Saudi Journal of Biological Sciences 29 (2022) 2989–2997

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Anti-biofilm potential of *Lactobacillus plantarum* Y3 culture and its cell-free supernatant against multidrug-resistant uropathogen *Escherichia coli* U12

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ARTICLE INFO

Article history:
Received 15 September 2021
Revised 8 January 2022
Accepted 10 January 2022
Available online 15 January 2022

Keywords:
Biofilms
Lactic acid bacteria
Adherence

ABSTRACT

Uropathogens develop biofilms on urinary catheters, resulting in persistent and chronic infections that are associated with resistance to antimicrobial therapy. Therefore, the current study was performed to control biofilm-associated urinary tract infections through assaying the anti-biofilm ability of lactic acid bacteria (LAB) against multidrug-resistant (MDR) uropathogens. Twenty LAB were obtained from pickles and fermented dairy products, and screened for their anti-biofilm and antimicrobial effects against MDR *Escherichia coli* U12 (ECU12). *Lactobacillus plantarum* Y3 (LPY3) (MT498405), showed the highest inhibitory effect and biofilm production. Pre-coating of a microtitre plate with LPY3 culture was more potent than co-incubation. Pre-coating with LPY3 culture generated a higher anti-biofilm effect with an adherence of 14.5% than cell free supernatant (CFS) (31.2%). Anti-biofilm effect of CFS was heat stable up to 100 °C with higher effect at pH 4–6. Pre-coating urinary catheter with LPY3 culture reduced the CFU/cm^2 of ECU12 attached to the catheter for up to seven days. Meanwhile, CFS reduced the ECU12 CFU/cm^2 for up to four days. Scanning electron microscope confirmed the reduction of ECU12 adherence to catheters after treatment with CFS. Therefore, *Lactobacillus plantarum* can be applied in medical devices as prophylactic agent and as a natural biointervention to treat urinary tract infections.

1. Introduction

Biofilms are composed of sessile growth of microbes found in the extracellular matrix and attached to a surface as aggregates. Biofilms formation protects pathogens from hard conditions, antimicrobial treatments in addition to the host immune responses. Furthermore, the principal factor for causing different chronic diseases is the pathogens biofilms that lowering the efficacy of the available antibiotics with higher minimum inhibitory concentrations. The antibiotics therapy changes normal flora and the resistant strains emergence, making biofilms treatment efficacy more difficult (Geerlings et al., 2014; Roy et al., 2018).

Catheterization with urinary catheters for longer periods leads to biofilms formation by pathogenic microorganisms and their colonization on indwelling urinary catheters. Pathogens biofilms formed on urinary catheters may be bacteria and yeasts (Percival et al., 2015). Catheterization causes serious recurrent problems, including catheter-associated urinary tract infection (CAUTI), and the prevalent preventive strategy for prevention of CAUTI is ineffective due to the biofilms formation on urinary catheters (Trautner et al., 2012; Werneburg et al., 2020).

Probiotics represent the best alternative strategy to reduce and eradicate biofilm-related medical devices such as catheters that cause serious infections (Panigrahy and Kumar, 2019). Probiotics are the natural, benign bacteria found in the healthy gut of all humans. Lactic acid bacteria represent the largest portion that pro-

Abbreviations: **LAB**, Lactic acid bacteria; **LPY3**, *Lactobacillus plantarum* Y3; **CAUTI**, catheter associated urinary tract infection; **MRS**, De Man, Rogosa, and Sharpe; **BHI**, brain heart infusion; **CRA**, congo red agar; **CV**, crystal violet; **PBS**, phosphate-buffered saline; **CFU**, colony forming unit; **SEM**, scanning electron microscope.

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Peer review under responsibility of King Saud University.

