Anticandidal activities of lactic acid bacteria isolated from the vagina

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1. Introduction
Human microbiota colonized in human body consists of trillions of microorganisms. Different microbial communities were located in the vagina, mouth, skin, gastrointestinal tract, nose, urethra, and other parts of the body (1).

Lactobacillus species are dominant in the vaginal microbiota of healthy women (2). Premenopausal healthy women have 10^7–10^8 colony forming unit/gram lactobacilli in their vaginal fluid (3). Furthermore, Staphylococcus spp., Ureaplasma spp., Corynebacterium spp., Streptococcus spp., Peptostreptococcus spp., Gardnerella vaginalis, Bacteroides spp., Mycoplasma spp., Enterococcus spp., Escherichia coli, Veillonella spp., Bifidobacterium spp., and Candida spp. are also found in the vagina (4).

The lactobacilli in the vaginal microbiota protect the flora against the colonization of other sexually transmitted infectious agents such as bacterial vaginosis, urinary tract infections, vulvovaginal candidiasis, and AIDS. It was suggested that this is carried out through adhesion to the vaginal epithelium cells by competing with the pathogens and by producing antimicrobials such as bacteriocin, hydrogen peroxide, and lactic acid (5).

Vulvovaginal candidiasis is a common infection seen in women throughout their lives. In some in vitro studies, some lactic acid bacterial strains were shown to inhibit the adhesion and development of Candida albicans (6). Drutz reported that oral administration of Lactobacillus acidophilus has a protective effect against Candida vaginitis (7).

In the present study, it was aimed to investigate the anticandidal activities of lactic acid bacteria isolated from the vagina of healthy women on some Candida species isolated from the vagina.

2. Material and methods
2.1. Isolation of microorganisms from the samples taken from the vagina
Lactic acid bacteria and yeast isolates were obtained through a physician from the samples taken from 30 healthy volunteer women aged between 20 and 40
years at the Gynecology Clinic of Medipol University Hospital, Istanbul. All were premenopausal and were not menstruating at the time of collection. All the studied women were clinically normal. They had not received antibiotics in the last 3 months. Vaginal samples were planted on the PDA (potato dextrose agar), chocolate agar, MRS (de Man, Rogosa, and Sharpe) agar, and M17 agar in the laboratory. For lactic acid bacteria isolation; MRS agar and M17 agar petri dishes were inoculated. The dishes were then incubated at 37 °C in 5% CO₂ for 48–72 h. For yeast isolation, the PDA was inoculated and then the petri dishes were incubated at 30 °C for 5 days. Isolation of colonies developed in the petri dishes after incubation was performed. Primarily, Gram stains and catalase tests of the isolated microorganisms were performed. Gram-positive, catalase-negative bacterial isolates were separated as lactic acid bacteria and oxidase; mobility tests, development at different temperatures (4, 15, and 45 °C), development at different salt concentrations (6%, 7.5%, and 10% NaCl), development at different pH levels (pH 3.9 and 9.6), H₂S formation, and ammonia formation from arginine were performed (8–9).

The microorganisms isolated from MRS agar, M17 agar, and PDA were stored at −85 °C. The permission of the ethical committee of our study was taken from the Ethics Committee of Non-Interventional Clinical Researches at the Istanbul Medipol University on Dec. 11, 04. All applicable international, national, and/or institutional guidelines for the care and use of human were followed.

2.1.1. Determination of the use of carbohydrates with API CHL 50 by lactic acid bacteria isolates
The test was carried out by sowing API CHL 50 kits in accordance with the administrator's instructions and the microorganisms were defined according to the carbohydrate sources they used. As a result of the test, the color change results of the isolates were entered into the database optimized by the management company and species identification was obtained as the % rates.

2.1.2. Definition of the yeast isolates by MALDI-TOF mass spectrometry
The colony sample was taken from the yeast isolate which was activated in PDA at 37 °C for 48 h and was planted on a 48-well plate. 0.3 µL of matrix solution was added onto the plate surface. In the device, the result obtained by performing the robust protein ionization and molecular weight measurement of the microorganism was based on the definition of microorganism by comparing it with the database. This part of the study was performed by BioMérieux Diagnostics Incorporated Company (Marcy-l’Étoile, France).

