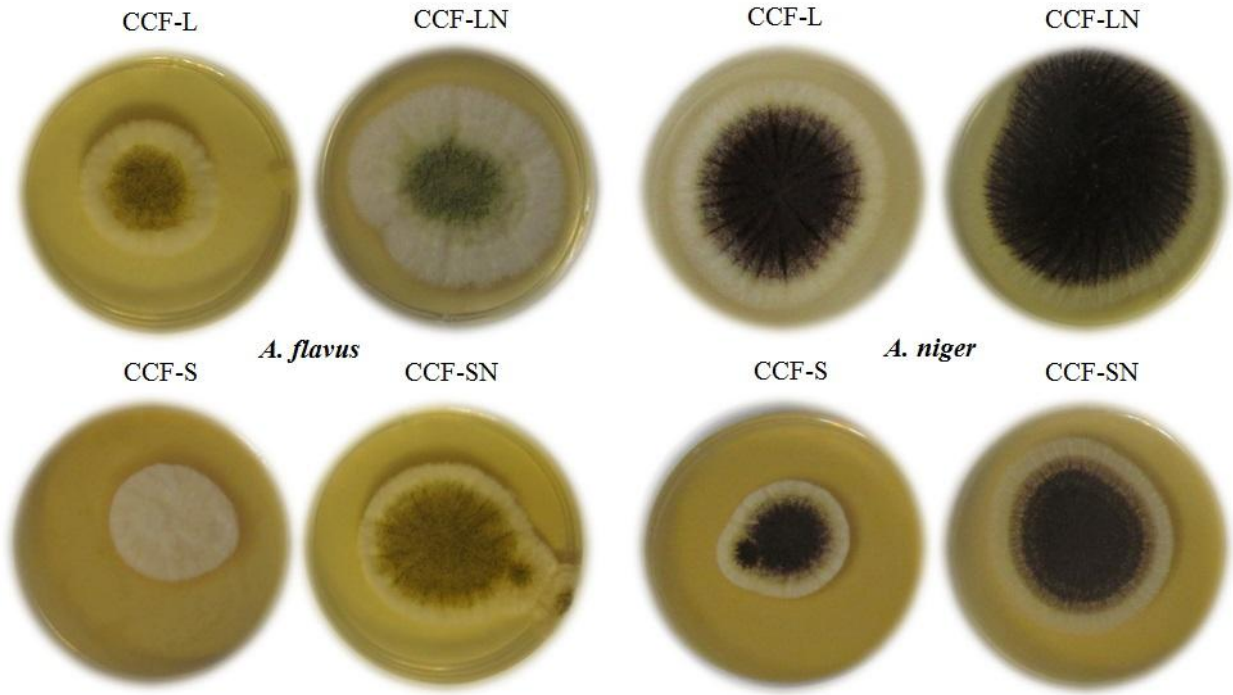

Fig. 3: Antifungal activities of *P. pentosaceus* isolate CCF-L, CCF-S, CCF-LN and CCF-SN against *A. niger* and *A. flavus* after 120 h incubation based on spore agar spot method. Abbreviated titles for mentioned CCF are including CCF-L (logarithmic phase, without any treatment), CCF-LN (logarithmic phase, neutralized pH), CCF-S (stationary phase, without any treatment), and CCF-SN (stationary phase, neutralized pH)

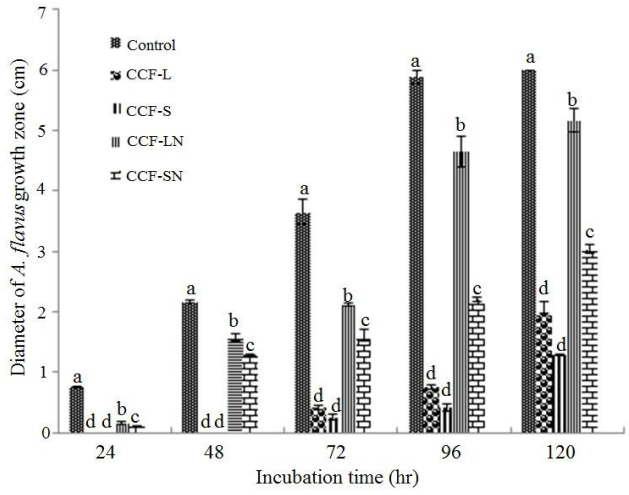

Fig. 4: Effects of *P. pentosaceus* isolate CCF on *A. flavus* growth during 120 h incubation. Columns with different letters at each incubation time are significantly different (p<0.05). Abbreviated titles for mentioned CCF are including CCF-L (logarithmic phase, without any treatment), CCF-LN (logarithmic phase, neutralized pH), CCF-S (stationary phase, without any treatment), and CCF-SN (stationary phase, neutralized pH)

