Research Article

POTENTIAL OF LACTIC ACID BACTERIA, ISOLATED FROM SEVERAL SOURCES, TO INHIBIT THE GROWTH OF *Candida albicans* ATCC10231

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ARTICLE HIGHLIGHTS

- Lactic acid bacteria have potential to control candidiasis or *Candida albicans*.
- The novelty of this study is to advance the potential of lactic acid bacteria to control candidiasis infection in human, with a view to develop novel LAB-based probiotic candidates with capability to inhibit/prevent infection by *C. albicans*, the causative agent of candidiasis.

ABSTRACT

The main aims of this research were to isolate and identify potential lactic acid bacteria (LAB) inhibitory to *Candida albicans*. The LAB sources were *kimchi*, honey and vaginal secrete of healthy women. They were isolated with a view to develop a novel alternative method with reduced use of antifungal agents in the treatment of patients infected by such fungal pathogen. Isolation of the LABs was conducted by applying dilution spread method on de Mann Rogosa Sharpe agar (MRSA) medium supplemented with bromo cresol purple (BCP) indicator. Once purified, they were tested for antagonism against *C. albicans* in dual culture assays. LAB isolates that showed significant inhibition against the pathogen were identified using 16s rDNA sequences and their sequences were aligned with those of known sequences deposited at the Gene Bank (http://www.ncbi.nlm.nih.gov). The results showed that 46 among more than 100 LABs isolated in this study significantly inhibited the growth of *C. albicans* in the *in vitro* dual culture assays, and all showed resistance property to antifungal agent (fluconazole). This indicated that they all have potential to be synergically applied with reduced use of fluconazole in the therapy. The most potential isolates (10 isolates) were closely related to three LAB species, namely *Lactobacillus paracasei, Lacticaseibacillus paracasei*, and *Pediococcus pentosaceus*, based on their 16s rDNA sequence similarities with those deposited in the GenBank.

Keywords:
candida albicans, candidiasis, diversity, probiotic, vaginal candidiasis

INTRODUCTION

In the last decade, candidiasis prevalence has been reported to increase, particularly among women with immune deficient (AIDS) or people under treatment with various types of antibiotics (Lauw *et al.* 2017). The main cause of this candidiasis infection is opportunistic fungal pathogens (particularly *Candida albicans* which is among myriads normal microbiota) of human inhabitants (Anh *et al.* 2021). This fungal pathogen is commonly found in the mucosa of oral cavity, skin surface, digestive tract, as well as in the reproductive tract of women. Under certain conditions, such as imbalance in the microbial composition of its hosts or immune system disturbance in its host, *C. albicans* tends to boost its growth and become an infectious pathogen (Richardson 2022; Macias-Paz *et al.* 2022). According to Wilson (2019) vulvovaginal and oral candidiasis are the most common cases due to *C. albicans* infection. Additionally, skins are also susceptible from this infection, if they are under long term exposure of humid conditions (less ventilated skins) (Qadir & Asif 2019). When the pathogen enters the blood stream and spread throughout the body via circulatory system, deep candidiasis may occur within the vulva of vagina as well as in the mucosa of oral cavity.

In the area of women genital (vagina and vulva), *C. albicans* infection may lead to abnormality in mucus secretion, inflammation, appearance of redness color, or burning-like feeling (Talapko *et al.* 2021). It was reported by Anh *et al.* (2021) that 75% women globally may have at least one infection of vulvovaginal candidiasis, and 40-50% among them may experience repeated/recurrent infections. Meanwhile, people with infection in their mouth mucosa may have pain, lose appetite, uncomfortable feeling within their mouth (Dangi *et al.* 2010; Singh *et al.* 2014).

Epidemiological survey on the prevalence of candidiasis indicates that most (approximately 85-95%) of the vaginal infection among women globally is caused by *C. albicans* (Makanjuola *et al.* 2018). It is further stated by this report that some non-*Candida albicans* species (such as *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. dubliniensis*) were also frequently found to be the cause of vulvovaginal candidiasis incidence worldwide. In Indonesia alone, it was reported by Wahyuningsih *et al.* (2021) that the prevalence and disease incidence caused by *C. albicans* infection has become a serious problem in last decade. Each year in Indonesia, approximately 5 million women (aged of between 15 and 50 years old) have been reported to experience recurrent vulvovaginal candidiasis. Therefore, research on the prevention and control of candidiasis is urgently needed to cope with such fungal infection.