2.1.3. 16s rRNA sequence analysis
16s rRNA sequence analysis was performed for genotypic identification of the bacteria isolate. The genomic DNA of the isolate was purified using GeneJET genomic DNA purification kit (Thermo Fisher Scientific, Waltham, MA, USA). The obtained genomic DNA was used as template DNA and PCR reaction was performed for 16s rRNA gene locus. 27F 5’ AGAGTTTGATCMTGGCTCAG-3’ and 1492R 5’TACGGYTACCTTGTACGACTT-3’ universal primers were used. PCR reaction components include 2.5 µL of 10X Taq buffer (+ KCl–MgCl₂), 2.5 µL of 25 mM MgCl₂, 2.5 µL of 2.5 mM dNTP mix, 2.5 µL of 2.5 mM 27F primer, 2.5 µL of 2.5 mM 1492R primer, 0.25 µL of Taq polymerase (5 U/µL), 11.75 µL of nuclease-free ddH₂O, and 1 µL of template DNA. PCR products obtained from the reaction were screened in 1% agarose gel. 1492R and 907R (5’-CCGTCAATTCMTTTRAGTTT - 3’) primers were used for the sequence analysis of nearly 1400 base pair region (10). The sequence analysis of isolate was performed by MedSanTek Laboratory Supplies Trade & Industry Ltd (İstanbul, Turkey).

2.1.4. Determination of lactic acid production of the lactic acid bacteria isolates
The lactic acid bacteria isolate was incubated in the MRS broth medium at 37 °C and 5% CO₂ for 48 h. Following the incubation, 1 mL of fresh lactic acid bacteria culture was transferred to a clean flask and filled up to 100 mL by sterile dH₂O. Two or three drops of phenolphthalein indicator were added and titrated with 0.1 M NaOH solution. The amount of NaOH was recorded. Acid produced by the culture was calculated as percent titrable acidity. Lactic acid amount produced by the bacteria was calculated by the formula below. The study was conducted in duplicate (11).

Acidity %: 0.1 N NaOH (mL) amount used × 0.9/mL

2.1.5. Determination of hydrogen peroxide production of the lactic acid bacteria isolates
5 mL of distilled water was added to the lactic acid bacteria cultures and centrifuged at 5000 rpm for 15 min. After centrifugation, the clear liquid formed on top was removed and filtered through Whatman No. 42 filter paper (Buckinghamshire, UK). After filtration, 4 mL of the obtained filtrate was taken into a separate tube. On top of this filtrate, 0.5 mL of sulfuric acid, 0.5 mL of ammonium molybdenum, and 0.5 mL of potassium iodide solution were added, and after each chemical addition, the samples were thoroughly vortexed. After all these processes were carried out, the optical densities of the obtained liquid were determined at 350 nm wavelength using a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan). The obtained optical density (OD) values were calculated in terms of µg/mL according to the previously prepared standard curve (12).

2.2. Determination of anticandidal activity
Anticandidal activity was investigated by agar spot technique. For this purpose, the lactic acid bacteria
3. Results

3.1. Isolation of microorganisms from the vaginal samples

Lactic acid bacteria and yeast isolates were obtained from samples taken from 30 healthy females aged between 20 and 40 years at the Gynecology Clinic of Medipol University Hospital, Istanbul. It was made sure that the healthy women were not pregnant and that they had not used antibiotics in the last 3 months. It was demonstrated in the study that the 49 isolates isolated from MRS and M17 agars were catalase-negative (−), gram-positive (+). 14 isolates (BMR11, 13P1, 18P1, 19P3, 21P2, 24P1, 30P1, 14P1, 27P2, 13P2, 17P2, 16P1, 1C3, 5MR2 isolates), on the other hand, were found to be yeast. The isolates isolated from MRS, M17, potato dextrose, and chocolate agars were named to include “MR”, “M”, “P”, and “C” codes, respectively.