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https://doi.org/10.1016/j.sjbs.2022.01.014
1319-562X/

vide benefits to the host, such as *Lactobacillus, Streptococcus, Leuconostoc, Streptococcus, and Lactococcus* (de Melo Pereira et al., 2018). These probiotics bacteria are applied in the medical field to stimulate the immune system, improve digestion, remediation of lactose intolerance, reducing of cholesterol levels, and reduce allergy (Nazir et al., 2018; Oak and Jha, 2019). Probiotics exert their beneficial effects against pathogenic microorganisms by different mechanisms such as competition, exclusion, and displacement. The competition involves both co-culture of probiotics or the secreted antimicrobial compounds with pathogens, and exclusion involves pre-coating with probiotics or the secreted antimicrobial compounds to prevent pathogen biofilms formation. Meanwhile, displacement involves the breakdown of preformed biofilms of pathogenic microorganisms by probiotics (Prabhurajeshwar and Chandrakanth, 2017).

Lactobacilli are benign bacteria and are considered as the most common natural microbiota safe for human health. *Lactobacilli* is used as a probiotic and an interfering bacterium that has good antimicrobial effect against different pathogenic bacteria and can interfere with the adhesion of pathogenic micro-organisms to urogenital tract cells (Markowiak and Śliżewska, 2017; Monteagudo-Mera et al., 2019); thus, these *lactobacilli* were used for treating several diseases, including urinary and vaginal tract infections. Hence, bacterial interference using *lactobacilli* represent the best intervention strategy for inhibition and eradication of biofilms from urinary catheter (Gupta et al., 2017; Ng et al., 2018; Barzegari et al., 2020). The purpose of the present study was to assess anti-biofilm and antimicrobial activities of LAB, obtained from pickles and fermented dairy products against multidrug-resistant uropathogen obtained from catheterized patients with urinary catheters. This study provides a good strategy by using potent probiotic to control and eradicate biofilm formation associated with serious diseases such as urinary tract infections.

1.1. Contributions. The following are our contributions to this study:

i) we did antibacterial and anti-biofilm activities of LPY3 against uropathogen ECU12. Our study showed that, the LPY3 isolated from yoghurt was the most suitable natural biocontrol agent that exhibited significant antimicrobial and anti-biofilm activities against ECU12.

ii) LPY3 efficiently reduced biofilm formation of ECU12 on urinary catheter. Therefore, pre-coating urinary catheters with LPY3 can be largely utilized as anti-biofilm agent as well as a prophylactic agent in medical fields to control and inhibit uropathogens biofilms formation. This study was applied in vitro that could be investigated further for actual use.

2. Materials and methods

2.1. Food specimen

Twenty LAB were obtained from various types of food as pickles (two samples from pepper, two from olive, two from lemon, one from carrot, and one from mixed specimens) and fermented dairy products (six samples from yogurt, three from mesh, and three from karessh cheese). These samples were obtained from different markets in Dakhlia Governorate, Egypt, from January 2019 till November 2019. These collected specimens were utilized for the LAB isolation.

2.2. The lactic acid bacteria isolation

About 25 g from every food specimen were separately mixed vigorously with 0.85% NaCl solution at 25 °C. After that, from each food specimen, appropriate dilutions from 10^{-2} to 10^{-6} were designed, and each serial dilution (0.1 mL) was spread onto De Man, Rogosa, and Sharpe 1960 (MRS) agar plates surface as performed by Shukla et al. (2008). Incubation of the plates was done at 35 °C for 24 h, and purification was carried out on the same media.

2.3. Indicator organisms

Uropathogens (50 isolate) were obtained from catheterized patients with urinary catheters. These collected uropathogens were identified based on morphological, biochemical characteristics (Holt et al., 1994), and tested against different groups of antibiotics and their ability to form a biofilm (Figs. S1and S2) (Tables S1, S2, S3 and S4). Therefore, *E. coli* U12 was selected as the most MDR uropathogen and as the most biofilm producer isolate that used in the current study as an indicator organism.

2.4. LAB antibacterial assay against E. coli U12

The agar well diffusion technique assessed the antimicrobial effect of CFS of LAB isolates against ECU12. Cell-free supernatant was prepared as the following; an inoculum of each overnight LAB culture (24 h age) was inoculated into 100 mL of MRS broth and kept for 48 h at 37 °C in a shaking incubator. After incubation, the cultures were centrifugated at 5000 g for 10 min at 4 °C. Then, the cells were removed and the CFS of each LAB isolate was collected. The inhibitory effect of acid was avoided through adjust the CFS of each isolate using 1 M NaOH to pH 6.5, then sterilized by a 0.22 mm Millipore filter. The targeted MDR ECU12 (10^6 CFU/mL) was inoculated in the BHI agar surface. By sterile cork poorer, a well with a diameter of 6 mm was performed, and 50 μL of CFS was added to every well. The plates were kept for 24 h at 37 °C, and the inhibition zones diameter (mm) was evaluated (Reda, 2018).