Until recently, control of *C. albicans* among infected patients has relied on application of anti-fungi, particularly fluconazole (Whaley *et al.* 2017). Long term antifungal application may result in bad side effects on the balance conditions of the intestinal normal microbiota. Many beneficial microbiota or probiotic microbes may be wiped out by broad spectrum of those antibiotics/anti-fungi and this may lead to health disturbance, such as diarrhea (Heng *et al.* 2021). Health level of intestinal tract has recently been reported to be correlated with health status of a person (Manor *et al.* 2020). Some researchers, such as Maiuolo *et al.* (2019), reported that there is a bidirectional communication between brain and gut microbiota which is well known as gut-brain axis. This implies that the balance of gut microbiota will have

significant effects on the brain function (Zhao *et al.* 2018). Bulgasem *et al.* (2016) also reported that resistance properties of the *C. albicans* had occurred as a result of improper application of fluconazole, and this may lead to an increase in the pathogenic capacity of the pathogen (Hossain *et al.* 2018)

To cope with negative side effects of the excessive/improper application of anti-fungi, alternative methods are urgently needed. Among many alternative methods, the use of antagonists (biocontrol agents) of the *C. albicans* appears to be the most promising method with the lowest risk. Biocontrol agents, with capability as well as with lowest risk to control the growth of *C. albicans*, are bacteria belong to the group of lactic acid bacteria (Zeise *et al.* 2021) as they are also being part of normal microbiota of human.

Lactic acid bacteria are Gram positive bacteria with capability to produce lactic acid as the main product in the metabolism of sugars. These LABs can easily be isolated from many sources, such as honey, fermented foods (kimchi and *tape ketan*), and various milk. These sources are ideal for the LABs as their main source of nutrition to support their growth is available in such products (Pasupuleti *et al.* 2017). Some genera of LABs successfully isolated from those sources (particularly honey) are *Lactobacillus, Streptococcus, Micrococcus*, and *Enterococcus* (Feizabadi *et al.* 2020). Ryu and Chang (2013) found 200 species of microorganisms from fermented kimchi, and among those LAB isolates, such as *Leuconostoc* spp. and *Lactobacillus* spp., were indicated to play the most significant role in the kimchi making.

Based on the above rational, the main aims of our research were to isolate and identify potential LAB isolates from several sources, such as kimchi, honey, and vaginal secrete of healthy women with a view to develop a novel method to cope with negative side effects of improper/excessive antifungal application in the conventional therapies of candidiasis. In this research, the isolates were identified molecularly on the basis of their 16s rDNA sequences which were aligned with those deposited at the Gene Bank (http://www.ncbi.nlm.nih.gov), following DNA extraction, DNA amplification in a PCR machine, and electrophoresis.

MATERIALS AND METHODS

Isolation of Potential Probiotics

Sources of lactic acid bacteria included fermented foods, such as *kimchi*, honey, and vaginal secrete of healthy women (ethical clearance Number: 1354/UN14.2.2.VII/LT/2022, approved by the Faculty of Medicine Udayana University). Dilution and spread method on MRSA (de Mann Rogosa Sharpe Agar) added with bromocresol purple (BCP) indicator was applied in the isolation of probiotic candidates. The samples were diluted to the rates of 10^{-1} to 10^{-6}. A volume of 100 µL samples with dilution rates of 10^{-3} to 10^{-6} were then spread on MRS agar with BCP indicator and incubated at 37 °C for 48 hours until distinct colonies appeared on this medium. Colonies with indication to produce acidic compounds were isolated, purified, and stored at -80 °C in MRS broth with 30% (v/v) glycerol in it for use in further studies.

Candida albicans ATCC10231 Isolate

The fungal pathogen (*C. albicans* ATCC10231) was obtained from Central Hospital, Sanglah-Denpasar, Bali. This pathogen was isolated from a patient with thrush disease. The identity of this pathogen was confirmed to be *C. albicans* following application of Biomerieux Vitex® 2 system. This pathogenic fungal isolate was maintained in sabouraud dextrose broth (SDB) with 30% (v/v) glycerol at -80 °C. For regular use, it was subcultured from the stock culture on sabouraud dextrose agar (SDA).

In Vitro Dual Culture Assay for Screening Potential Probiotic Candidates

In vitro dual culture assays on MRS agar medium were applied in the screening of the 46 potential LAB isolates antagonistic against *C. albicans*. A volume of 200 µL *C. albicans* suspension with cell density of 10^8 cfu/mL or equivalent to 0.5 McFarland scale was spread onto MRS agar medium and let dry for 5 minutes. This *C. albicans* lawn was then spot inoculated with LAB isolates, incubated at 37 °C overnight, and observed for inhibition zones around the LAB isolates. Five replicate experiments were done with sterile MRS broth and fluconazole as negative and positive controls, respectively. Those showed inhibition activity on *C. albicans* were purified (streak for single colony) and stored in MRS broth medium at -80 °C prior to further studied. Similar method was also applied in the antifungal resistance of LAB isolates obtained in our study.

