Ann Microbiol (2014) 64:1333–1346
DOI 10.1007/s13213-013-0777-8

ORIGINAL ARTICLE

In vitro antifungal, probiotic and antioxidant properties of novel *Lactobacillus plantarum* K46 isolated from fermented sesame leaf

Mariadhas Valan Arasu · Da Hye Kim · Pyoung Il Kim · Min Woong Jung · Soundarrajan Ilavenil · Mariamichael Jane · Kyung Dong Lee · Naif Abdullah Al-Dhabi · Ki Choon Choi

Received: 28 July 2013 / Accepted: 18 November 2013 / Published online: 5 December 2013
© Springer-Verlag Berlin Heidelberg and the University of Milan 2013

Abstract This study aimed to describe the diversity of antifungal lactic acid bacteria (LAB) in popular traditional Korean fermented food. A total of 22 LAB strains was selected and subjected to a monophasic identical approach using 16S rRNA gene sequence analysis. Antifungal LAB associated with fermented food was identified as *Lactobacillus plantarum* (9), *Lactobacillus graminis* (5), *Lactobacillus pentosus* (4), *Lactobacillus sakei* (2), *Lactobacillus paraplantarum* (1), and *Leuconostoc mesenteroides* subsp. *mesenteroides* (1). Novel *Lactobacillus plantarum* strain K46 exhibited comparatively better antifungal activity against several spoilage fungi, and was deposited in the Korean Collection for Type Cultures (KACC91758P). Antifungal substances from the spent medium in which K46 was cultivated were extracted with ethyl acetate. Antifungal activity was assessed using the broth micro dilution technique. Compounds were characterized based on infrared, ^{13}C nuclear magnetic resonance (NMR), and ^{1}H NMR spectral data. The minimum inhibitory concentration (MIC) of the compounds against *Aspergillus clavatus*, *Aspergillus oryzae*, *Penicillium chrysogenum* and *Penicillium roqueforti* was 2.5 mg/mL and that against *Aspergillus fumigatus*, *Aspergillus niger*, *Curvularia lunata* and *Gibberella moniliformis* was 5.0 mg/mL. K46 was able to survive gastrointestinal conditions simulating the stomach and the duodenum passage with the highest percentage of hydrophobicity. In addition, its resistance to hydrogen peroxide and highest hydroxyl radical and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activities, with inhibition rates of 43.53 % and 56.88 %, respectively, were to its advantage. An antimicrobial susceptibility pattern was an intrinsic feature of this strain, thus consumption does not represent a health risk to humans. The results showed the potential of K46 strain as an antifungal, probiotic and antioxidant culture, and hence it was determined to be suitable for application in functional foods.

Keywords *Lactobacillus plantarum* · Antifungal compound · Probiotic properties · Antioxidant activities

M. V. Arasu · M. W. Jung · S. Ilavenil · M. Jane · K. C. Choi (✉)
Grassland and Forage Division, National Institute of Animal Science, RDA, Seonghwan-Eup, Cheonan-Si, Chungnam 330-801, Republic of Korea
e-mail: choiwh@korea.kr

D. H. Kim
The United Graduate School of Agricultural Sciences, Tottori University, Tottori-Shi 680-8553, Japan

D. H. Kim
Faculty of Life and Environmental Science, Shimane University, Matsue-Shi 690-8504, Japan

P. I. Kim
Jeonnam Bio Control Center, Im-myeon, Gokseong-gun, Jollanam-do 516-944, Republic of Korea

K. D. Lee
Department of Oriental Medicine Materials, Dongsin University, Naju 520-714, Republic of Korea

N. A. Al-Dhabi
Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

Introduction

Korean traditional fermented foods such as fermented cabbage, fermented radish, fermented sesame leaf and kimchi are gaining popularity as a functional foods. These lactic acid

fermented vegetable products are also considered to be a good source of potentially beneficial lactic acid bacteria (LAB). Food spoilage fungi present in these fermented foods are the main factors responsible for the production of gas, alcohol and undesirable aromatic compounds leading to flavor and economic losses (Torkar and Vengust 2008). Certain molds present in these fermented foods also synthesize a wide panel of mycotoxins that are harmful to consumer health (Pitt 2000). Filtenborg et al. (1996) reported that a very limited number of fungal species have been associated with the spoilage of food, mainly *Penicillium*, *Aspergillus*, and *Fusarium*. To avoid fungal spoilage, numerous chemical preservatives are used (Smith and Hong-Shum 2003). However, the increasing resistance of fungi toward chemical preservatives, as well as consumer demand for healthy and natural products and legislative requirements, have led the industry to search for new means of preservation through the promotion of bioprotective cultures, such as LAB (Brul and Coote 1999).

LAB are a group of Gram-positive microorganisms that are involved mainly in the fermentation of dairy food products and food preservation. They are present naturally in yogurts, creams, fresh plants and fermented foods such as sourdoughs, as well as in meat and alcoholic beverages (Corsetti and Settanni 2007; Pfeiler and Klaenhammer 2007; Garofalo et al. 2008). Moreover, most of them belong to the qualified presumption of safety (QPS) and generally recognized as safe (GRAS) lists, ensuring their safe use in food (Rossetti et al. 2009). In particular, *L. plantarum* is a member of the facultative hetero-fermentative group of lactobacilli. Strains of *L. plantarum* are capable of surviving gastric transit (amylases in the oral cavity, low pH in the stomach, bile secretions and pancreatic juice in the duodenal section of the small intestine) in humans and other mammals (Georgieva et al. 2009). Furthermore, it has been observed that there are significant species- and strain-specific variability in the functional probiotic properties of *L. plantarum* (Strahinic et al. 2007). Therefore, the putative beneficial effect is strain-dependent. Thus, it is essential that potential probiotic strains are well characterized prior to their use, as not all LAB possess the ability to confer health benefits to the host. Therefore, it becomes necessary to screen and characterize numerous strains to obtain ideal probiotic strains. So far, little attention has been paid to the antioxidant activities of *L. plantarum* strains isolated from traditional Korean fermented foods. Therefore, the objective of this work was to isolate antifungal LAB strains from fermented foods such as fermented cabbage, radish, sesame leaf and kimchi. This study also involved in analysis of the 16S rRNA gene based phylogenetic affiliation, extraction and identification of antifungal metabolites, and a bio prospective study against various food spoilage fungi. The probiotic and anti-oxidant characteristics were also tested in vitro.

Materials and methods

Isolation of *Lactobacillus* strains

For isolation of the LAB strains, fermented cabbage, fermented radish, fermented sesame leaf and commercial kimchi were purchased from a market (Home plus) in South Korea. The samples were mixed with sterile distilled water and centrifuged at 8,000 rpm for 10 min to remove heavy particulates. After appropriate dilution, the supernatant was spread on MRS agar for plating. The plates were incubated at 30 °C for 48 h, and a few isolates with a morphology similar to that of *Lactobacillus* were selected. After purification, the selected strains were numbered K1–K54, and stored at −80 °C in MRS broth with 20 % glycerol. The stock cultures were propagated twice in MRS broth for 18 h before each experiment.

Fungal strains and preparation of spore suspensions

Ubiquitous fungi commonly encountered in food spoilage were chosen as indicator microorganisms for the antifungal assays. *Aspergillus clavatus* (KCTC 40071), *Aspergillus fumigatus* (KCTC 40080), *Aspergillus niger* (KCTC 40280), *Curvularia lunata* (KCTC 40392), *Fusarium culmorum* (KCTC 42099), *Fusarium oxysporum* (KCTC 40051), *Gibberella moniliformis* (KCTC 44022), *Penicillium chrysogenum* (KCTC 40399) and *Penicillium roqueforti* were obtained from the culture collection at the Rural Development Administration (RDA) Suwon, Republic of Korea. They were cultivated on Sabrous dextrose agar (SDA, Sigma, St. Louis, MO) slants for 96 h at 30 °C until spores were formed. Spores were then harvested with sterile saline water and the suspension was spread on SDA in a Roux flask to increase spore production. A final concentration of 10^7 spores/mL suspension was stored at −20 °C in glycerol (10 %, v/v) for further experiments.