2.5. Estimation of biofilm formation by LAB

The detection of biofilm production was conducted using congo red agar (CRA) (Bose et al., 2009) and crystal violet (CV) assay (Castelijn et al., 2012) methods.

In CRA method, medium ingredients were designed as the following; brain heart infusion (37 g/L), agar (10 g/L), sucrose (50 g/L), and CR stain (8 g/L). The concentrated aqueous solution from CR stain was designed, autoclaved at 121 °C for 15 min. After that, it was added to the autoclaved BHI agar when cooled at 55 °C then, media poured in petridishes and streaked with each LAB isolate, then kept at 37 °C for 24 h. The appearance of dry black crystalline colonies indicated that LAB were strong biofilm producers, when the appearance of colonies without crystalline and dry structure indicated as weak biofilm bacteria, and the appearance of pink colonies was indicated as negative biofilm bacteria. The test was repeated three times.

In the crystal violet (CV) assay, all the obtained LAB cultures were screened for their biofilm formations. The microtiter plate wells were filled with BHI broth medium and inoculated with 200 μL of overnight LAB cultures; then, kept at 30 °C for 48 h. Then, the wells were rinsed with phosphate-buffered saline (PBS) pH 7.1 (10 mM Na_2HPO_4, 1 mM KH_2PO_4, 3 mM KCl, 140 mM NaCl) three times. Biofilms that formed in wells were fixed for 30 min with methanol (99 %) and stained with CV (0.1% (W/V)). The wells were rinsed with PBS 3 times. After that, 70% (V/V) of ethanol was added to every well and kept for 30 min to solubilize adhered uropathogen. The optical density (OD) in every well was estimated at 595 nm by ELISA (Microplate reader, Molecular Devices, LLC, San Jose, CA, USA).

2.6. Identification of selected bacteria

Lactic acid bacteria isolates that give the potent antimicrobial effect, and strong biofilm formation were selected for further study. Genomic DNA was extracted (Hyronimus et al., 1998) and the identification of the selected LAB and uropathogen ECU12 was confirmed by 16S *rRNA* gene sequencing using universal primers; F (5-AGAGTTTGATCMTGGCTCAG-3′) and R (5-TACGGY TACCTTGTTACGACTT-3′) (Cibik et al., 2000). These isolates were subjected to sequence analysis by the Basic Local Alignment Search Tool program and submission was made in GenBank.

2.7. Assay of anti-biofilm effect of the selected LAB Y3 against E. coli U12

A biofilm inhibition test with the LAB Y3 culture and its CFS against ECU12 was conducted with co-incubation and pre-coating tests as described by Gudiña et al. (2010). In pre-coating tests, the microtiter plate wells were separately filled with LAB Y3 culture and its CFS (200 µL), and kept at 4 °C for 18 h. Then, the wells were rinsed 3 times with PBS (pH 7.2). Overnight ECU12 cultures (200 µL) (10^6 CFU/mL) was added to the wells and kept at 37 °C for 48 h. The biofilm formation of ECU12 was detected using ELISA Microplate Reader as described previously. ECU12 culture without LAB Y3 culture and its CFS was designed as a control. The percentage of microbial adhesion was calculated by the equation of Fracchia et al. (2010):

$$\%\text{Microbial adhesion}_C = (A_C/A_0) \times 100$$

where; A_C represents the absorbance of the well with LAB culture or CFS and A_0 represents the absorbance of the control well.

Meanwhile, in co-incubation tests, ECU12 (10^6 CFU/mL) was added to the wells together with LAB Y3 culture or CFS and kept at 37 °C for 48 h. Then, the wells were rinsed with PBS (pH 7.2). The following steps were done as mentioned above in pre-coating experiment.