Molecular 16s rDNA Sequencing for the Identification of Most Potential Bacterial Isolates

Some potential LAB antagonists were identified by sequencing components of their 16s rDNA and comparing these with their counterparts in a clone library of known bacteria (http://www.ncbi.nlm.nih.gov). The followings are procedures of extraction, purification, amplification, and sequencing of the LAB's 16s rDNA.

Isolation of Genomic DNA

A volume of 1 mL suspension of LABs in MRSB medium was added into an Eppendorf tube, centrifuged at 8,000 *xg* for 3 minutes at a temperature of 4 °C, and decanted. The pellet was then washed twice with sterile saline solution, centrifuged at 8,000 *xg* at 4 °C for 3 minutes, and its supernatant was decanted. This procedure was repeated several times until at least 50 mg pellet or bacterial cell mass was obtained. The pellet was subsequently re-suspended in 200 µL of saline solution to obtain densed cell suspension. The DNA extraction followed the procedures as specified in the *Quick*-DNA™ *Microprep Kit* (Zymo Research, USA). The cell suspension obtained (200 µL) and 750 µL BashingBeadTM Buffer was added into a ZR BashingBead™ Lysis Tube, shaken with a bead beater (TOMY micro Smash™ MS-100) at the speed of 4,500 rpm for 6 minutes and centrifuged at 10,000 *xg* for 1 minute at 4 °C. The supernatant was transferred into a Zymo-Spin™ III-Filter in a collection tube and centrifuged at 8,000 *xg* for 1 minute at a temperature of 4 °C. A volume of 1,200 µL of Genomic Lysis Buffer was then added to the filtrate in a collection tube. A volume of 800 µL of this mixture was next pipetted into a Zymo-Spin™ IICR Column in a new collection tube, centrifuged at 10,000 *xg* (at 4 °C) for 1 minute, and its supernatant was decanted. This procedure was repeated twice. This was followed by the addition of 200 µL DNA Pre-Wash Buffer into a new collection tube of Zymo-Spin™ IICR Column, centrifuged at 10,000 *xg* (at 4 °C) for 1 minute, and decanted. A volume of 500 µL g-DNA Wash Buffer was added into a Zymo-Spin™ IICR Column, further centrifuged at 10,000 *xg* (at 4 °C) for 1 minute, and 35 µL DNA Elution Buffer was added into a Zymo-Spin™ IICR Column in a 1.5 ml capacity of Eppendorf tube. This mixture was again centrifuged at 10,000 *xg* (at 4 °C) for 30 seconds in the DNA elution. The DNA obtained was quantified with a nanophotometer P-300

(Implen) at the wavelength of 260 nm and 280 nm, stored at -20 °C prior to use in the subsequent analysis.

Amplification of 16s rDNA using HotStart Mastermix PCR Kit, Qiagen

Primers of 27-F (5' AGA GTT TGA TCC TGG CTC AG3') dan 1492-R (5' TAC GGY TAC CTT GTT ACG ACTT 3') were used to amplify the isolated 16s rDNA of the LAB isolates. The total volume of the reaction was 50 µL, consisted of 48 µL PCR master mix (5 µL dNTPs, 5 µL PCR buffer, 3.5 µL MgCl$_2$, 1 µL of 1 pmol primer 27-F, 1 µL of 1 pmol primer 520-R, 0.25 µL taq polymerase, and 34 µL deionized water) and 2 µL DNA sample. Amplification (30 cycles) was conducted in an infinigen thermocycler machine. The DNA samples were first denatured at 94 °C and followed by 30 cycles of PCR with each cycle conditions of: denaturing of DNA for 30 seconds, 2 minutes of annealing at 55 °C, and 2 minutes elongation at 72 °C. The cycle was ended with a cycle of elongation for 5 minutes at 72 °C. Following this, electrophoresis was run on a gel of 1% (w/v) agarose, containing 1 µg/mL ethidium bromide in TAE buffer to confirm whether or not the target sequence was successfully amplified. Electrophoresis was conducted for 45 minutes at 80 Volt and the correct size of appeared bands were visualized under a UV trans-illuminator. Purification of the PCR product followed once of these bands were visualized.

Purification of PCR product and sequencing of purified 16s rDNA

Purification of PCR product was conducted using SUPRC™ PCR (Takara Biomedicals, Otsu, Japan) followed by sequencing with Big Dye Primer Cycle Sequencing FS Ready Reaction Kit (Applied Biosystem) using automated sequencing 3100 Genetic Analyzer (PE Applied Biosystems). This was conducted at the 1st base, Malaysia via the PT Genetika Science Indonesia. The 16s rDNA sequences of our isolates were then aligned with their counterparts in a clone library of known bacteria deposited at the Gen Bank (http://www.ncbi.nlm.nih.gov), so that their molecular identity could be recognized. Following multiple sequence alignment of the 16s rDNA sequences of the SV, MD, and KIM isolates, with eight bacterial species previously reported as antagonistic to *C. albicans*, using Clustal W2 program, a phylogenetic tree was constructed using Neighbor-Joining methods with

1,000 bootstrap replicates and *p-distance* method in MEGA11 software (Tamura *et al.* 2021). The phylogenetic analysis involved 18 nucleotide sequences. All positions containing gaps and missing data were eliminated. There was a total of 664 positions in the final dataset.