Antifungal activity

The inhibition spectrum of test organisms was determined by using the overlay method as well as the diffusion method, as described by Strom et al. (2002), on MRS agar plates, using *G. moniliformis* and *A. fumigatus* as an indicator with slight modification. Briefly, the LAB was grown in the form of 2 cm streaks on MRS agar plates, and incubated under micro aerobic conditions at 30 °C for 48 h. The plates were overlaid with semi-solid malt extract agar (1.0 %), seeded with 10^4 spores/mL *G. moniliformis* and *A. fumigatus*, and incubated aerobically at 30 °C for 24–72 h. Clear zones of inhibition were recorded and scored.

Identification of LAB cultures

Twenty two antifungal LAB strains were selected for identification using 16S rRNA gene sequence analysis. Briefly, the 16S rRNA gene was amplified from the genomic DNA of the 22 antifungal LAB strains by PCR with *Taq* DNA polymerase, using the following primers: 27 forward primer (FP) (5′ AGA GTT TGA TCG TGG CTC AG 3′) and 1492 reverse primer (RP) (3′ GGT TAC CTT GTT ACG ACT T 5′) (Arasu et al. 2013). Each PCR mixture in a final volume of 20 μL contained 50 ng genomic DNA (2 μL), 10X *Taq* buffer (2 μL), 200 μM dNTP (1 μL), 10 pmol FP primer (1 μL), 10 pmol RP primer (1 μL), 1U *Taq* DNA polymerase (0.5 μL), and water (12.5 μL). The conditions for thermal cycling were as follows: initial denaturation of the target DNA at 95 °C for 10 min followed by 30 cycles of amplification, denaturation at 95 °C for 2 min, primer annealing at 58 °C for 1 min and primer extension at 72 °C for 2 min. At the end of the cycle, the reaction mixture was held at 72 °C for 10 min and cooled to 4 °C. Amplified DNA was visualized at 100 V and 400 mA for 25 min on agarose gel [1 % (w/v) in TAE buffer 1x, 0.1 μL ethidium bromide solution (stock; 10 mg/mL)]. The amplified PCR products were purified by a QIAquick® PCR purification Kit (Qiagen, Crawley, UK). A length of 1,500 base pairs were sequenced by Solgent (Seoul, Korea). The obtained sequences were subjected to a BLAST search at http://www.ncbinlm-nih.gov/ in the NCBI database. The evolutionary history was inferred using the neighbor-joining (NJ) method. Evolutionary analyses were conducted in MEGA5.

Identification of the K46 isolate

Biochemical tests

To prepare the inoculum for the identification experiments, one glycerol stock vial was used to inoculate a flask containing 50 mL MRS medium. The activation culture was grown at 30 °C for 24 h. K46 isolate was propagated twice before being tested in experiments. API 50 CHB (BioMérieux, Marcy l'Etoile, France) test kits were used to characterize phenotypes. The API test strips were prepared according to the manufacturer's instructions, and scored after 48 h of incubation at 30 °C.

Scanning electron microscopy

Isolate K46 was grown exponentially in MRS medium under aerobic conditions, centrifuged at 8,000 rpm for 10 min, and the pellet was then washed twice with 0.1 M phosphate buffer saline (PBS), pH 7.2. The cells were then re-suspended in a primary fixative solution containing glutaraldehyde (2.5 %) and PBS (0.1 M). After dehydration through a graded ethanol series (50, 60, 70, 80, 90, and 95 %), the fixed cells were dried twice with hexamethyldisilazane. Finally, the dehydrated cells were sputter-coated with gold at 25 mA for 250 s, and observed by scanning electron microscopy (SEM; JSM-6460 LV; JEOL, Tokyo, Japan) at 12 kV.

Determination of antibiotic sensitivity and resistance pattern

The antibiotic sensitivity and resistance pattern of isolate K46 were assayed by a disc diffusion method as described by Arasu et al. (2013). Briefly, cells were prepared by growing in MRS medium for 24 h at 30 °C. The test cultures (100 μL) of suspension containing 10^8 CFU/mL bacteria) were swabbed on the top of the solidified medium and allowed to dry for 10 min. Discs loaded with different antibiotics were placed on the surface of the medium and left for 30 min at room temperature to allow diffusion of the antibiotics. After incubation, the organisms were classified as sensitive or resistant to antibiotics, according to the diameter of the inhibition zone as determined by a standard antibiotic disc chart.

Mass cultivation and extraction of antifungal metabolites

Isolate K46 was cultivated at 30 °C for 3 days in a sterile Erlenmeyer flask containing 3.5 L MRS medium with 5 % glucose. At the end of the fermentation cycle the culture broth was filtered and the supernatant was separated by centrifuging at 8,000 rpm for 15 min. A total of 10 L culture broth was collected and the supernatant was extracted with ethyl acetate (1:3 ratio). The solvent phase was concentrated by using vacuum at 40 °C each to obtain the crude extract for antifungal bioassay. The ethyl acetate extract was purified by silica gel column and the various fractions were collected. The purity of the compounds was checked in HPLC by eluting through a 300×7.8 mm Aminex HPX-87H (Bio-Rad; Hercules, CA) column at 60 °C using 5.0 mM H_2SO_4. The HPLC analysis was carried out at a flow rate of 0.5 mL/min at a column wavelength of 220 nm. The compound was further identified by spectroscopic analysis by ^{1}H nuclear magnetic resonance (NMR) (300 MHz). The ^{13}C NMR spectrum was measured on an AL-300 JEOL instrument (75.45 MHz), and the electrospray ionization mass spectra (MS) were recorded. The MS operating conditions were as follows: ion spray voltage, 5.5 kV; curtain gas, 20 psi; nebulizing gas, 50 psi; heating gas, 50 psi; high purity nitrogen (N_2); heating gas temperature, 550 °C; declustering potential (100 V); entrance potential (10 V) and spectra scanning range, m/z 100–800 (scan time 4.8 s). The infrared spectrum was also recorded using the KBr pellet method.

Antifungal activity

The antifungal activity and minimum inhibitory concentration (MIC) was determined according to the standard reference

method (NCCLS 1999). The compounds were dissolved in water, together with 2 % dimethyl sulfoxide (DMSO). The initial test concentration was serially diluted two-fold. Each well was inoculated with 5 μL suspension containing 10^4 spore/mL fungi. The antifungal agent ketoconazole was included in the assays as a positive control. Plates were incubated for 24, 48 or 72 h at 30 °C. MIC was determined as the lowest concentration of the extracts inhibiting the visual growth of test cultures. Three replications were maintained in order to confirm the antifungal activity.

Probiotic properties of K46 isolate

Tolerance to low pH

Tolerance to low pH was determined using the plate count method. Briefly, active K46 isolate was grown in MRS broth and was inoculated (1 %) in 10 mL fresh MRS broth adjusted to pH 2.5 with HCl (1.0 *N*) and incubated at 30 °C for 3 h. Samples were determined for their initial bacterial population and residual cell population at 0 and 3 h, respectively, by plating suitable dilutions on MRS agar plates. The plates were incubated at 30 °C for 48 h, and the number of colonies that grew was counted. The experiment was performed in triplicate.

Bile tolerance

The ability of K46 isolate to grow in the presence of two different bile salts was studied according to the method of Vinderola and Reinheimer (2003) with slight modification. MRS-thiobroth (MRS supplemented with 0.2 % sodium thioglycollate) and MRS-thiobroth supplemented with 0.3 % (w/v) Oxgall were freshly prepared and inoculated overnight with 1 % suspensions of K46 isolate. Samples without Oxgall were used as a control. After 24 h incubation at 30 °C, the bacterial concentration was checked by a viable count determination on MRS agar by plating suitable dilutions. The experiment was performed in triplicate.

Biogenic amine production

The production of biogenic amines was assessed by the method described by Bover-Cid and Holzapfel (1999). Briefly, a 0.5 optical density at 600 nm (OD_{600}) aliquot of freshly prepared K46 isolate cells was inoculated on MRS agar medium supplemented with 1.0 % each of lysine, tyrosine, ornithine, and histidine. Tween 80 (0.1 %) was included in the medium to enhance bacterial growth. Bromocresol purple (0.006 %) was used as the pH indicator. The formation of a clear purple halo was considered a positive reaction, indicating the presence of the amino acid decarboxylase. Medium without supplemented amino acids was used as a negative control, and the experiment was performed in triplicate.