In Table 1, the test results of 49 gram-positive (+) bacilli and catalase-negative (−) isolates for oxidase activity, growth at different temperatures, development at different salt concentrations, formation of hydrogen sulphide, and formation of ammonia from arginine are illustrated. According to these results, it was determined that all of the 49 isolates were oxidase-negative and immobilized. If we look at the development at different pH levels, 42 isolates developed at pH 3.9, while 7 isolates showed no improvement. Furthermore, 45 isolates showed a pH of 9.6 and 4 of them did not. Regarding the developments in different salt concentrations, 19 isolates were produced in the medium containing 6% NaCl and 30 isolates were not produced. 12 isolates were produced in the medium containing 7.5% NaCl, and 37 isolates were isolated. In the medium containing 10% NaCl, 9 isolates were produced and 40 isolates were not produced. If we look at the developments at different temperatures, 13 isolates grew at 4 and 15 °C and 36 did not show any growth. At 45 °C, 43 isolates showed growth, 6 did not show any growth. As for the formation of hydrogen sulphide, no hydrogen sulphide or gas formation was observed in any of the 49 isolates. Ammonia was also formed from 18 isolate arginine and no ammonia was formed from 31 isolate arginine (Table 1).

3.1.1. Determination of carbohydrate utilization status of the lactic acid bacteria isolates with API CHL 50 system

Identification results of 49 gram-positive (+) bacilli and catalase-negative (−) bacterial isolates isolated with the API CHL50 system are illustrated in Table 2. According to these results, of the 49 isolates, 8 were identified as Lactobacillus acidophilus, 3 as Lactobacillus plantarum, 9 as Lactobacillus pentosus, 8 as Lactobacillus fermentum, 17 as Lactobacillus paraescei subsp. paraescei, 13 as Lactobacillus crispatius, and 2 as Lactobacillus delbrueckii subsp. delbrueckii.

3.1.2. Definition of yeast isolates by the MALDI-TOF mass spectrometry

By the MALDI-TOF Mass Spectrometry, 10 of 14 yeast isolates were Candida albicans, 3 was C. glabrata, and 1 was C. tropicalis.

3.1.3. Results of the 16s rRNA sequence analysis

The 5MR1, 5MR6, and 10MR5 isolates with high anticandidal activity were identified as Lactobacillus jensenii (GenBank accession no: MH327499), Enterococcus faecalis (GenBank accession no: MH327502) with 99% similarity according to genotypic characterization results by 16S RNA sequence analysis, and L. jensenii (GenBank accession no: MH327501), respectively.

3.1.4. Lactic acid and hydrogen peroxide production of the lactic acid bacteria isolates

Table 3 illustrates the amounts of lactic acid and hydrogen peroxide in the lactic acid bacteria isolated from the vagina. While the lactic acid production of 49 isolates was between %0.91 and %2.684, it was found that the 10M7 isolate produced the lowest and the 5MR6 isolate produced the highest amount of lactic acid. As for the production of hydrogen peroxide, it was established that the production of hydrogen peroxide of 72 isolates was between 0.308 and 0.863 μg/mL. Furthermore, it was found that the 5MR8 isolate produced the lowest and 10M3 isolate produced the highest amount of hydrogen peroxide.

3.2. Anticandidal activities of the lactic acid bacteria isolates

Table 4 illustrates the anticandidal activity results of 49 lactic acid bacteria isolated and identified from the vagina against the 14 Candida species (C. tropicalis 1C3, C. glabrata, C. albicans, C. krusei, C. dubliniensis, C. parapsilosis, C. glabrata, C. paracasei, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. glabrata, C. gla...
5MR2, C. glabrata 16P, C. glabrata 17P2, C. albicans 8MR11, C. albicans 13P1, C. albicans 13P2, C. albicans 14P1, C. albicans 18P1, C. albicans 19P3, C. albicans 21P2, C. albicans 24P1, C. albicans 27P2, C. albicans 30P) isolated from the vagina again. As far as the results are concerned, most of the lactic acid bacteria had variation of 8–44 mm of zone formation against the 14 Candida isolates. It was found that the lowest zone formation (8 mm) and the L. acidophilus 10MR14 isolate took place on the C. glabrata 8MR11 isolate, and the highest zone formation (44 mm), on the other hand, L. acidophilus 45°C isolate took place on the C. glabrata 16P isolate.