2.8. Determination of different physical conditions of antimicrobial compounds secreted by LAB Y3

The aliquots of sterilized CFS was treated with different temperature values; 25, 30, 35, 40, 45, 50, 55, 70, 80, 90, and 100 °C for 30 min and at 121 °C for 15 min, to test the thermal stability of anti-biofilm effect of CFS. In addition, the CFS stability at different pH values (4, 5, 6, 7, 8, 9 and 10) was investigated. pH values of CFS were adjusted by HCl (1 N) and NaOH (1 N), and kept for 1 h at 25 °C, then readjusted at pH 7. The anti-biofilm effect of CFS against ECU12 was observed by the pre-coating experiment as mentioned above (Gudiña et al., 2010).

2.9. Determination of biofilm formation of E. coli U12 on urinary catheter

The counting colony-forming units' method was utilized to investigate the anti-biofilm effects of the selected LAB Y3 culture and its CFS against the adherence of ECU12 to urinary catheter pieces for seven consecutive days according to Ezeonu and Kanu (2016) with modifications. The catheter cutted into pieces 1 cm and placed in a 250 mL flask. After that, catheter pieces were separately coated with LAB Y3 culture and its CFS. Catheter pieces were rinsed twice with PBS pH 7.2. Afterward, overnight culture of ECU12 (10^6 CFU/mL) was added to the flask and kept at 37 °C for seven days. Additionally, untreated catheter parts were immersed in ECU12 cultures as a control. Viable cell count methodologies were used to determine the establishment of biofilms on catheter fragments. Each day, a catheter piece was cleaned with sterile distilled water and biofilms formation on catheter were collected using a sterile swab. Then, serial dilutions were done, and viable counting was carried out on MacConkey agar.

2.10. Study of scanning electron microscopy (SEM)

The adherence of ECU12 on the treated urinary catheter with CFS, after the first, second, and sixth day and control, was visualized by SEM. The adhered ECU12 on the urinary catheter specimens was fixed with 2.5% glutaraldehyde for at least three hours and rinsed twice in PBS. Graded concentrations of ethanol from 30% to 100% were added to urinary catheter specimens for dehydration. Each specimen was gold-coated and inspected with JEOL JSM 6510 IV SEM (Li et al., 2015).

3. Statistical analysis

One Way ANOVA test was utilized for statistical analysis of the attained data. IBM SPSS Corp did all statistical analysis. The significance of the obtained findings was estimated ($p < 0.05$). Each value of the obtained results is the mean of three replicates ± standard deviation.

4. Results

4.1. Antibacterial effect by LAB

Twenty LAB were obtained from pickles and fermented dairy products, and their antagonistic activities were tested against MDR ECU12 (Table 1). The results in Table 1 showed that the highest inhibitory effect was exhibited by LAB isolate code Y3 against ECU12 isolate (Fig. S3).

Table 1
Antimicrobial effect of CFS of LAB isolates against *E. coli* U12.

LAB isolate code	Diameter of inhibition zone (mm)
Y1	20 ± 0.52bc
Y2	18 ± 0.32de
Y3	25 ± 0.45a
Y4	21 ± 0.21b
Y5	19 ± 1.2 cd
Y6	18 ± 1.05d
CK7	19 ± 1.1 cd
CK8	0 ± 0
CK9	18 ± 0.54de
CM10	17 ± 0.32e
CM11	0 ± 0
CM12	21 ± 0.25b
PL13	19 ± 0.45 cd
PL14	17 ± 0.44e
PO15	0 ± 0
PO16	17 ± 1.27e
PM17	21 ± 1.21b
PC18	0 ± 0
PP19	20 ± 2.5bc
PP20	0 ± 0.0
L.S.D	1.22

Y: Yogurt, **CK:** Cheese (Karessh)), **CM:** Cheese (mesh), **PL:** Pickles (lemon), **PO:** Pickles (olive), **PM:** Pickles (mixed), **PC:** Pickles (carrot), **PP:** Pickles (pepper). Statistical analysis was performed by One- way ANOVA and L.S.D. The data are mean of three replicates ± standard deviation. Means with different letters within column are significant difference, $P \leq 0.05$–0.01. Means with the same letters within column are non significant difference. OD value. Biofilm formation.
< 0.120. No biofilm (-).
0.120:0.240. Moderate (+++).
> 0.240. Strong (++++).