Screening of enzymatic activities

Screening for the production of different enzymes, such as alkaline phosphatase, esterase, esterase lipase, lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, caphthol-AS-Biphophohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-gannosidase and α-glucosidase, in K46 isolate was performed using the kit method according to the manufacturer's instructions (BioMerieux). Freshly prepared cells at 0.5 OD_{600} were centrifuged at 8,000 rpm for 10 min, and the pellets were suspended in sterile distilled water for screening. Enzyme activities of each LAB strain were evaluated by transferring 50 μL active cell suspension into each well, followed by 4 h incubation at 30 °C. After the incubation, 20 μL ZYMA and ZYM-B reagent was added to each well and incubated at 30 °C for 5 min to measure enzyme activity.

Hemolytic activity

For evaluation of hemolytic activity, ingredients including heart infusion, peptone, sodium chloride and agar were mixed with water and sterilized in an autoclave. After sterilization, 5 % (w/v) blood at about 40–50 °C was added to the medium, which was then poured into sterile Petri plates. After solidification, freshly prepared K46 isolate cells were streaked on agar plates, and incubated for 48 h at 30 °C (Maragkoudakis et al. 2006).

Proteolytic activity

Proteolytic activity was measured by growing the K46 isolate in 10 % skim milk at 30 °C for 42 h. The absorbance was read at 650 nm with an ELISA reader (Bio-Rad, Hercules, CA) (Citi et al. 1963). The results were expressed as milligrams/milliliter tyrosine by means of reference to a calibration curve.

Evaluation of cell surface hydrophobicity

The cell surface hydrophobicity assay was conducted according to the method described by Lee et al. (2011), with slight modifications. Briefly, freshly prepared cells were centrifuged at 8,000 rpm for 10 min. The cells were washed twice with PBS (pH 7.0). One milliliter of this suspension was used to determine the absorbance at OD_{580}. In duplicate assessments, 1 mL suspension was added to an equal volume of n-hexadecane (Sigma) and thoroughly mixed for 2 min using a vortex. The phases were allowed to separate at room temperature for 30 min, after which 1 mL of the upper phase was removed and the absorbance was determined at OD_{580}. Percentage hydrophobicity was calculated as follows:

Hydrophobicity (%) = (OD_{580} reading 1 − OD_{580} reading 2 / OD_{580} reading 1) 100.

In vitro antioxidant activity of K46 isolate

Resistance to hydrogen peroxide

The method of Buchmeier et al. (1997) was used with some modifications. Briefly, 0.3 OD_{600} of K46 isolate was grown in 500 mL Erlenmeyer flasks containing 100 mL MRS broth supplemented with 0.2, 0.4, 0.6, 0.8 and 1.0 mM hydrogen peroxide, at 30 °C on an orbital incubator shaker. Cell growth was measured spectrophotometrically at 600 nm, and increase in cell growth was measured as increase in optical density (OD).

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging assay was conducted by a Fenton reaction method (He et al. 2004). Briefly, the reaction mixture containing 1.0 mL brilliant green (0.435 mM), 2.0 mL $FeSO_4$ (0.5 mM), 1.5 mL H_2O_2 (3.0 %, w/v), and either 1.0, 1.5, 2.0 or 2.5 mL freshly prepared K46 isolate cells (10^9 CFU/mL) was incubated at room temperature for 15 min, and the absorbance was then measured at 624 nm. The change in absorbance of the reaction mixture indicated the scavenging ability of the K46 isolate for hydroxyl radicals.

$$\text{Scavenging activity}(\%) = [(A_s - A_0)/(A - A_0)] \times 100,$$

Where A_s is the absorbance in the presence of the sample, A_0 is the absorbance of the control in the absence of the sample, and A is the absorbance without the sample and Fenton reaction system.

2,2-Diphenyl-1-picrylhydrazyl free radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging capacity was determined according to the method described by Li et al. (2012) with some modifications. Briefly, 1.0, 1.5, 2.0 and 2.5 mL freshly prepared K46 isolate cells (10^9 CFU/mL), was added to 1.0 mL methanolic DPPH radical solutions (0.05 mM). The mixture was mixed vigorously and incubated at room temperature in the dark for 30 min. The controls included only deionized water and DPPH solution. The blanks contained only methanol and the cells. The absorbance of the resulting solution was measured in triplicate at 517 nm, after centrifugation at 12,000 rpm for 10 min.

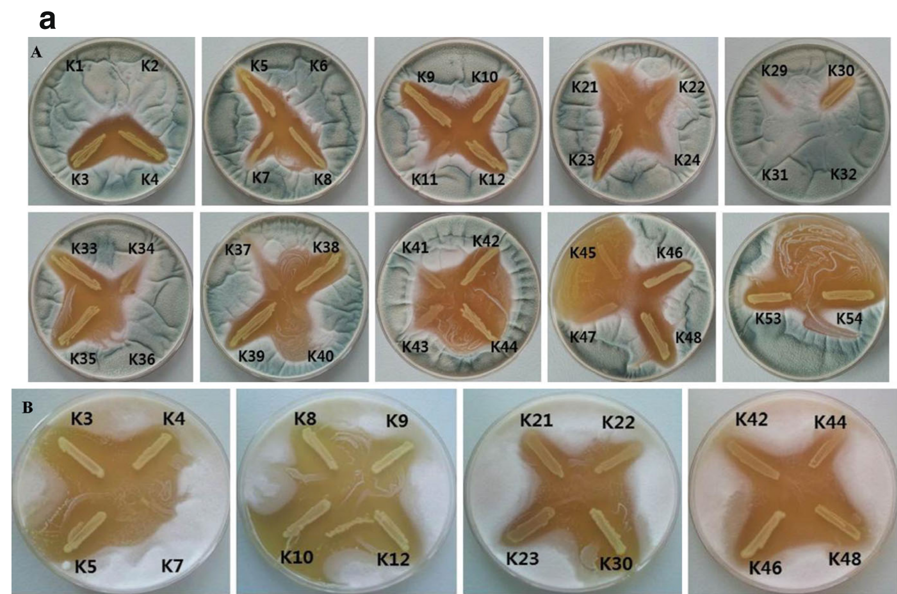
$$\text{Scavenging activity}(\%) = \left[1 - \left(A_{\text{sample}} - A_{\text{blank}}\right)/A_{\text{control}}\right] \times 100$$

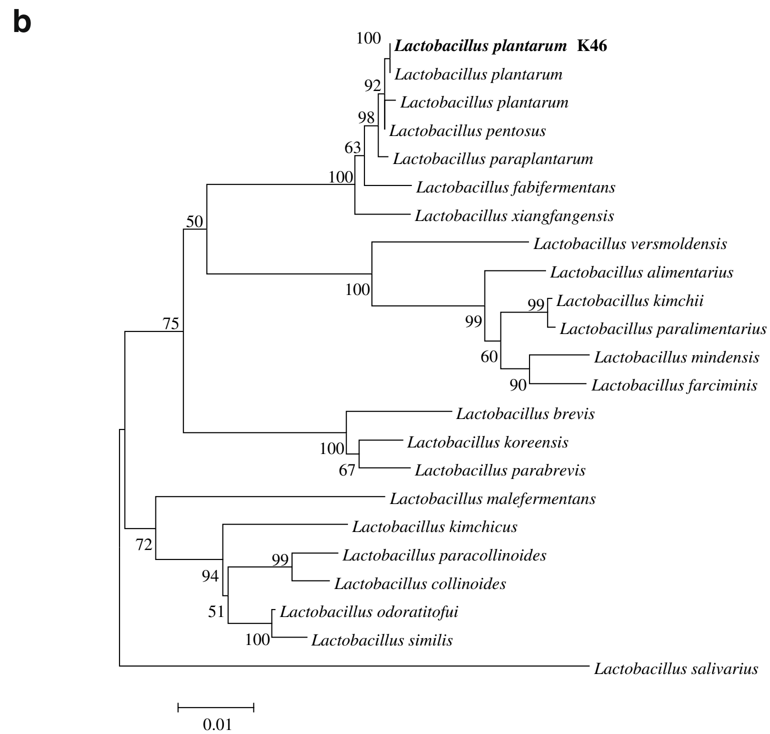
Results

Isolation, antifungal activity and identification of LAB cultures

The present investigation involved the isolation and characterization of antifungal LAB strains from fermented foods obtained from commercial markets of the Republic of Korea. Fifty four presumptive LAB strains were isolated and purified based on their capability to grow on MRS agar medium and BCP medium. All the strains were screened for their antifungal activity against various food spoilage fungi. Good antifungal activity was recorded against *A. fumigatus*. Among the 54 LAB strains, 33 strains exhibited activity against *A. fumigatus*, whereas 16 LAB strains revealed activity against *G. moniliformis* (Fig. 1a). From the primary screening, 22 LAB strains were selected for further identification. The 16S rRNA gene of 22 LAB strains was completely sequenced and analyzed for similarities. The NCBI BLAST search program showed that all the sequence data had high identity (98 %) to *Lactobacillus* species. Based on the results of the BLAST search program, strains K3, K4, K7, K9, K10, K23, K33, K46 and K54 were identified as *Lactobacillus plantarum*, and strains K8, K39, K48 and K53 showed close similarity to *Lactobacillus pentosus* (Table 1). Strain K5 was identified as *Leuconostoc mesenteroides* (100 %) and strains K21, K22, K30, K35 and K42 shared close similarity with *Lactobacillus graminis*. The sequence data of strain K46 had high identity (100 %) to *L. plantarum*, with a bits score and E value of 2,617 and 0, respectively. A phylogenic tree was constructed using the NJ method (Fig. 1b). The percentage of replicate trees showed that the associated taxa clustered together in the bootstrap test (1,000 replicates). The tree was drawn to scale with branch lengths in the same units as those of evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method and were based on units of the number of base substitutions per site.