Table 1. Biochemical activity tests of gram-positive (+) bacilli and catalase-negative (−) bacteria. (+): positive, (−): negative.

| Microorganism                  | Isolate name                  | Oxidase | Mobility | Growing at pH 3.9 | Growing at pH 9.6 | Growing at 6% NaCl | Growing at 7.5% NaCl | Growing at 10% NaCl | Growing at 4°C | Growing at 15°C | Growing at 45°C | H₂S formation | Ammonia formation from arginine |
|-------------------------------|-------------------------------|---------|----------|-------------------|-------------------|-------------------|---------------------|---------------------|---------------|----------------|----------------|----------------|-----------------------------|
| Lactobacillus crispatus       | 7MR5, 7MR7.4                  | (−)     | (−)      | (+)               | (+)               | (−)               | (−)                 | (−)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
|                               | 7MR1                          | (−)     | (−)      | (+)               | (+)               | (+)               | (+)                 | (+)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
|                               | 8MR19, 10MR3                  | (−)     | (−)      | (+)               | (+)               | (+)               | (+)                 | (+)                 | (−)           | (−)           | (+)           | (−)           | (−)                        |
|                               | 8MR20, 8MR4, 8MR9, 8MR3       | (−)     | (−)      | (+)               | (+)               | (+)               | (+)                 | (+)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
|                               | 7MR4                          | (−)     | (−)      | (+)               | (+)               | (+)               | (+)                 | (+)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
|                               | 8MR1                          | (−)     | (−)      | (+)               | (+)               | (+)               | (+)                 | (+)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
| Lactobacillus fermentum       | 5MR3, 5MR4                    | (−)     | (−)      | (+)               | (+)               | (+)               | (+)                 | (+)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
|                               | 1MR1.1, 5MR6                  | (−)     | (−)      | (+)               | (+)               | (+)               | (+)                 | (+)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
|                               | 11MR4                         | (−)     | (−)      | (+)               | (+)               | (+)               | (+)                 | (+)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
|                               | 5MR8, 5MR1                    | (−)     | (−)      | (+)               | (+)               | (+)               | (+)                 | (+)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
|                               | 11MR20                        | (−)     | (−)      | (+)               | (+)               | (+)               | (+)                 | (+)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
| Lactobacillus acidophilus      | 10MR5, 10MR15, 10MR4, 8MR2, 10MR14, 10MR6 | (−) | (−) | (+) | (+) | (−) | (−) | (−) | (−) | (−) | (+) | (+) | (−) |
|                               | 1MR3                          | (−)     | (−)      | (+)               | (+)               | (+)               | (+)                 | (+)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
| Lactobacillus paracasei subsp. paracasei | 10MR2, 8M1                    | (−)     | (−)      | (+)               | (+)               | (+)               | (+)                 | (+)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
|                               | 10MR7, 10MR8, 10MR9, 10MR18   | (−)     | (−)      | (+)               | (+)               | (−)               | (−)                 | (−)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
|                               | 3M2, 3M6, 4M10, 4M5            | (−)     | (−)      | (+)               | (+)               | (+)               | (+)                 | (+)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
|                               | 2M2                           | (−)     | (−)      | (+)               | (+)               | (+)               | (+)                 | (+)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
|                               | 8M4                           | (−)     | (−)      | (+)               | (+)               | (+)               | (+)                 | (+)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
| Lactobacillus pentosus        | 11MR12, 11MR19                | (−)     | (−)      | (+)               | (+)               | (+)               | (+)                 | (+)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
|                               | 10M3, 10M10                   | (−)     | (−)      | (+)               | (+)               | (−)               | (−)                 | (−)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
|                               | 9M8                           | (−)     | (−)      | (+)               | (+)               | (−)               | (−)                 | (−)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
| Lactobacillus plantarum       | 9M1, 9M3                      | (−)     | (−)      | (+)               | (+)               | (−)               | (−)                 | (−)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
|                               | 9M7                           | (−)     | (−)      | (+)               | (+)               | (−)               | (−)                 | (−)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
| Lactobacillus delbrueckii subsp. delbrueckii | 10MR12                      | (−)     | (−)      | (+)               | (+)               | (−)               | (−)                 | (−)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |
|                               | 10MR13                        | (−)     | (−)      | (+)               | (+)               | (−)               | (−)                 | (−)                 | (−)           | (−)           | (−)           | (−)           | (−)                        |

MR: MRS agar, M: M17 agar. The bacteria isolated from MRS and M17 agars were named to include “MR” and “M” codes, respectively. NaCl: Sodium chloride, H₂S: hydrogen sulphide.
Lactobacillus species are dominant in the vaginal microbiota (14). It was reported that Lactobacillus acidophilus, L. plantarum, L. casei, L. cellobiotus, L. oris, L. reuteri, L. ruminis, L. salivarius, L. brevis, L. delbrueckii, and L. vaginalis species were commonly isolated from the vagina (15). The Lactobacillus in the vaginal microbiota protected the microbiota against the colonization of other sexually transmitted infections such as bacterial vaginosis, urinary tract infections, vulvovaginal candidiasis, and HIV (5).