Data Analysis

The data of our current research was analyzed descriptively, elaborated the meaning of each data, and compared it with similar phenomena that have been reported by other researchers in previous studies.

Ethical statement: The ethical clearance of human sample use for this study was provided by the Faculty of Medicine Udayana University (clearance Number: 1354/UN14.2.2.VII/LT/2022).

RESULTS AND DISCUSSION

More than 100 isolates were successfully isolated in our study with colony morphologies on MRSA medium as shown in Figure 1 (A-C). The initial indication that those isolates belong to lactic acid bacteria (LABs) was their ability to change the color (from purple to yellow) of MRSA medium with BCP indicator in it (Figs. 1D-F). This indication which is supported by results of several important tests toward to LAB identity (such as Gram positive, catalase negative, homo fermentative) confirmed that they all belong to LAB group.

Lactic acid bacteria have been widely used as probiotics to maintain the balance of human and animal intestinal tract. They are abundant in various sources, such as fermented foods, honey or vaginal secrete of healthy women, as isolated in our current research. These are in line with those reported by Taye *et al.* (2021), Soemarie *et al.* (2022), Lin *et al.* (2020), Amabebe *et al.* (2018), Elzeini *et al.* (2021), and Munandar *et al.* (2022) who successfully isolated LABs from milk, fermented foods, sea grass, vaginal secrete of healthy women, honey, and intestinal tract, respectively.

In dual culture assays, the 46 isolates showed their antagonistic activity, and these were indicated by formation of clear zones around the LAB isolates on lawns of *C. albicans*, with various diameters of inhibition (Tabel 1; Fig. 2). Similar results were also reported by Bulgasem et al. (2016) who found several LAB isolates, such as *L. plantarum, L. curvatus, Pediococcus acidilactici*, and *P. pentosaceus* with capability to inhibit the in vitro growth of *C. albicans.*
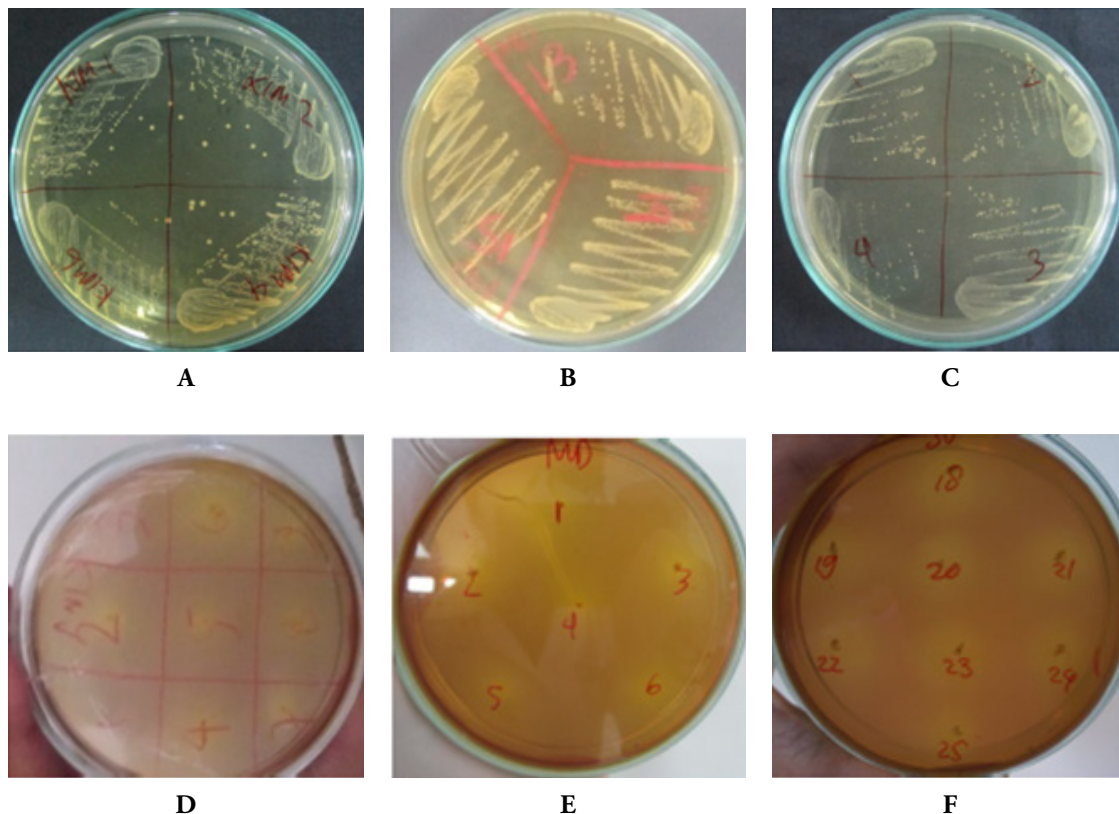
Figure 1 Colony morphologies of LAB isolated from kimchi (A), honey (B), and vaginal secrete (C) on MRSA medium. LABs isolated from kimchi (D), honey (E), and vaginal secrete of healthy women (F) with ability to change the color (from purple to yellow) of MRSA medium supplemented with BCP indicator