Primary screening indicated that the 22 LAB strains were the potential candidates for broad range antifungal metabolites. *L. plantarum* K46 exhibited better activity against the tested fungi, so this strain was identified by biochemical and physiological characteristics. The 16S rRNA gene sequence of *L. plantarum* K46 was deposited in the NCBI nucleotide sequence database under the accession number KC430920. A *L. plantarum* K46 pure culture was deposited in the Korean Collection for Type Cultures, Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology, Republic of South Korea and was assigned the accession number KACC91758P. It was further cultivated in large scale, and novel bioactive metabolites were extracted and characterized. The in vitro probiotic and antioxidant properties of *L. plantarum* K46 were also determined.





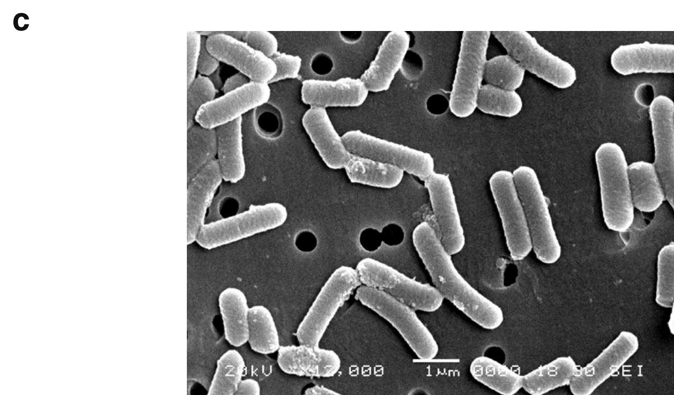


Fig. 1 **a** Antifungal activities of *Lactobacillus* strains against food spoilage fungus. *A* Against *A. fumigatus*, *B* against *G. moniliformis*. **b** Phylogenetic tree based on 16S rRNA gene sequence, showing the relationship between *Lactobacillus plantarum* K46 and species belonging to the genus *Lactobacillus*, constructed using the neighbor joining (NJ) method. Bootstrapping values >50 are not mentioned. **c** Scanning electron microscope (SEM) image of *Lactobacillus plantarum* K46

Biochemical characterization of *L. plantarum* K46

A bacterium designated as K46 was recovered from the fermented sesame leaf sample; it exhibited good antifungal activity against *A. fumigatus* and *G. moniliformis*. Biochemical tests revealed that K46 isolate was a Gram positive, mesophilic, catalase negative and rod rod-shaped (0.7–0.9 μm width and 2.0–3.2 μm length) bacterium; a SEM photograph is shown in Fig. 1c. These micro-morphological characters suggested strongly that strain K46 belonged to the genus *Lactobacillus*. The optimal pH and temperature for growth were 6.0–7.0 and 30–37 °C. *L. plantarum* K46 isolate displayed positive results for amylase, protease and gelatinase enzyme production test. It did not produce CO_2 from glucose, but produced gas from gluconate. Biochemical identifications presented in the API 50 CHB micro tests were determined. *L. plantarum* K46 cells could grow on glucose, lactose, rhamnose, sucrose, arabinose, fructose, galactose, and sorbose. However, they could not grow on inositol, xylose, trehalose, melibiose, glycerol or arabitol. Utilization of various carbon sources indicated a wide pattern of carbon source assimilation. m-Diaminopimelic acid was observed in cell wall hydrolysates. Biochemical and physiological studies indicated that the strain belongs to the *Lactobacillus* genus.

Antibiotic sensitivity pattern of *L. plantarum* K46

An antibiotic sensitivity test was conducted against the most commonly used antibiotics for bacterial infection by means of the disc diffusion method. *Lactobacillus* sp. K46 exhibited a similar sensitivity pattern towards all tested antibiotics (Table 2). The results indicated that the pathogenicity of *L. plantarum* K46 was low as the strain was sensitive to most of the tested antibiotics, and exhibited highest sensitivity towards amino glycoside inhibitor tobramycin, followed by fluroquinolone inhibitor levofloxacin and sparfloxacin respectively. Polyketides, macrolide and fluroquinolone antibiotics: tetracycline, erythromycin and nalidixic acid, did not inhibit growth of the strain. Tetracycline resistance indicated its typical LAB feature. The observed higher sensitivity to antibiotics makes this strain more promising for various applications.

Characterization, spectral analysis and antifungal activity

The crude ethyl acetate extract was re-extracted with methanol and ethyl acetate at different ratios: 0:100, 25:75, 50:50 and 75:25, to obtain different fractions. The fractions were concentrated and re-checked for their purity by TLC and bioautography. The fractions were washed thoroughly with 100 % methanol to remove impurities, and the active fraction

Table 1 Different antifungal *Lactobacillus* strains and their 16S rRNA gene sequence similarities

Strain No.	Source of isolation	Identity (%)[a]	Closest relative	Accession no.[b]
K3	Fermented cabbage	99.9	*L. plantarum* JCM 1558^T	ACGZ01000098
K4	Fermented cabbage	99.9	*L. plantarum* ATCC 14917^T	ACGZ01000098
K5	Fermented cabbage	100	*L. mesenteroides* ATCC 8293^T	CP000414
K7	Fermented radish	100	*L. plantarum* ATCC 14917^T	ACGZ01000098
K8	Fermented radish	99.9	*L. pentosus* JCM 1558^T	D79211
K9	Fermented sesame leaf	100	*L. plantarum* ATCC 14917^T	ACGZ01000098
K10	Fermented sesame leaf	99.9	*L. plantarum* ATCC 14917^T	ACGZ01000098
K12	Fermented cabbage	100	*L. paraplantarum* DSM 10667^T	AJ306297
K21	Fermented radish	98.5	*L. graminis* DSM 20719^T	AM113778
K22	Fermented radish	98.5	*L. graminis* DSM 20719^T	AM113778
K23	Fermented cabbage	100	*L. plantarum* ATCC 14917^T	ACGZ01000098
K30	Fermented cabbage	98.5	*L. graminis* DSM 20719^T	AM113778
K33	Fermented sesame leaf	100	*L. plantarum* ATCC 14917^T	ACGZ01000098
K35	Fermented sesame leaf	98.5	*L. graminis* DSM 20719^T	AM113778
K38	Fermented cabbage	100	*L. sakei* DSM 20017^T	AY204893
K39	Fermented cabbage	100	*L. pentosus* JCM 1558^T	D79211
K42	Fermented cabbage	98.5	*L. graminis* DSM 20719^T	AM113778
K44	Fermented radish	100	*L. sakei* DSM 20017^T	AY204893
K46	Fermented sesame leaf	100	*L. plantarum* ATCC 14917^T	ACGZ01000098
K48	Fermented sesame leaf	100	*L. pentosus* JCM 1558^T	D79211
K53	Kimchi	100	*L. pentosus* JCM 1558^T	D79211
K54	Kimchi	100	*L. plantarum* ATCC 14917^T	ACGZ01000098

[a] The percentage of identical nucleotides in the sequence obtained from the isolates and the sequence of the closest relative found in the GenBank database