4.2. Biofilm formation by LAB

All LAB isolates were assayed for their capability to form biofilms. The results in Table 2 showed that, LAB isolate code Y3 formed a strong biofilm. Consequently, this isolate code Y3 was selected as the most potent biofilm producer isolate for further studies.

4.3. Bacterial identification

Identification of LAB isolates Y3 and ECU12 were confirmed as *Lactobacillus plantarum* Y3 and *E. coli* U12, respectively, by PCR amplification of 16S *rRNA* gene. The partial nucleotide sequence of the amplified genes from *Lactobacillus plantarum* Y3 and ECU12 were submitted in GenBank with accession numbers MT498405, and MT498270, respectively. The phylogenetic trees are demonstrated in (Fig. 1 a, b).

4.4. Anti-biofilm effect of L. plantarum Y3 against E. coli U12

The percentage of biofilm formation by ECU12 was significantly ($P < 0.05$) decreased by its pre-coating either with LPY3 or with CFS and their co-incubation with LPY3 or with CFS represented percentages; 14.5, 31.2, 38.8, and 47.2%, respectively (Figs. 2 and S4). Moreover, pre-coating the microtitre plate with LPY3 culture was found to be more potent than co-incubation. Pre-coating with LPY3 culture generated high anti-biofilm effect against ECU12 isolate than its CFS.

4.5. Effect of different physical conditions on the anti-biofilm effect of CFS of LPY3

In this experiment, the anti-biofilm effect of CFS of LPY3 towards ECU12 was tested using a pre-coating experiment after exposure to different temperatures and pH values. The results showed that the anti-biofilm effect of CFS was heat-stable till 100 °C, whereas at 121 °C no effect was observed (Fig. 3). Anti-biofilm effect of the CFS was high at pH values from 4 to 6, and lower effect at alkaline pH values (Fig. 4).

Table 2
Biofilm production by LAB isolates.

LAB isolate code	OD of LAB biofilm formation
Y1	0.412 ± 0.002b
Y2	0.24 ± 0.001
Y3	0.611 ± 0.005a
Y4	0.411 ± 0.004b
Y5	0.312 ± 0.002bcd
Y6	0.39 ± 0.002bc
CK7	0.305 ± 0.001bcd
CK8	0.014 ± 0.005
CK9	0.34 ± 0.008bcd
CM10	0.223 ± 0.001 cd
CM11	0.005 ± 0.001
CM12	0.453 ± 0.004b
PL13	0.311 ± 0.005bcd
PL14	0.235 ± 0.008 cd
PO15	0.065 ± 0.001e
PO16	0.129 ± 0.001e
PM17	0.21 ± 0.001d
PC18	0.078 ± 0.001e
PP19	0.469 ± 0.001b
PP20	0.041 ± 0.003e
L.S.D	0.161

Statistical analysis was performed by One- way ANOVA and L.S.D. The data are mean of three replicates ± standard deviation Means with different letters within column are significant difference, $P \leq 0.05–0.01$. Means with the same letters within column are non significant difference.
OD value. Biofilm formation.
< 0.120. No biofilm (-).
0.120:0.240. Moderate (+++).
> 0.240. Strong (++++).

4.6. Inhibition of biofilm formation of E. coli U12 on urinary catheter

This experiment was applied in vitro to control biofilm formation of ECU12 on a urinary catheter using LPY3 culture and its CFS. The results in Table 3 and Fig. 5 indicated that the pre-coating urinary catheter pieces with LPY3 culture significantly ($P < 0.05$) lowered the CFU/cm^2 of ECU12 attached on urinary catheter after 1, 2, 3, 4, 5, and 6 days of incubation by about 3.9, 5.2, 6.2, 7.1, 7.9 and 8.3 log cycles, respectively, with complete inhibition being achieved at the seventh day. Meanwhile, CFS generated anti-biofilm effect by reducing the CFU/cm^2 of ECU12 on urinary catheter pieces for four days by about 6.1 log cycles.