In more recent study, Er *et al.* (2019) reported similar phenomenon following bioassays of their isolates (vaginal secrete isolates of healthy women) on lawns of *C. albicans*. According to Vieco-Saiz *et al.* (2019) formation of clear zones around the LAB isolates indicated that toxic compounds inhibitory to *C. albicans*, such as organic acids, bacteriocin, or hydrogen peroxides could be released.

Organic acids (lactic acid in particular) released by LABs to their surroundings will lead to a decrease in pH level (lower than 4) of their environment, and could inhibit the growth of their competitors, especially pathogenic bacteria (Ayivi *et al.* 2020). Under such condition, the H^+ ion level in their surrounding become very high that lead to

excessive passive diffusion of such ions (due to high level of H^+ ion gradient concentration) into the cell cytoplasm of the residing microbes exposed to such low pH condition (Guan & Liu 2020). This will subsequently result in a significant decrease of pH within the cell cytoplasm. Microbes (pathogens in particular) which are not equipped with proton pump in such condition will not be able to survive due to failure to maintain their cytoplasm pH condition higher than that of their surrounding (Ayivi *et al.* 2020). Diameter of inhibition zones formed on the lawns of *C. albicans* as shown in Table 1 and Figure 2, may have been due to this type of mechanism, although it needs to be further elucidated.

Table 2 Diameter of inhibition zone (mm) of LAB isolates (kimchi, honey, and vaginal secrete of healthy women) on lawns of *C. albicans* on MRSA medium

No.	Codes	Inhibition zone (mm)*	No.	Codes	Inhibition zones (mm)*
1	MD 1	23.05±0.10	25	KIM 6	5.11±0.08
2	MD 2	21.37±0.18	26	KIM 7	5.10±0.06
3	MD 3	23.93±0.08	27	KIM 8	9.94±0.17
4	MD 4	18.92±0.11	28	KIM 9	13.95±0.16
5	MD 5	21.38±0.19	29	KIM 10	5.08±0.08
6	MD 7	20.99±0.18	30	KIM 11	10.03±0.09
7	MD 8	22.94±0.18	31	SV 6	5.09±0.08
8	MD 9	22.99±0.15	32	SV 7	8.37±0.08
9	MD 10	19.43±0.26	33	SV 8	5.11±0.04
10	MD 11	18.67±0.15	34	SV 9	5.09±0.09
11	MD 12	25.95±0.10	35	SV 10	9.08±0.20
12	MD 13	24.96±0.19	36	SV 21	5.13±0.06
13	MD 14	24.03±0.13	37	SV 23	5.06±0.06
14	MD 15	19.87±0.16	38	SV 24	5.11±0.05
15	MD 16	21.38±0.09	39	SV 26	9.04±0.09
16	MD 17	9.11±0.17	40	SV 27	9.26±0.09
17	MD 18	10.05±0.13	41	SV 28	5.08±0.08
18	MD 19	9.99±0.10	42	SV 29	10.50±0.09
19	MD 20	10.12±0.13	43	SV 30	9.19±0.14
20	KIM 1	19.01±0.12	44	SV 31	9.99±0.16
21	KIM 2	5.15±0.06	45	SV 32	5.12±0.07
22	KIM 3	15.67±0.12	46	SV 33	10.22±0.15
23	KIM 4	23.92±0.18	47	K (+)	36.08±0.07
24	KIM 5	8.99±0.15	48	K (-)	0.00±0.00

Notes: *Values in Table 1 ± standard deviations are averages of 5 replicates. K(-) is negative control (sterile broth medium used to produce LAB suspension), K(+) is anti-fungus fluconazole. KIM, MD, and SV are codes for isolates of Kimchi, honey, and vaginal secrete origin, respectively.