[b] Accession numbers of the sequence of the closest relative strains found by BLAST

was subjected to HPLC to check its purity (Fig. 2a). The homogeneity of the antibiotic was further confirmed by preparative reversed phase HPLC, in which the antibiotic was eluted from the column as a single peak. IR, ^{1}H NMR and ^{13}C NMR spectral data of the identified compounds guided the identification of the chemical structure of the compounds, which shared close similarity to the known compound 3-phenyl lactic acid (Fig. 2b–d). The antifungal profile of the isolated compound is shown in Table 3. It exhibited moderate activity against *A. clavatus*, *A. oryzae*, *P. chrysogenum* and *P. roqueforti* (MIC: 2.5 mg/mL), whereas it showed less activity towards *A. fumigatus*,

Table 2 Comparative sensitivity pattern of *Lactobacillus plantarum* K46 towards various antibiotics

Antibiotic group	Antimicrobial agent	Disc potency (μg)	Diameter of inhibition zone (mm)[a]	Status[b]
Aminoglycoside	Amikacin	30	19	S
	Gentamicin	10	25	S
	Kanamycin	30	20	S
	Streptomycin	10	18	S
	Tobramycin	10	33	S
Carboxypenicillin	Carbenicillin	50	18	S
	Ampicillin	50	15	S
	Amoxyclav	10	13	S
	Augmentin	30	21	S
	Dicloxacillin	1	20	S
β-lactamase inhibitor	Imipenem	10	21	S
	Meticillin	5	29	S
	Penicillin	100 U	20	S
	Sulbactam	15	18	S
	Ticarcillin	75	25	S
	Ciprofloxacin	5	22	S
	Gatifloxacin	5	25	S
	Levofloxacin	5	32	S
	Moxifloxacin	5	19	S
Fluroquinolone	Nalidixic acid	30	0	R
	Norfloxacin	10	25	S
	Ofloxacin	5	29	S
	Sparfloxacin	5	30	S
	Cefpodoxime	10	31	S
	Ceftriaxone	30	21	S
	Ceftazidime	30	25	S
Cephalosporin	Cephalothin	30	25	S
	Cefpodoxim	30	19	S
	Cetriaxone	30	25	S
	Cephaloridine	30	22	S
Cephamycin antibiotic	Cefoxitin	30	11	S
Glycopeptide antibiotic	Vancomycin	30	0	R
Polymixin	Colistin	10	26	S
Polyketides	Tetracyclin	100	0	R
	Co-Trimoxazole	25	29	S
Sulphonamide	Sulphafurazole	300	28	S
Lincosamide antibiotic	Clindamycin	2	19	S
	Lincomycin	30	20	S
Macrolide antibiotic	Erythromycin	15	0	R
	Oleandomycin	15	25	S
Nitrofuran antibiotic	Nitrofurantoin	50	28	S

[a] Zones of inhibition were measured after incubating the strains for 48 h at 30 °C in MRS agar medium (MRSA)

[a] *S* Sensitive, *R* resistant

A. niger, *C. lunata* and *G. moniliformis* (MIC: 5.0 mg/mL). The compound showed comparatively higher MIC values (MIC: 10.0 mg/mL) against other fungal strains.

Probiotic properties of *L. plantarum* K46

The ability of microorganisms to survive in the gastrointestinal tract is one of the most important characteristics of effective probiotic bacteria. Therefore, the viability at low pH and bile salts conditions were studied. The viabilities of *L. plantarum* K46 at pH 3.0 and 4.0 are presented in Table 4. The results indicated that the cell viability was slightly decreased at pH 3.0, whereas at pH 4.0 the strain exhibited more stable growth. The survival ability of the strain in the presence of bile salts (Oxgall (0.3 %) and sodium taurocholate (0.3 %) is presented in Fig. 3. The results revealed that the strain was able to grow in the presence of sodium taurocholate and was slightly sensitive to oxgall. The strain showed negative results for hemolysis of agar, and was therefore determined to be non-virulent in nature. Proteolytic activity was determined as 0.073 mg/mL tyrosine liberation. A positive result for decarboxylase activity by tyrosine was observed, exhibiting high hydrophobicity (100 %). It showed weak-to-moderate levels of leucine arylamidase and β-glucosidase activity, but did not reveal a positive response to alkaline phosphatase, α-fucosidase or α-mannosidase activities (Table 5). Therefore, this strain is considered to be safe for use as a probiotic.

Antioxidant activity

Resistance to hydrogen peroxide

The effect of hydrogen peroxide on the viability of K46 is shown in Fig. 4a. *L. plantarum* K46 strain exhibited a moderate to strong resistance towards different concentrations of H_2O_2 when compared with the control. Results revealed that the strain was able to tolerate 0.6–0.8 mM H_2O_2, whereas at 1.0 mM H_2O_2 the growth was reduced with, optical densities less than 0.65 after incubation for 8 h.

Hydroxyl radical scavenging activity

The results for hydroxyl scavenging assay of the *L. plantarum* K46 are shown in Fig. 4b. Hydroxyl radical scavenging

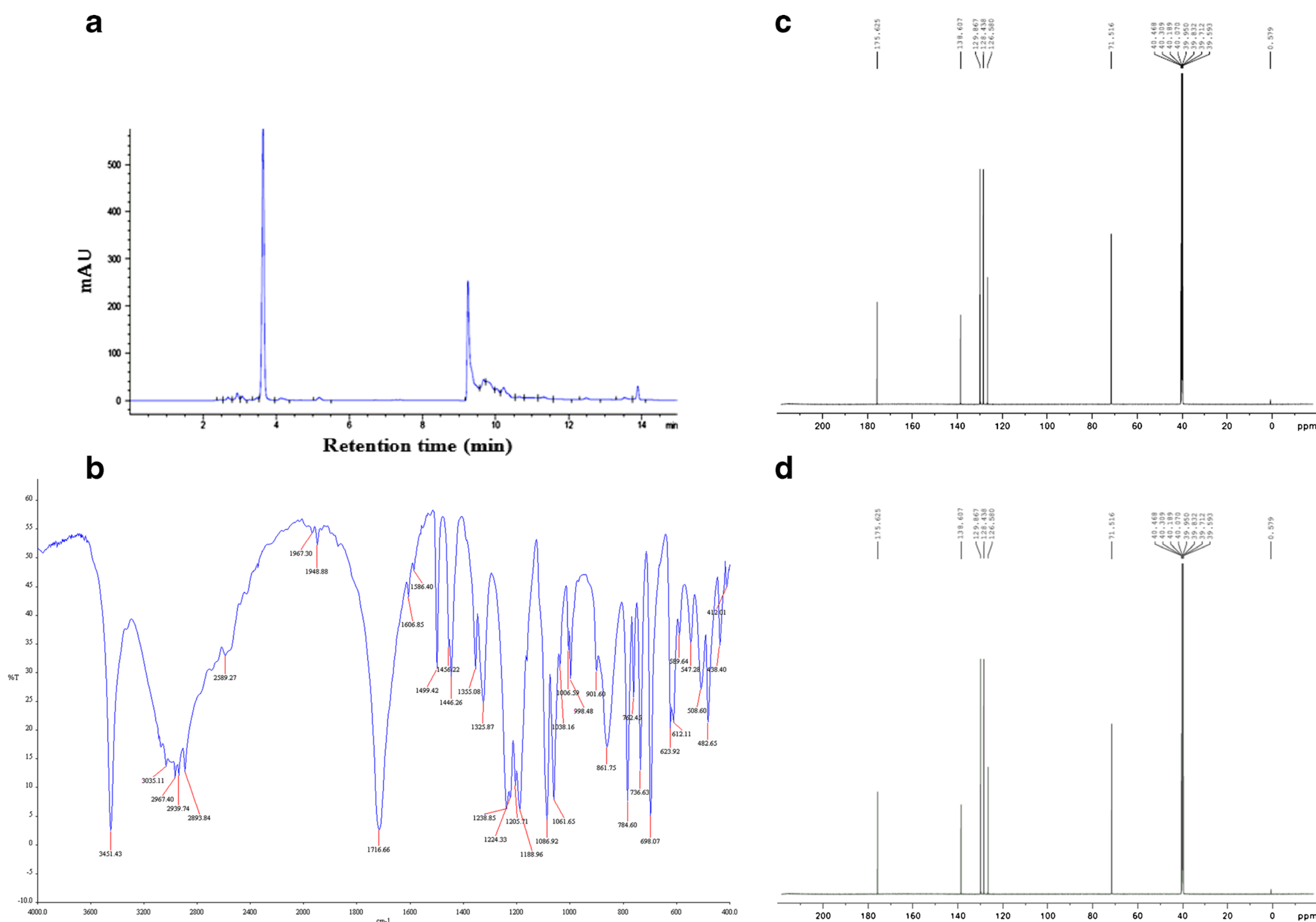


Fig. 2a–d Analysis of antifungal compound isolated from *L. plantarum* K46. **a** High performance liquid chromatography separation. **b** Fourier-transform infrared spectrum. **c** [1]H nuclear magnetic resonance spectrum. **d** [13]C nuclear magnetic resonance spectrum