4.7. Scanning electron microscopy (SEM)

SEM was used to test and confirm bacterial-loaded cell aggregation on urinary catheters following exposure to CFS of LPY3. The findings presented that the CFS arrested the bacterial growth and reduced the adhesion of ECU12 to the urinary catheter at the first and third day compared with the positive control (Fig. 6).

5. Discussion

Probiotics were utilized to lower the pathogenic bacteria growth related to the urinary tract, oral cavity, and human gut. Several medical studies recorded the inhibition of biofilm formation using probiotics, especially *Lactobacillus* species, to interfere with uropathogenic bacteria that cause serious diseases such as CAUTI (Darouiche and Hull 2012; Barzegari et al., 2020). Therefore, the control of biofilm formation by uropathogens using LAB was investigated. In the present study, LAB isolate code Y3 exhibited the highest inhibitory effect against ECU12 and formed a potent biofilm. This observation may be attributed to the inhibitory substances' production by probiotics like hydrogen peroxide, lactic acid, diacetyl, carbon dioxide, and bacteriocin that possess antimicrobial effect; during the growth of probiotic bacteria, the medium acidity increases, which destroyed the pathogenic bacteria because they were naturally sensitive to the acidic conditions (Vieco-Saiz et al., 2019).

Similarly, Ogunbanwo et al. (2003) indicated that CFS of the two probiotics *L. plantarum* and *L. brevis* had good antimicrobial effect and inhibited the *E. coli* growth, *Bacillus cereus,* and *Yersinia enterocolitica*. Also, Heredia-Castro et al. (2015) stated that *Lactobacillus* spp. produce bacteriocin that had antibacterial effect against *Salmonella typhimurium, Escherichia coli,* and *Staphylococcus aureus*. Drprabhurajeshwar and Chandrakanth (2019) reported that the *Lactobacillus* strains obtained from yogurt exhibited antagonistic effects against *E. coli, S. aureus, K. pneumonia, E. faecalis, Pseudomonas aerogenosa, Shigella* spp., and *S. typhii.* Reda and Refaie (2019) demonstrated that the CFS of *Pediococcus pentosaceus* N33 obtained from pickles showed the best inhibitory effects towards both *Lysinibacillus fusiformis* and *Brevibacillus brevis*. El-Mokhtar et al. (2020) reported that the CFS of *L. acidophilus* obtained from yogurt displayed potent antagonistic effect against *P. aeruginosa* and *K. pneumoniae*. Hashem and Abd El-Baky (2021) recorded that the CFS of *Lactobacilli* that was obtained from breastfed infants (healthy infants) showed a strong antimicrobial effect against uropathogenic MDR *E. coli*.

Catheter-associated urinary tract infections are associated with the production of biofilms on the urinary catheter, resulting in the