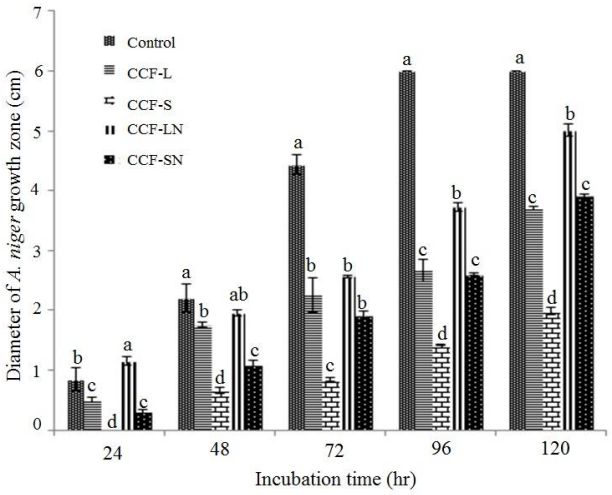

Fig. 5: Effects of *P. pentosaceus* isolate CCF on *A. niger* growth during 120 h incubation. Columns with different letters at each incubation time are significantly different (p<0.05). Abbreviated titles for mentioned CCF are including CCF-L (logarithmic phase, without any treatment), CCF-LN (logarithmic phase, neutralized pH), CCF-S (stationary phase, without any treatment), and CCF-SN (stationary phase, neutralized pH)

When pH of CCF was neutralized, the antifungal activity was also observed (CCF-SN and CCF-LN had significant inhibitory effect on fungi growth in comparison to control samples) which showed that except organic acids, this isolate has produced some antifungal compounds both in logarithmic and stationary phases.

Cabo et al. (2002) have suggested that antifungal activity of LAB is due to a synergistic effect of organic acids especially lactic acid produced by the bacteria and acetic acid from the MRS growth medium. Lactic acid as the major homofermentative LAB metabolite, reduces pH and the undissociated acid, collapses the electrochemical proton gradient. In heterofermentative LAB, acetic and propionic acids (e.g. lactic acid), interact with cell membranes to neutralize the electrochemical proton gradient, but the effect of these organic acids is related to the reduction in pH by lactic acid. Acetic and propionic acids have higher pK_a values than lactic acid and so have a greater proportion of undissociated acid at a similar pH. On the other hand, at pH below 4.5, propionic acid reduces fungal growth by effect on fungal membranes. Studies on the effect of LAB on fungi are complicated by considering the differences in sensitivity observed among the mould species to the organic acids. These differences may be related to fungi capacity to change the cell metabolism in response to organic acids stress conditions (Rouse et al., 2008; Schnurer and Magnusson, 2005).

Studying specific interactions between LAB and fungi is more complicate and usually after eliminating of organic acids, the antifungal activity will be observed, too. This shows that beside organic acids, there are some other antifungal metabolites in LAB. The majority of antifungal substances purified from LAB have been low molecular mass compounds such as phenyllactic acids, cyclic dipeptides and short chain fatty acids. As organic acids, the rate of dissociation of these metabolites is mostly dependent on the pH degree. At low pH, the undissociated form of these compounds can easily pass across the cell membrane and then accumulate, thereby causing loss of viability and fungal cell destruction. Furthermore, there are synergistic interactions between organic acids and other antifungal compounds. These interactions will affect on antifungal potential of CCF compounds in different pH. From this point of view, it would be expected different antifungal activity for different metabolites and so on; different mode of action would be involved in the antifungal effect. In fact, the precise mechanism of antifungals can often not be defined because of a complex interaction between the different compounds produced during cell growth and the frequently synergistic effects among them (Dalie et al., 2010; Magnusson et al., 2003).

Fungi included in this study, *A. niger* and *A. flavus*, are important spoilage organisms in cereal products such as bread and also can produce mycotoxins in cereal grains. Antagonistic activity of *P. pentosaceus* isolate against these indicator fungi indicates that it is possible to use of this isolate as antifungal starter in processing of fermented cereal products such as sourdough breads (Gerez et al., 2009). Results showed that *P. pentosaceus* isolate (Fig. 1), strongly prevents from mycelial growth of indicator fungi. Some LABs are able to inhibit the conidial germination, while others inhibit the mycelial growth. By considering the fact that, the conidia germination is the most sensitive growth stage to inhibition, comparison of the potency of LAB strains as a novel biocontrol strategy of germination with their activity in a mycelial growth assay can provide preliminary information on their mode of action (Li et al., 2014).