Table 3 Antifungal activities of compound obtained from *L. plantarum* K46. *MIC* Minimum inhibitory concentration

Food pathogen	MIC	
	mg/mL	CS[a]
Aspergillus clavatus	2.50	50
Aspergillus fumigatus	5.00	50
Aspergillus niger	5.00	25
Aspergillus oryzae	2.50	25
Curvularia lunata	5.00	37.5
Fusarium culmorum	5.00	37.5
Fusarium oxysporum	7.50	25
Gibberella moniliformis	5.00	75
Penicillium chrysogenum	2.50	50
Penicillium roqueforti	2.50	25

[a] *C* Compound, *S* fungal control reference (Ketoconazole: μg/mL)

activity increased with increasing cell concentration. The result revealed that the strain had the highest hydroxyl radical scavenging ability, with an inhibition rate of 43.53 % at 2 mL cells at 10^9 CFU/mL.

DPPH free radical scavenging activity

The methanol extract of *L. plantarum* K46 exhibited a significant dose-dependent inhibition of DPPH activity, with the highest radical scavenging activity (59.88 %) at 2 mL cells at 10^9 CFU/mL. The results are presented in Fig. 4c.

Discussion

The present study was aimed primarily at evaluating the antifungal, probiotic and antioxidant properties of *L. plantarum* K46 recovered from fermented sesame leaf. The discovery of bioactive compounds is part of the never-ending process to meet the demand for novel biomolecules with antifungal properties to combat pathogens and food spoilages. There are many reports on the production of antimicrobial compounds by LAB (Lindgren and Dobrogosz 1990; Stiles 1996), but the number of reports on antifungal LAB is still low and the majority describe only the primary screening of LAB. Until now, very few studies have characterized antifungal compounds from LAB and their application in the field of food processing although in recent years these continue to attract the interest of researchers because of their wide application in the food industry (O'Sullivan et al. 2002; Schnürer and Magnusson 2005; Dal Bello et al. 2007; Zhang et al. 2010; Coda et al. 2011; Garofalo et al. 2012).

Fifty four LAB strains were isolated from fermented food and were screened for their antifungal properties. Preliminary results clearly indicated that *L. plantarum* K46 exhibited good activity against food spoilage fungi *G. moniliformis* and *A. fumigatus*, which often cause spoilage in bakery products (Legan 1993). The antifungal activity of *L. plantarum* strains has also been reported by other investigators (Gourama and Bullerman 1995; Lavermicocca et al. 2000). Karunaratne et al. (1990) reported that commercial *L. plantarum* strains had significant antifungal activity against *A. flavus* subsp. *parasiticus* in liquid culture. Antifungal activities depended on microbial community composition as well as environment and growth conditions (Schillinger and Villarreal 2010). Other environmental isolates with potent antifungal activity belonging to *L. plantarum* from fermented foods have been characterized (Magnusson et al. 2003). In this study, *L. plantarum* was identified as the dominant antifungal strain (41 %). During the isolation of antifungal metabolite producers, *Lactobacillus* sp. K46 was identified and showed strong antifungal activity against spoilage fungus, and was therefore further subjected to a series of in vitro analyses. It was found that the strain is Gram positive, with a smooth surface when grown on solid agar medium. It utilized most of the sugars that were provided, indicating a wide pattern of carbon assimilation. These results were in close agreement with the findings of Lee et al. (2011).

In many reports, the antifungal activity of LAB has often been evaluated only with the agar well diffusion method. Strom et al. (2002) reported four antifungal metabolites from LAB as active only in agar medium, but not in fermentation broth, whereas *L. plantarum* K46 showed good antifungal activity in solid medium and also in fermented spent broth, indicating that the production of antimicrobial compounds was active in both solid and liquid phase. The results confirmed that the antifungal metabolites were extracellular in

Table 4 Cell viability of *L. plantarum* K46 under low pH. Values are means of triplicate determinations ± standard deviations; (+) positive activity; (−) negative activity

pH	Viable count (log CFU/mL)					
	Tolerance level					
	Control	0 min	30 min	60 min	120 min	180 min
3.0	8.85±0.22	8.67±0.35	8.72±0.03	8.82±0.03	8.01±0.07	8.27±0.06
4.0	8.44±0.07	8.47±0.02	8.54±0.02	8.61±0.00	8.61±0.02	8.65±0.04

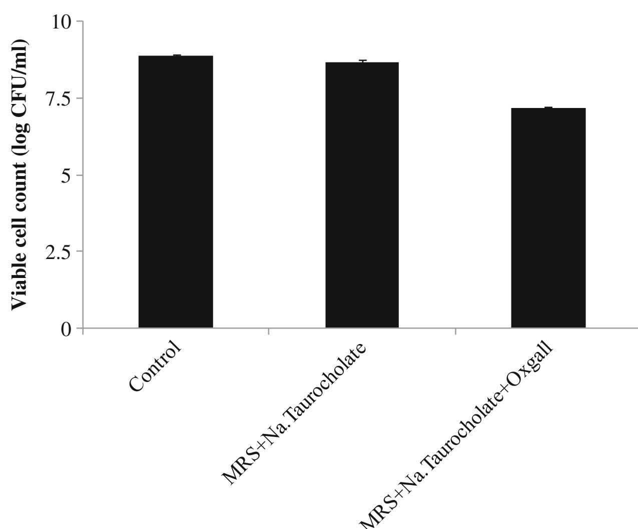


Fig. 3 Cell viability of *L. plantarum* K46 in the presence of bile salts

nature (Lee et al. 2011). The antifungal metabolite was purified in a silica column, and the compound was characterized chemically by ^{1}H NMR and ^{13}C NMR. The antifungal compound was identified as being acidic in nature. HPLC retention times (min): 15.10; ^{1}H NMR; 7.31 (H-5 and H-9), 7.25 (H-6 to H-8), 4.16 (H-2); ^{13}C NMR; 175.59 (C-1), 71.52 (C-2), 40.47 (C-3), 138.60 (C-4), 128.43 (C-5 and C-9), 129.85 (C-6 and C-8), 126.57 (C-7) and ESI-MS (m/z):165 $(M+H)^{+}$ were characteristic of 3-phenyllactic acid (Wang et al. 2012). From the results obtained, the antifungal action of the compound was determined to be highly active against the tested spoilages. The MIC of the compound ranged from 2.5 to 7.5 mg/mL, and it varied for each fungal strain. Phenyllactic acid from *L. plantarum* has been reported previously in fungal inhibition (Lavermicocca et al. 2000), and also found to exhibit antibacterial properties (Dieuleveux et al. 1998). Likewise, the MIC values of the tested antifungal

Table 5 Extracellular enzyme activities of *L. plantarum* K46. The enzyme activities of *L. plantarum* K46 were assayed using the API ZYM kit method, and measured as approximate number of nanomoles of substrate hydrolyzed during a 4 h incubation at 30 °C. *L. plantarum* K46 without enzyme activity was represented as 0; Enzyme activities are represented as 5, 10 and 20 nM hydrolyzed substrate

Nos	Intra cellular and extracellular enzymes	*L. plantarum* K46
1	Alkaline phosphatase	0
2	Esterase (C_4)	5
3	Esterase lipase (C_8)	5
4	Lipase (C14)	0
5	Leucine arylamidase	10
6	Valine arylamidase	5
7	Cystine arylamidase	5
8	Trypsin	5
9	α-Chymotrypsin	0
10	Acid phosphatase	0
11	Naphthol-AS-Biphophohydrolase	5
12	α-Galactosidase	10
13	β-Galactosidase	0
14	β-Glucuronidase	20
15	α-Glucosidase	0
16	β-Glucosidase	10
17	N-Acetyl-β-glucosaminidase	10
18	α-Mannosidase	0
19	α-Fucosidase	0