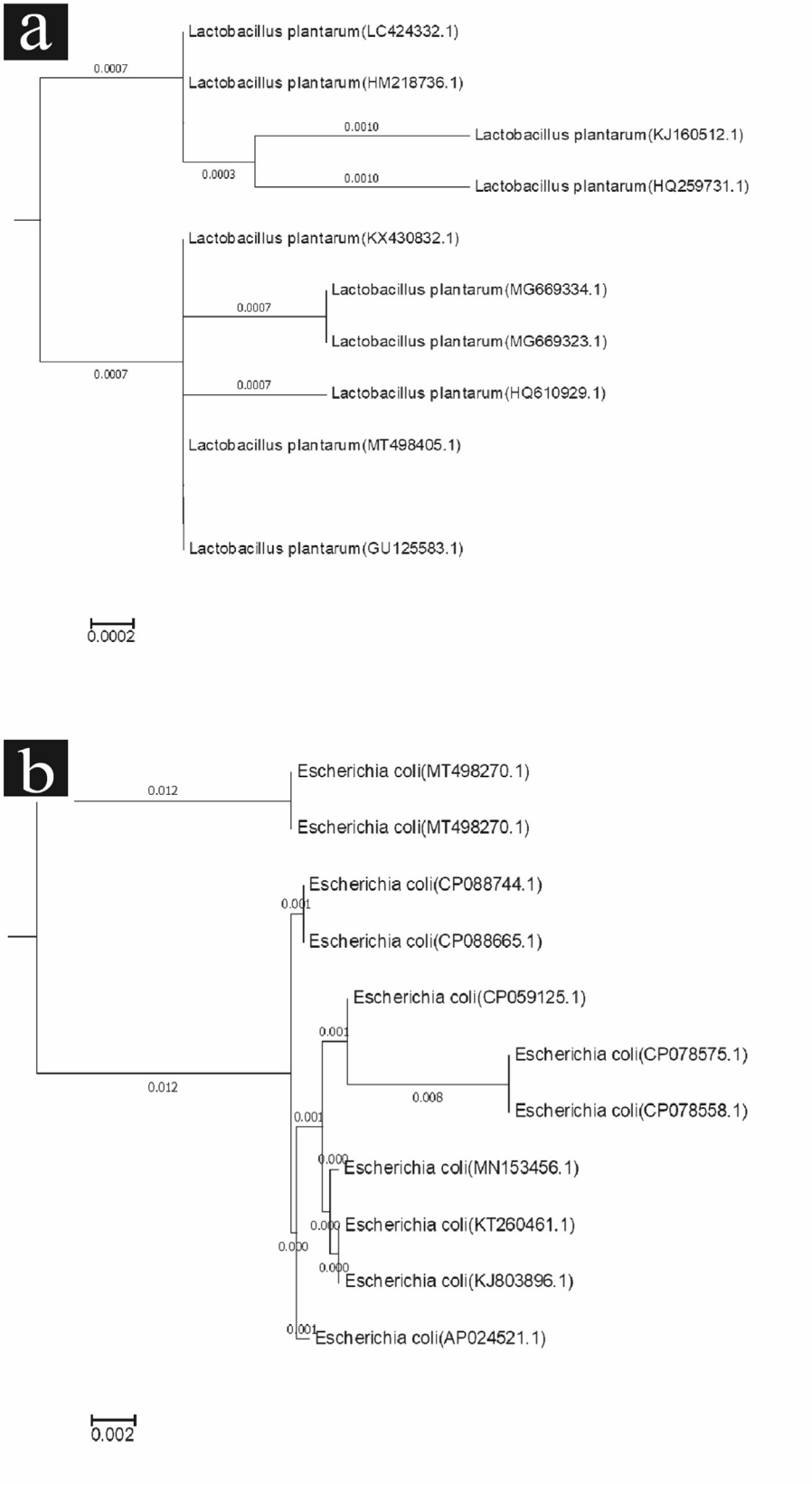

Fig. 1. Phylogenetic tree of *L. plantarum* Y3 (a), and *E. coli* U12 (b).

spread of uropathogens to the bladder and cause serious problems (Summers and Goeres, 2019; Almalki and Varghese, 2020).

Bacterial interference involves using a non-pathogenic bacterial biofilm and acting as an alternate strategy to fight biofilm-producing bacteria associated with devices. This strategy is considered a protective barrier to prevent uropathogens colonization (Chen et al., 2017; Mishra et al., 2020). In the current study, an alternative strategy was used that involved the use of probiotic