The antagonistic activity of *P. pentosaceus* against *A. niger* was higher than *A. flavus* evinced that the antifungal ability of LAB isolate was dependent on the fungus species. This might be due to the differences in sensitivity level to organic acids or/and other antifungal compounds. Our finding showed also that *P. pentosaceus* isolate CCF, especially CCF-S (Fig. 2), may contain potent anti-mould preservative compounds with potential to use in different area. The research field of antifungal LAB is still novel and most publications in this area have merely illustrated their inhibitory effects, but the reasons for the inhibitory activity have been seldom identified. By considering synergistic interactions between organic acids and other antifungal compounds, the inhibitory effects will continue to complicate studies on antifungal properties of LAB, unless rigorous further purification and characterization of substances is applied. So, further analyzing of the compounds with antifungal activity and their mode of action, and also finding proper applications, could have a perfect potential for application of *P. pentosaceus* isolate in order to control of the spoilage fungi in fermented cereal products.

Conclusion

The current study showed that dominant LAB isolated from whole barley sourdough and its CCF can exhibit antifungal activity against *A. flavus* and *A. niger*. For using mentioned antifungal LAB as a novel biopreservative instead of chemical preservatives, it is so necessary to design more additional studies on purification, characterization as well as contribution of the bioactive molecules having considerable antifungal activity.

Conflicts of interest

All authors of this article declare that they have no conflict of interest in this research.

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References

AACC International. (2010). Approved Methods of Analysis. American association of cereal chemists.11th edition. AACC International, St. Paul, MN, U.S.A.

Altschul S.F., Madden T.L., Schaffer A.A., Zhang J., Zhang Z., Miller W., Lipman D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*. 25: 3389-3402.

Arendt E.K., Ryan L.A.M., Dal Bello F. (2007). Impact of sourdough on the texture of bread. *Food Microbiology*. 24: 165-174.

Cabo M.L., Braber A.F., Koenraad P.M.F.J. (2002). Apparent antifungal activity of several lactic acid bacteria against *Penicillium discolor* is due to acetic acid in the medium. *Journal of Food Protection*. 65: 1309-1316.

Chavan R.S., Chavan S.R. (2011). Sourdough technology, a traditional way for wholesome foods: a review. *Comprehensive Reviews in Food Science and Food Safety*. 10: 169-182.

Clarke C.I., Arendt E.K. (2005). A review of the application of sourdough technology to wheat breads. *Advances in Food and Nutrition Research*. 49: 137-161.

Corsetti A., Settanni L., Van Sinderen D. (2004). Characterization of bacteriocin-like inhibitory substances (BLIS) from sourdough lactic acid bacteria and evaluation of their *in vitro* and *in situ* activity. *Journal of Applied Microbiology*. 96: 521-534.

Dal Bello F., Clarke C.I., Ryan L.A.M., Ulmer H., Schober T.J., Strom K., Sjogren J., van Sinderen D., Schnurer J., Arendt E.K. (2007). Improvement of the quality and shelf life of wheat bread by fermentation with the antifungal strain *Lactobacillus plantarum* FST 1.7. *Journal of Cereal Science*. 45: 309-318.

Dalie D.K.D., Deschamps A.M., Richard-Forget F. (2010). Lactic acid bacteria; potential for control of mould growth and mycotoxins: a review. *Food Control*. 21: 370-380.

De Vuyst L., Vancanneyt M. (2007). Biodiversity and identification of sourdough lactic acid bacteria. *Food Microbiology*. 24: 120-127.

Fazeli M.R., Shahverdi A.R., Sedaghat B., Jamalifar H., Samadi N. (2004). Sourdough-isolated *Lactobacillus fermentum* as a potent anti-mould preservative of traditional Iranian bread. *European Food Research and Technology*. 218: 554-556.

Ferchichi M., Valcheva R., Prevost H., Onno B., Dousset X. (2007). Molecular identification of the microbiota of French sourdough using temporal temperature gradient gel electrophoresis. *Food Microbiology*. 24: 678-686.

Gerez C.L., Torino M.I., Rollan G., Font de Valdez G. (2009). Prevention of bread mould spoilage by using lactic acid bacteria with antifungal properties. *Food Control*. 20: 144-148.

Gobbetti M., Rizzello C.G., Di Cagno R., De Angelis M. (2014). How the sourdough may affect the functional features of leavened baked goods. *Food Microbiology*. 37: 30-40.

Golshan Tafti A., Peighambardoust S.H., Hejazi M.A., Moosavy M.H. (2014). Diversity of *Lactobacillus* strains in Iranian traditional wheat sourdough. *Journal of Food Quality and Hazards Control*. 1: 41-45.