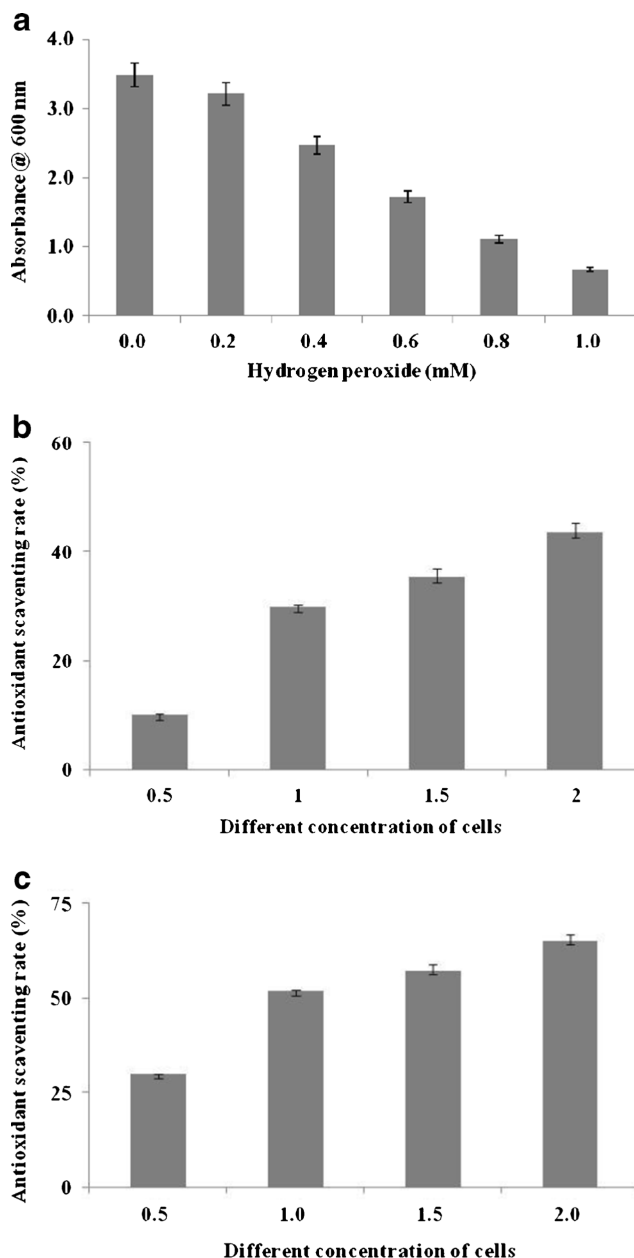


Fig. 4a–c Antioxidant activities of *L. plantarum* K46. **a** Resistance at different hydrogen peroxide concentrations. **b** Scavenging activities on hydroxyl radicals. **c** Scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical

compound were compared to the metabolite produced by commercial *L. plantarum* 21B, which ranges from 50 to 166 mg/mL (Lavermicocca et al. 2000). Strom et al. (2002) identified antifungal metabolites, such as cyclic dipeptides and 3-phenyllactic acid, from *L. plantarum* as being active against, *Pantoea agglomerans* VTTE-90396 and *Fusarium avenaceum* VTTD-80147. Prema et al. (2008) reported, *L. plantarum* strain displayed growth inhibition against common fungal strains such as *Aspergillus niger*, *A. fumigatus*, *A. terreus*, *A. flavus*, *A. nidulans*, *Penicillium roqueforti*, *P. corylophilum*, *P. expansum* and *P. camemberti*.

Probiotics are live microbial supplements that beneficially alter the host animal by improving its intestinal microbial balance (Fuller 1992). Acid tolerance is a property that any strain is expected to possess to have an effect in the gastrointestinal tract (Salminen et al. 1996). *L. plantarum* K46 isolated from fermented food was able to grow at pH 3.0 and 4.0. Lee et al. (2011) reported that most strains showed a high survival rate at pH 3.0 after 1 h and the survival rates were more than 90 %. Compared to the report described above, our strain had much better acid tolerance. Through analysis, the sources of our strains (fermented sesame leaf) may be considered as the major factors responsible for high acid tolerance. Bile salts are produced in the liver from the catabolism of cholesterol. Therefore, when evaluating the potential of using LAB as effective probiotics, it is necessary to evaluate their ability to resist the effects of bile acids (Lee et al. 2011). The exposure to low buffering conditions was more destructive to the strains than to the bile salts, whereas *L. plantarum* K46 was able to tolerate low pH and bile salts. The survival of LAB strains at pH 3.0 up to 2.0 h was reported by Succi et al. (2005) and Charteris et al. (1998) demonstrated strain-dependent resistance of probiotic strains to bile salts. The antibiotic sensitivity pattern was found to be susceptible to most of the commonly used antibiotics, which inhibit protein synthesis, which coincides with the observations of Goldstein et al. (2000), of resistance to nalidixic acid and tetracycline. The antibiotic sensitivity study agrees well with similar studies conducted on *L. plantarum* isolated from probiotic products currently used in the European market (Temmerman et al. 2003). Jamaly et al. (2011) studied the LAB strains in relation to hydrophobicity and bacterial adhesion. *L. plantarum* K46 exhibited high hydrophobicity (100 %) and negative results for hemolysis, which is observed only rarely in food LAB (Maragkoudakis et al. 2006).

Hydroxyl radical are primarily responsible for the oxidative injury of bio-molecules, and these originate mainly from the Fenton reaction in the presence of transition metals such as iron (Fe^{2+}) and copper (Cu^{2+}). Chelation of these ions by certain antioxidants may inhibit the generation of hydroxyl radicals (Kao and Chen 2006). There are many reports claiming the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases (Rose et al. 1972). In this study, *L. plantarum* K46 was able to tolerate a moderate level of H_2O_2 for 9 h, and exhibited good free radical scavenging activity. Our results are similar to those of Kullisaar et al. 2003; Lee et al. 2005, who also demonstrated H_2O_2 resistance in their respective probiotic *Lactobacillus* strains at 0.4 and 1 mM concentrations, respectively. Some probiotic strains, such as *Streptococcus thermophilus*, *Bifidobacterium longum*, *Lactobacillus plantarum* and *Lactobacillus casei*, possess strong antioxidative activity, and are able to decrease the risk of accumulation of reactive oxygen species (ROS) during the ingestion of food (Lee et al. 2005). In the DPPH test, different concentrations of *L. plantarum* K46 were able to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. A DPPH free radical scavenging study indicated that the cell surface active compounds of *L. plantarum* K46 may be involved in antioxidant activity, and that it is directly proportional to cell concentration. The reason for the activity may be due to the existence of enzymes, such as NADH-oxidase, SOD, NADH peroxide, and non-heme catalases.

In conclusion, 22 antifungal strains of LAB were identified from the traditional fermented food of Korea by 16S rRNA gene sequence analysis. *Lactobacillus plantarum* was the predominant strain. Among all LAB, *L. plantarum* K46 had high antifungal activity against food spoilage fungi. IR, ^{13}C NMR, ^{1}H NMR and MS spectrum identified that the antifungal metabolite was phenolic in nature. The strain had good functional probiotic properties, such as high tolerance to low pH and bile salts. An antimicrobial susceptibility pattern was an intrinsic feature of this strain; thus, consumption does not represent a health risk to humans or animals. In addition, the strain exhibited strong hydrogen peroxide resistant ability, hydroxyl radical and DPPH free radical scavenging activity which might be helpful in preventing or slowing the progress of various oxidative stress related diseases. Therefore, it is confirmed that this *L. plantarum* K46 strain possesses several suitable characteristics that make it appropriate for the production of various products.

Acknowledgments We would like to thank Minister for Food, Agriculture, Forestry and Fisheries, for their support to carry out this project under the grant No. PJ008445. We also extend our gratitude to NIAS, RDA for their support.