In the present study, antifungal activities of various lactic acid bacteria isolated from the vagina of healthy volunteer women on the 14 vaginal Candida isolates were investigated. For this purpose, it was firstly determined that all the 49 lactic acid bacteria isolates were oxidase-negative and immobilized. It was observed that most isolates grew at pH 3.9 and pH 9.6. In addition, it was found that most isolates did not grow in medium containing 6%, 7.5%, and 10% NaCl and at 4, 15, and 45 °C. As for the formation of hydrogen sulphide, no hydrogen sulphide or gas formation was observed in any of the 49 isolates (Table 1). It was reported by Pektaş that not all of the 136 lactic acid bacteria isolates produced H₂S (16). Similarly in our study, it was found that 18 of the 49 isolates did not form ammonia from arginine of 31 isolates, which formed ammonia from arginine (17).

As a result of the identification of the isolated lactic acid bacteria by the API CHL 50 system, it was found that the 49 lactic acid bacteria isolates mostly belonged to L. paracasei subsp. paracasei (at the ratio of 27%) and to L. crispatus (at the ratio of 22.4%), followed by L. fermentum (16.3%), L. acidophilus (14.3%), L. pentosus (10.2%), L. plantarum (6.1%), and L. delbrueckii subsp. delbrueckii (4.1%) (Table 2). It was determined that 10 out of 14 yeast isolates isolated from the vagina by MALDI-TOF mass spectrometry belonged to C. albicans (8MR11, 13P1, 13P2, 14P1, 18P1, 19P3, 21P2, 24P1, 27P2, 30P), 3 isolates to C. 

Table 2. Identification of lactic acid bacteria with API CHL 50 system.

| Isolate name | API CHL 50 result | % of similarity |
|--------------|-------------------|----------------|
| 7MR4         | Lactobacillus crispatus | 99.9 |
| 7MR5, 7MR1, 8MR9, 10MR3, 8MR3, 8MR4, 8MR1 | L. crispatus | 99.7 |
| 7MR7.4       | L. crispatus | 99.3 |
| 8MR19, 8MR20 | L. crispatus | 98.4 |
| 5MR3, 5MR1, 5MR8, 5MR4, 5MR6, 11MR4, 11MR20 | L. fermentum | 95.3 |
| 1MR1.1       | L. fermentum | 88.9 |
| 10MR5, 10MR15, 10MR4, 10MR14 | Lactobacillus acidophilus | 97.4 |
| 1MR3         | L. acidophilus | 98.6 |
| 8MR2, 10MR6  | L. acidophilus | 95.3 |
| 10MR7, 4M5, 8M4 | Lactobacillus paracasei subsp. Paracasei | 99.9 |
| 10MR2, 10MR8, 3M2, 3M6, 10M7 | L. paracasei subsp. paracasei | 99.6 |
| 8M1          | L. paracasei subsp. paracasei | 97.7 |
| 10MR9, 10MR18 | L. paracasei subsp. paracasei | 80.5 |
| 2M2          | L. paracasei subsp. paracasei | 78.1 |
| 4M10, 11MR12, 11MR19 | Lactobacillus pentosus | 99.9 |
| 10M10, 10M3  | L. pentosus | 92.0 |
| 9MR4         | L. pentosus | 88.9 |
| 9M3          | Lactobacillus plantarum | 99.2 |
| 9M7          | L. plantarum | 80.5 |
| 9M1          | L. plantarum | 72.6 |
| 10MR12, 10MR13 | L. delbrueckii subsp. delbrueckii | 92.8 |

MR: MRS agar, M: M17 agar. The bacteria isolated from MRS and M17 agars were named to include “MR” and “M” codes, respectively.

4. Discussion

Lactobacillus species are dominant in the vaginal microbiota (14). It was reported that Lactobacillus acidophilus, L. plantarum, L. casei, L. cellobiotus, L. oris, L. reuteri, L. ruminis, L. salivarius, L. brevis, L. delbrueckii, and L. vaginalis species were commonly isolated from the vagina (15). The Lactobacillus in the vaginal microbiota protected the microbiota against the colonization of other sexually transmitted infections such as bacterial vaginosis, urinary tract infections, vulvovaginal candidiasis, and HIV (5).