The roles of acidic compounds to inhibit several types of *Candida* spp. have also previously been reported by many researchers. Lourenço *et al.* (2019) for example reported that acetic acid and lactic acid had significant role to inhibit the growth of *C. albicans* in their *in vitro* assays. Application of such acids synergically with anti-fungi belong to azole groups was found by these authors to be more effective to inhibit *C. albicans*. Similar phenomenon was also observed by Wang *et al.* (2021) who found that application of asiatic acid, separately or in combination with anti-fungi fluconazole effectively inhibited the *in vitro* growth of *C. albicans*. In the *in vivo* experiments, asiatic acid increased the effectiveness of fluconazole to control the growth of *C. albicans*. It was concluded in the report of Wang *et al.* (2021) that the asiatic acid had significant role to decrease environmental pH by releasing higher level of H⁺ ions (through acidic compound dissociation) so that increasing the proton gradient between the cell cytoplasm and its surrounding.

Growth inhibition in our *in vitro* dual culture assays could also be due to bacteriocin or bacteriocin-like compounds released by our LAB isolates. Various types of LABs have been reported to produce such compounds and play significant role to inhibit the growth of *C. albicans*. Mohsin and Ali (2021) for example reported some LABs isolated from yoghurt with capability to produce bacteriocin, inhibitory to the growth of *C. albicans*. In more recent study, Hefzy *et al.* (2021) also reported some LABs (*L. pentosus, L. paracasei* subsp. *paracasei, L. rhamnosus, L. delbrueckii* subsp. *lactis*) with capability to produce potential bacteriocin-like compounds, active against *C. albicans*, the causative agent of candidiasis.
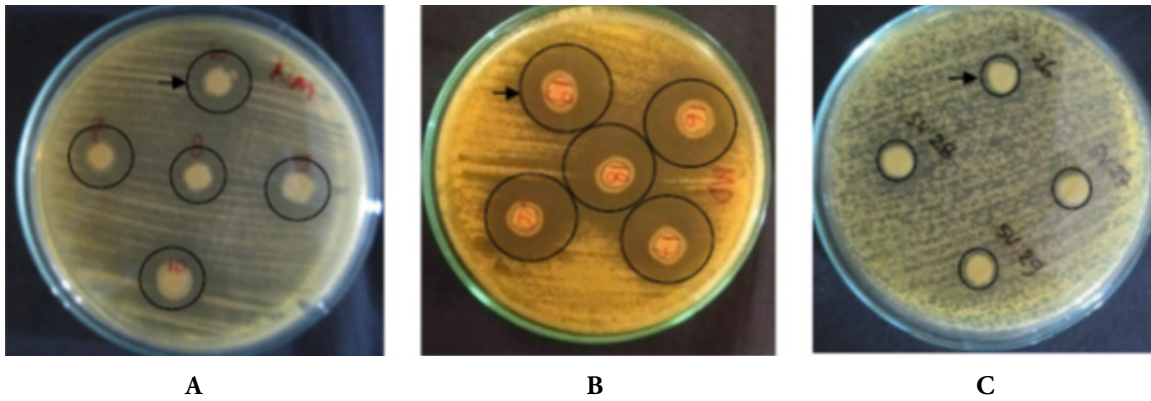
A   **B**   **C**

Figure 2 Inhibition zones of LABs isolated from kimchi (A), honey (B), and vaginal secrete of healthy women (C) on MRSA medium

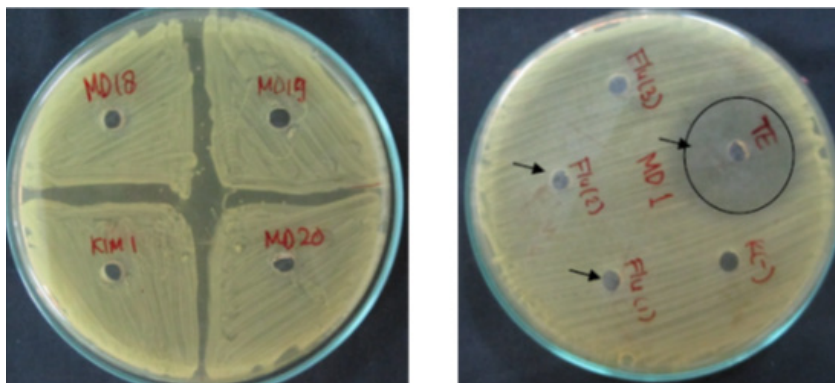Note: Clear inhibition zones are indicated by arrow heads.

Figure 3 Some examples of resistance tests of LAB isolates against antifungal fluconazole

Notes: All tested isolates were resistance against fluconazole, but not against tetracycline (Arrow heads). Sterile distilled water was used as negative control in these assays.

Bacteriocins are heat resistant antimicrobial peptides produced by LABs. The spectrum of control of this compound may either be wide (targeting various species other than LABs) or narrow (targeting only species closely related to LABs) (Collins *et al.* 2019). Plataricin, an example of bacteriocin produced by *L. plantarum,* initiates pore formation on cell membrane, and resulting in an increase in permeability and alteration of cell morphology of the *C. albicans* (Collins *et al.* 2019; Vazquez-Munoz & Dongari-Bagtzoglou 2021).