References

Arasu MV, Duraipandiyan V, Ignacimuthu S (2013) Antibacterial and antifungal activities of polyketide metabolite from marine *Streptomyces* sp. AP-123 and its cytotoxic effect. Chemosphere 90:479–487

Bover-Cid S, Holzapfel WH (1999) Improved screening procedure for biogenic amine production by lactic acid bacteria. Int J Food Microbiol 53(1):33–41

Brul S, Coote P (1999) Preservative agents in foods: mode of action and microbial resistance mechanisms. Int J Food Microbiol 50:1–17

Buchmeier N, Bossie S, Chen CY, Fang FC, Guiney DG, Libby SJ (1997) SlyA, a transcriptional regulator of *Salmonella typhimurium*, is required for resistance to oxidative stress and is expressed in the intracellular environment of macrophages. Infect Immun 65:3725–3730

Charteris WP, Kelly PM, Morelli L, Collins JK (1998) Development and application of an in vitro methodology to determine the transit tolerance of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in the upper human gastrointestinal tract. J Appl Microbiol 84: 759–768

Citi JE, Sandime WE, Eliker PR (1963) Some observation on the Hull method for measurement of proteolysis in milk. J Dairy Sci 46:337–345

Coda R, Cassone A, Rizzello CG, Nionelli L, Cardinali G, Gobbetti M (2011) Antifungal activity of *Wickerhamomyces anomalus* and *Lactobacillus plantarum* during sourdough fermentation: identification of novel compounds and long-term effect during storage of wheat bread. Appl Environ Microbiol 77(10):3484–3492

Corsetti A, Settanni L (2007) Lactobacilli in sourdough fermentation. Food Res Int 40:539–558

Dal Bello F, Clarke CI, Ryan LAM, Ulmer H, Schober TJ, Strom K, Sjogren J, van Sinderen D, Schnurer J, Arendt EK (2007) Improvement of the quality and shelf-life of wheat bread by fermentation with the antifungal strain *Lactobacillus plantarum* FST 1.7. J Cereal Sci 45:309–318

Dieuleveux V, Lemarinier S, Gueguan M (1998) Antimicrobial spectrum and target site of D-3-phenyl lactic acid. Int J Food Microbiol 40: 177–183

Filtenborg O, Frisvad J, Thrane U (1996) Moulds in food spoilage. Int J Food Microbiol 33:85–102

Fuller R (1992) Probiotics: the scientific basis. Chapman and Hall, London

Garofalo C, Silvestri G, Aquilanti L, Clementi F (2008) PCR-DGGE analysis of lactic acid bacteria and yeast dynamics during the production processes of three varieties of Panettone. J Appl Microbiol 105:243–254

Garofalo C, Zannini E, Aquilanti L, Silvestri G, Fierro O, Picariello G, Clementi F (2012) Selection of sourdough lactobacilli with antifungal activity for use as biopreservatives in bakery products. J Agric Food Chem 60(31):7719–7728

Georgieva R, Iliev I, Haertlé T, Chobert JM, Ivanova I, Danova S (2009) Technological properties of candidate probiotic *Lactobacillus plantarum* strains. Int Dairy J 19:696–702

Goldstein EJC, Citron DM, Merriam CV, Warren Y, Tyrrell LL (2000) Comparative in vitro activities of ertapenem (MK-0826) against 1001 anaerobes isolated from human intra-abdominal infections. Antimicrob Agents Chemother 44:2389–2394

Gourama H, Bullerman LB (1995) Antimycotic and antiaflatoxigenic effect of lactic acid bacteria: a review. J Food Prot 57:1275–1280

He ZS, Luo H, Cao CH, Cui ZW (2004) Photometric determination of hydroxyl free radical in Fenton system by brilliant green. Am J Clin Med 6:236–237

Jamaly N, Benjouad A, Bouksaim M (2011) Probiotic potential of *Lactobacillus* strains isolated from known popular traditional moroccan dairy products. Br Microbiol Res 1(4):79–94

Kao T, Chen B (2006) Functional components in soybean cake and their effects on antioxidant activity. J Agric Food Chem 54:7544–7555

Karunaratne A, Wezenberg E, Bullerman LB (1990) Inhibition of mould growth and aflatoxin production by *Lactobacillus* spp. J Food Prot 53:30–236

Kullisaar T, Songisepp E, Mikelsaar M, Zilmer K, Vihalemm T, Zilmer M (2003) Antioxidative probiotic fermented goats' milk decreases oxidative stress-mediated atherogenicity in human subjects. Br J Nutr 90:449–456

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

Lee J, Hwang K, Chung M, Cho D, Park C (2005) Resistance of *Lactobacillus casei* KCTC 3260 to reactive oxygen species (ROS): role for a metal ion chelating effect. J Food Sci 70: 388–391

Lee H, Yoon H, Ji Y, Kim H, Park H, Lee J, Shin H, Holzapfel W (2011) Functional properties of *Lactobacillus* strains isolated from kimchi. Int J Food Microbiol 145:155–161

Legan JD (1993) Mold spoilage of bread: the problem and some solutions. Int J Biodeterior Biodegrad 32:33–53

Li S, Zhao Y, Zhang L, Zhang X, Huang L, Li D, Niu C, Yang Z, Wang Q (2012) Antioxidant activity of *Lactobacillus plantarum* strains isolated from traditional Chinese fermented foods. Food Chem 135: 1914–1919

Lindgren SE, Dobrogosz WJ (1990) Antagonistic activities of lactic acid bacteria in food and feed fermentations. FEMS Microbiol Rev 87: 149–164

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

Maragkoudakis PA, Zoumpopoulou G, Miaris C, Kalantzopoulos G, Pot B, Tsakalidou E (2006) Probiotic potential of *Lactobacillus* strains isolated from dairy products. Int Dairy J 16:189–199

National Committee for Clinical Laboratory Standards (NCCLS) (1999). Document M31-A performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, approved standard NCCLS, Villanova, p.57

O'Sullivan L, Ross RP, Hill C (2002) Potential of bacteriocin- producing lactic acid bacteria for improvements in food safety and quality. Biochimie 84:593–604

Pfeiler EA, Klaenhammer TR (2007) The genomics of lactic acid bacteria. Trends Microbiol 15:546–553

Pitt J (2000) Toxigenic fungi and mycotoxins. Br Med Bull 56:184

Prema P, Smila D, Palavesam A, Immanuel G (2008) Production and characterization of an antifungal compound (3-phenyllactic acid) produced by *Lactobacillus plantarum* strain. Food Bioprod Technol 3:379–386

Rose WM, Creighton MO, Stewart DHPJ, Sanwal M, Trevithick GR (1972) In vivo effects of vitamin E on cataractogenesis in diabetic rats. Can J Ophthalmol 17:61–66

Rossetti L, Carminati D, Zago M, Giraffa G (2009) A qualified presumption of safety approach for the safety assessment of Grana Padano whey starters. Int J Food Microbiol 130:70–73

Salminen S, Isolauri E, Salminen E (1996) Clinical use of probiotics for stabilizing the gut mucosal barrier: successful strains and future challenges. Antonie Van Leeuwenhoek 70:347–358

Schillinger U, Villarreal JV (2010) Inhibition of *Penicillium nordicum* in MRS medium by lactic acid bacteria isolated from foods. Food Control 21:107–111

Schnürer J, Magnusson J (2005) Antifungal lactic acid bacteria as biopreservatives. Trends Food Sci Technol 16:70–78

Smith J, Hong-Shum L (2003) Food additives data book. Blackwell Science, Oxford

Stiles ME (1996) Biopreservation by lactic acid bacteria. Antonie Van Leeuwenhoek 70:331–345

Strahinic I, Busarcevic M, Pavlica D, Milasin J, Golic N, Topisirovic L (2007) Molecular and biochemical characterizations of human oral lactobacilli as putative probiotic candidates. Oral Microbiol Immunol 22:111–117

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

Succi M, Tremonte P, Reale A, Sorrentino E, Grazia L, Pacifico S, Coppola R (2005) Bile salt and acid tolerance of *Lactobacillus rhamnosus* strains isolated from Parmigiano Reggiano cheese. FEMS Microbiol 244:129–137

Temmerman R, Pot B, Huys G, Swings J (2003) Identification and antibiotic susceptibility of bacterial isolates from probiotic products. Int J Food Microbiol 81:1–10

Torkar KG, Vengust A (2008) The presence of yeasts, moulds and aflatoxin M1 in raw milk and cheese in Slovenia. Food Control 19:570–577

Vinderola CG, Reinheimer JA (2003) Lactic acid starter and probiotic bacteria: a comparative in vitro study of probiotic characteristics and biological barrier resistance. Food Res Int 36:895–904

Wang HK, Yan YH, Wang JM, Zhang HP, Qi W (2012) Production and characterization of antifungal compounds produced by *Lactobacillus plantarum* IMAU10014. PLOS ONE 7(1)

Zhang C, Brandt JM, Schwaba C, Gänzle GM (2010) Propionic acid production by cofermentation of *Lactobacillus buchneri* and *Lactobacillus diolivorans* in sourdough. Food Microbiol 27(3): 390–395