Gulahmadov S.G., Abdullaeva N.F., Guseinova N.F., Kuliev A.A., Ivanova I.V., Dalgalarondo M., Chobert J.M., Haertlee T. (2009). Isolation and characterization of bacteriocin-like inhibitory substances from lactic acid bacteria isolated from Azerbaijan cheeses. *Applied Biochemistry and Microbiology*. 45: 266-271.

Heuer H., Krsek M., Baker P., Smalla K., Wellington E.M. (1997). Analysis of actinomycete communities by specific amplification of genes encoding *16S rRNA* and gel-electrophoretic separation in denaturing gradients. *Applied and Environmental Microbiology*. 63: 3233-3241.

Katina K., Arendt E., Liukkonen K.H., Autio K., Flander L., Poutanen K. (2005). Potential of sourdough for healthier cereal products. *Trends in Food Science and Technology*. 16: 104-112.

Katina K., Sauri M., Alakomi H.L., Mattila-Sandholm T. (2002). Potential of lactic acid bacteria to inhibit rope spoilage in wheat sourdough bread. *LWT- Food Science and Technology*. 35: 38-45.

Lavermicocca P., Valerio F., Visconti A. (2003). Antifungal activity of phenyllactic acid against moulds isolated from bakery products. *Applied and Environmental Microbiology*. 69: 634-640.

Leroy F., De Vuyst L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science and Technology*. 15: 67-78.

Li H., Zhang S., Lu J., Liu L., Uluko H., Pang X., Sun Y., Xue H., Zhao L., Kong F., Lv J. (2014). Antifungal activities and effect of *Lactobacillus casei* AST18 on the mycelia morphology and ultrastructure of *Penicillium chrysogenum*. *Food Control*. 43: 57-64.

Magnusson J., Schnurer J. (2001). *Lactobacillus coryniformis* sub sp. coryniformis strain Si3 produces a broad-spectrum proteinaceous antifungal compound. *Applied and Environmental Microbiology*. 67: 1-5.

Magnusson J., Strom K., Roos S., Sjogren J., Schnurer J. (2003). Broad and complex antifungal activity among environmental isolates of lactic acid bacteria. *FEMS Microbiology Letters*. 219: 129-135.

Mariotti M., Garofalo C., Aquilanti L., Osimani A., Fongaro L., Tavoletti S., Hager A.S., Clementi F. (2014). Barley flour exploitation in sourdough bread-making: a technological, nutritional and sensory evaluation. *LWT- Food Science and Technology*. 59: 973-980.

Messens W., De Vuyst L. (2002). Inhibitory substances produced by *Lactobacilli* isolated from sourdoughs: a review. *International Journal of Food Microbiology*. 72: 31-43.

Paterson A., Piggott J.R. (2006). Flavour in sourdough breads: a review. *Trends in Food Science and Technology*. 17: 557-566.

Poutanen K., Flander L., Katina K. (2009). Sourdough and cereal fermentation in a nutritional perspective. *Food Microbiology*. 26: 693-699.

Rieder A., Holtekjolen A.K., Sahlstrom S., Moldestad A. (2012). Effect of barley and oat flour types and sourdoughs on dough rheology and bread quality of composite wheat bread. *Journal of Cereal Science*. 55: 44-52.

Rouse S., Harnett D., Vaughan A., Sinderen D.V. (2008). Lactic acid bacteria with potential to eliminate fungal spoilage in foods. *Journal of Applied Microbiology*. 104: 915-923.

Sadeghi A. (2008). The secrets of sourdough; a review of miraculous potentials of sourdough in bread shelf life. *Biotechnology*. 7: 413-417.

Schnurer J., Magnusson J. (2005). Antifungal lactic acid bacteria as biopreservatives. *Trends in Food Science and Technology*. 16: 70-78.

Simsek O., Con A.H., Tulumoglu S. (2006). Isolating lactic starter cultures with antimicrobial activity for sourdough processes. *Food Control*. 17: 263-270.

Wang H., Yan Y., Wang J., Zhang H., Qi W. (2012). Production and characterization of antifungal compounds produced by *Lactobacillus plantarum* IMAU10014. *Plos One*. 7: 1-7.

Yang E., Fan L., Jiang Y., Doucette C., Fillmore S. (2012). Antimicrobial activity of bacteriocin-producing lactic acid bacteria isolated from cheeses and yogurts. *AMB Express*. 2: 1-12.

Zannini E., Garofalo C., Aquilanti L., Santarelli S., Silvestri G., Clementi F. (2009). Microbiological and technological characterization of sourdoughs destined for bread-making with barley flour. *Food Microbiology*. 26: 744-753.