Another metabolite of LAB (*L. reuteri*) affecting the growth *C. albicans* is reuterin (Collins *et al.* 2019). Aldehyde group of the reuterin may interact with *tiol* group of various compounds and causes oxidative stress on *Candida albicans* (Vazquez-Munoz & Dongari-Bagtzoglou 2021). Other inhibitory compounds of LABs (*Lactobacillus*), such as hydrogen peroxide (H_2O_2) causing high oxidative stress and genotoxicity have also been reported being products of LABs inhibitory against *C. albicans* (Vazquez-Munoz & Dongari-Bagtzoglou 2021).

In the resistance tests against fluconazole, all potential isolates, presented in Table 1, showed resistance properties against this antifungal compound, and the results are shown in Table 2 and Figure 3. All tested isolates showed their resistance against fluconazole, but not against tetracycline, indicating that they all have possibility to be applied synergically with fluconazole in the therapies of patients infected by *C. albicans*.

Fluconazole is an antifungal agent belong to *azole* class, targeting the 1,4-α-*demethylase* enzyme involved in the biosynthesis of ergosteol (an important compound of fungal cell membrane) (Bassetti *et al.* 2016; Bhattacharya *et al.* 2020). Inhibition of this enzyme by the fluconazole results in growth disturbance on *C. albicans*.

Investigations on the use LABs in combination with conventional anti-fungi have been conducted worldwide with a view to reduce antifungal dose in the therapy of *C. albicans* infection (Shenoy & Gottlieb 2019). Implementation of LAB in the therapy of *C. albicans* infection is also expected

to improve the effectiveness of conventional anti-fungi as well as to reduce risk of excessive use of such anti-fungi (Bhattacharya *et al.* 2020). The effectiveness of fluconazole was reported to increase when it was combined with *L. acidophilus, B. bifidum,* and *B. longum* (Davar *et al.* 2016), or with *L. rhamnosus* dan *L. gasseri* (Pendharkar *et al.* 2015). The effectiveness of *L. gasseri,* however, was not improved when it was combined with itraconazol in the therapy of vulvovaginal candidiasis (Shenoy & Gottlieb 2019).

Isolates of LABs (10 isolates) that showed the best inhibition zones in dual culture assays against *C. albicans* (Table 1) were molecularly identified (sequencing their 16s rDNA), and the results are displayed in Figure 4. Based on the local alignment

of the 16s rDNA nucleotide sequence (BLASTn) with those recorded in the GenBank, our isolates were closely related to three species of LABs, *Lactobacillus paracasei* (accession ID LC463234), *Lacticaseibacillus paracasei* (accession ID PP210106), and *Pediococcus pentosaceus* (accession ID MT604839). Previous studies also reported that *L. paracasei* (Rossoni *et al.* 2017; De Gregorio *et al.* 2019), *Lacticaseibacillus paracasei* (Leska *et al.* 2022), and *Pediococcus petosaceous* (Bulgasem *et al.* 2016) had antagonistic effect on the growth of *C. albicans*. This indicates that our isolates have potential to be developed as probiotic candidates for use in the therapies of infections caused by *C. albicans*.
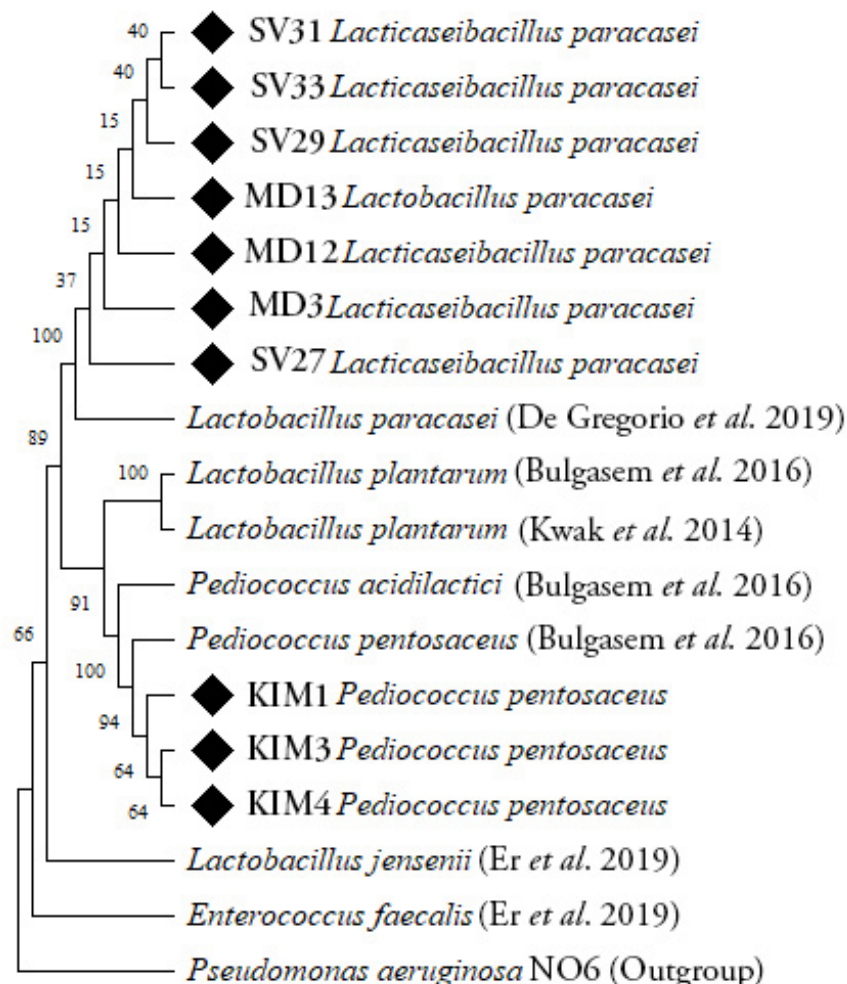