In the present study, antifungal activities of various lactic acid bacteria isolated from the vagina of healthy volunteer women on the 14 vaginal Candida isolates were investigated. For this purpose, it was firstly determined that all the 49 lactic acid bacteria isolates were oxidase-negative and immobilized. It was observed that most isolates grew at pH 3.9 and pH 9.6. In addition, it was found that most isolates did not grow in medium containing 6%, 7.5%, and 10% NaCl and at 4, 15, and 45 °C. As for the formation of hydrogen sulphide, no hydrogen sulphide or gas formation was observed in any of the 49 isolates (Table 1). It was reported by Pektaş that not all of the 136 lactic acid bacteria isolates produced H₂S (16). Similarly in our study, it was found that 18 of the 49 isolates did not form ammonia from arginine of 31 isolates, which formed ammonia from arginine (Table 1). It was reported that Lactobacilli such as L. hilgardii, L. buchneri, and L. brevis could hydrolyze the arginine (17).

As a result of the identification of the isolated lactic acid bacteria by the API CHL 50 system, it was found that the 49 lactic acid bacteria isolates mostly belonged to L. paracasei subsp. paracasei (at the ratio of 27%) and to L. crispatus (at the ratio of 22.4%), followed by L. fermentum (16.3%), L. acidophilus (14.3%), L. pentosus (10.2%), L. plantarum (6.1%), and L. delbrueckii subsp. delbrueckii (4.1%) (Table 2). It was determined that 10 out of 14 yeast isolates isolated from the vagina by MALDI-TOF mass spectrometry belonged to C. albicans (8MR11, 13P1, 13P2, 14P1, 18P1, 19P3, 21P2, 24P1, 27P2, 30P), 3 isolates to C.
Table 3. The amounts of lactic acid and hydrogen peroxide of the lactic acid bacteria.

| Microorganism                  | Isolate name | % of acidity | The amount of hydrogen peroxide (µg/mL) |
|--------------------------------|--------------|--------------|----------------------------------------|
| L. crispatus                   | 7MR5         | 2.366        | 0.629 ± 0.034                           |
|                                | 7MR7.4       | 2.093        | 0.584 ± 0.166                           |
|                                | 7MR1         | 2.184        | 0.680 ± 0.025                           |
|                                | 8MR19        | 2.138        | 0.620 ± 0.064                           |
|                                | 10MR3        | 2.184        | 0.473 ± 0.074                           |
|                                | 8MR20        | 2.502        | 0.682 ± 0.019                           |
|                                | 8MR4         | 1.82         | 0.502 ± 0.123                           |
|                                | 8MR9         | 1.911        | 0.607 ± 0.123                           |
|                                | 8MR3         | 1.41         | 0.406 ± 0.001                           |
|                                | 7MR4         | 2.275        | 0.589 ± 0.000                           |
|                                | 8MR1         | 1.547        | 0.705 ± 0.080                           |
| L. fermentum                   | 5MR3         | 1.911        | 0.389 ± 0.106                           |
|                                | 5MR4         | 1.956        | 0.572 ± 0.006                           |
|                                | 1MR1.1       | 2.093        | 0.499 ± 0.199                           |
|                                | 5MR6         | 2.684        | 0.555 ± 0.164                           |
|                                | 11MR4        | 1.797        | 0.602 ± 0.079                           |
|                                | 5MR8         | 2.002        | 0.308 ± 0.079                           |
|                                | 5MR1         | 2.229        | 0.620 ± 0.060                           |
|                                | 11MR20       | 2.093        | 0.633 ± 0.049                           |
| L. acidophilus                 | 10MR5        | 2.366        | 0.663 ± 0.079                           |
|                                | 10MR15       | 1.638        | 0.628 ± 0.077                           |
|                                | 10MR4        | 1.911        | 0.773 ± 0.000                           |
|                                | 8MR2         | 1.365        | 0.389 ± 0.030                           |
|                                | 10MR14       | 1.82         | 0.597 ± 0.066                           |
|                                | 10MR6        | 2.548        | 0.584 ± 0.043                           |
|                                | 1MR3         | 1.41         | 0.569 ± 0.016                           |
| L. paracasei subsp. paracasei  | 10MR2        | 2.275        | 0.730 ± 0.000                           |
|                                | 8M1          | 1.001        | 0.756 ± 0.019                           |
|                                | 10MR7        | 1.274        | 0.392 ± 0.008                           |
|                                | 10MR8        | 1.456        | 0.699 ± 0.068                           |
|                                | 10MR9        | 1.683        | 0.607 ± 0.017                           |
|                                | 10MR18       | 1.729        | 0.652 ± 0.071                           |
|                                | 3M2          | 1.183        | 0.808 ± 0.040                           |
|                                | 3M6          | 1.41         | 0.778 ± 0.022                           |
|                                | 4M5          | 1.592        | 0.807 ± 0.025                           |
|                                | 4M10         | 1.228        | 0.770 ± 0.026                           |
|                                | 2M2          | 1.092        | 0.829 ± 0.030                           |
|                                | 10M7         | 0.91         | 0.822 ± 0.059                           |
|                                | 8M4          | 1.183        | 0.764 ± 0.036                           |
| L. pentosus                    | 11MR12       | 1.865        | 0.672 ± 0.001                           |
|                                | 11MR19       | 1.365        | 0.411 ± 0.000                           |
|                                | 10M3         | 1.274        | 0.863 ± 0.096                           |
|                                | 10M10        | 1.137        | 0.834 ± 0.027                           |
|                                | 9M8          | 1.046        | 0.805 ± 0.046                           |
| L. plantarum                  | 9M1          | 1.092        | 0.793 ± 0.001                           |
|                                | 9M3          | 1.092        | 0.836 ± 0.034                           |
|                                | 9M7          | 0.955        | 0.802 ± 0.047                           |
| L. delbrueckii subsp. delbrueckii | 10MR12 | 1.638        | 0.707 ± 0.160                           |
|                                | 10MR13       | 1.911        | 0.364 ± 0.041                           |