Figure 4 Relatedness of LABs isolated from kimchi (KIM), honey (MD), and vaginal secrete (VS) of healthy women, along with those previously reported to be antagonistic against *C. albicans*

Notes: The phylogenetic tree was built using Neighbor-Joining method with 1000 bootstrap replicates and *p-distance* substitution in MEGA11 (Tamura *et al.* 2021) following multiple sequence alignment of the 16s rDNA sequences of the SV, MD, dan KIM isolates, with the previously reported bacteria antagonistic to *C. albicans* recorded in the GenBank. the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches. the tree is a rooted-cladogram type. Our LAB isolates are those marked with black diamonds.

Table 2 Resistance of LAB isolates against antifungal fluconazole commonly used in the candidiasis therapies

No	Codes	Diameters of inhibition zones (mm)*	No	Codes	Diameter of inhibition zones (mm)*
1	MD 1	0.00±0.00	25	KIM 6	0.00±0.00
2	MD 2	0.00±0.00	26	KIM 7	0.00±0.00
3	MD 3	0.00±0.00	27	KIM 8	0.00±0.00
4	MD 4	0.00±0.00	28	KIM 9	0.00±0.00
5	MD 5	0.00±0.00	29	KIM 10	0.00±0.00
6	MD 7	0.00±0.00	30	KIM 11	0.00±0.00
7	MD 8	0.00±0.00	31	SV 6	0.00±0.00
8	MD 9	0.00±0.00	32	SV 7	0.00±0.00
9	MD 10	0.00±0.00	33	SV 8	0.00±0.00
10	MD 11	0.00±0.00	34	SV 9	0.00±0.00
11	MD 12	0.00±0.00	35	SV 10	0.00±0.00
12	MD 13	0.00±0.00	36	SV 21	0.00±0.00
13	MD 14	0.00±0.00	37	SV 23	0.00±0.00
14	MD 15	0.00±0.00	38	SV 24	0.00±0.00
15	MD 16	0.00±0.00	39	SV 26	0.00±0.00
16	MD 17	0.00±0.00	40	SV 27	0.00±0.00
17	MD 18	0.00±0.00	41	SV 28	0.00±0.00
18	MD 19	0.00±0.00	42	SV 29	0.00±0.00
19	MD 20	0.00±0.00	43	SV 30	0.00±0.00
20	KIM 1	0.00±0.00	44	SV 31	0.00±0.00
21	KIM 2	0.00±0.00	45	SV 32	0.00±0.00
22	KIM 3	0.00±0.00	46	SV 33	0.00±0.00
23	KIM 4	0.00±0.00	47	K (+)	31.06±0.11
24	KIM 5	0.00±0.00	48	K (-)	0.00±0.00

Notes: *Values in Table 2±standard deviations are averages of 5 replicates. Tetracycline and fluconazole were used as positive control while the solvent of the antifungal agent was used as negative control.

CONCLUSION

LABs with potential to control the growth of *C. albicans* could be isolated from many sources, such as fermented foods (kimchi), honey, or vaginal secrete of healthy women. Among more than 100 isolates successfully isolated in our current study, 46 isolates showed *in vitro* antagonistic activity against *C. albicans*, and they also showed resistance property to fluconazole (an antifungal agent commonly used in the therapy of candidiasis), indicating that they all have potential to be synergically applied with this antifungal agent in the therapies of such infection. The most potential isolates (10 isolates) were closely related to three LAB species, namely *Lactobacillus paracasei, Lacticaseibacillus paracasei*, and *Pediococcus petosaceous*, based on their 16s rDNA sequence similarity with those deposited in the GenBank (http://www.ncbi.nlm.nih.gov).

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