MR: MRS agar, M: M17 agar. The bacteria isolated from MRS and M17 agars were named to include “MR” and “M” codes, respectively.
Table 4. Anticandidal activities of the lactic acid bacteria.

| Microorganism                          | Isolate name             | 1C3 (C. tropicalis) | 5MR2 (C. glabrata) | 16P (C. glabrata) | 17P2 (C. glabrata) | 8MR11 (C. albicans) | 13P1 (C. albicans) | 13P2 (C. albicans) | 14P1 (C. albicans) | 18P1 (C. albicans) | 19P3 (C. albicans) | 21P2 (C. albicans) | 24P1 (C. albicans) | 30P (C. albicans) |
|----------------------------------------|--------------------------|---------------------|--------------------|-------------------|-------------------|---------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| L. crispatus                           | 7MR5, 7MR1, 8MR4, 7MR7.4, 8MR19, 8MR20 | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
|                                        | 10MR3                    | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
|                                        | 7MR4                     | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
|                                        | 8MR3, 8MR1, 8MR9         | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
| L. fermentum                           | 5MR3                     | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
|                                        | 1MR1, 1.1, 11MR4         | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
|                                        | 5MR8, 5MR6, 5MR4, 5MR1   | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
|                                        | 11MR20                   | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
| L. acidophilus                         | 10MR5                    | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
|                                        | 10MR15, 10MR4            | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
|                                        | 8MR2                     | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
|                                        | 10MR14, 10MR6            | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
|                                        | 11MR3                    | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
| L. paracasei subsp. paracasei          | 10MR2, 10MR7, 10MR8      | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
|                                        | 3M2, 3M6, 4M10           | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
|                                        | 2M2, 4M5, 8M1, 10M7      | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
|                                        | 8M4                      | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
|                                        | 10MR9, 10MR18            | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
| L. pentosus                            | 11MR12                   | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
|                                        | 11MR19                   | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
|                                        | 10M10, 10M3              | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
|                                        | 9M8                      | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
| L. plantarum                          | 9M3, 9M7                 | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
|                                        | 9M1                      | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |
| L. delbrueckii subsp. delbrueckii      | 10MR12, 10MR13           | (+)                 | (+)                | (+)                | (+)                | (+)                 | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                | (+)                |

The microorganisms isolated from MRS, M17, Potato dextrose, and chocolate agars were named to include "MR", "M","P", and “C” codes, respectively. Zone diameters are given in mm. (+):2 to ≥13 mm; (++) 14 to ≥25 mm; (+++) 26 to ≥38 mm.
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