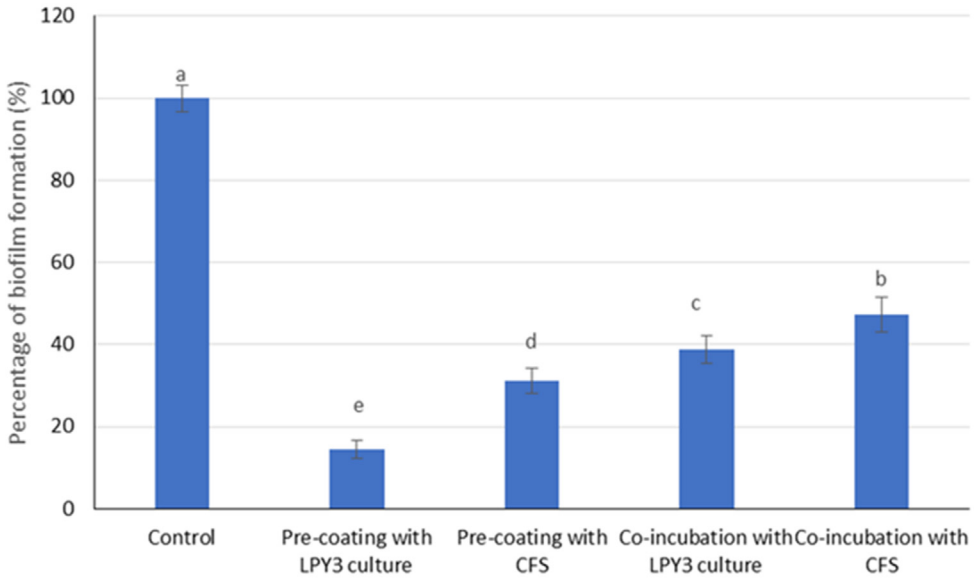

Fig. 2. Effect of pre-coating and co-incubation with LPY3 culture and its CFS on biofilm formation of ECU12. Statistical analysis was performed by One- way ANOVA and L.S.D. Means with different letters within column are significant difference, P ≤ 0.05–0.01. Means with the same letters within column are non significant difference.

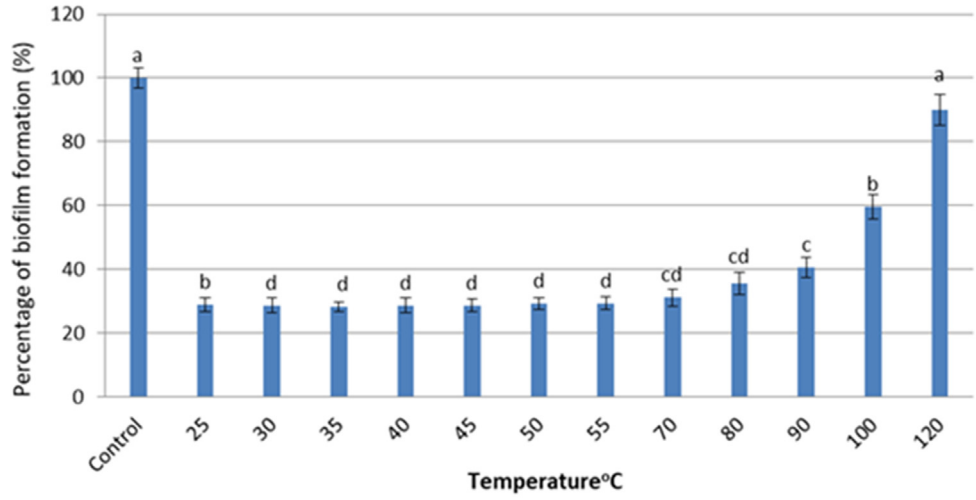

Fig. 3. Effect of different temperatures on anti-biofilm effect of CFS of LPY3 against ECU12. Statistical analysis was performed by One- way ANOVA and L.S.D. Values represent the mean of three replicates. Error bars are the standard deviation of the mean. Means with different letters within column are significant difference, P ≤ 0.05–0.01. Means with the same letters within column are non significant difference.

bacteria LPY3 culture that was useful to form biofilm and prevent biofilm-producing uropathogens. Pre-coating the microtitre plate with LPY3 culture generated a higher anti-biofilm effect against ECU12 isolate than its CFS. The reduced biofilm of ECU12 may be associated with the interference of LPY3 culture and its metabolites with cell assembly and cell–cell interaction and the reduction of quorum sensing required for biofilm production, as in agreement with Sharma et al. (2018). They recorded that the biofilm formation of *P. aeruginosa* PAO1 reduced after pre-coating either with exopolysaccharides or with CFS and their combination for seven days. Barzegari et al. (2020) recorded the benefits of probiotics in preventing quorum-sensing signals that are needed for biofilm production and the pathogen's survival through the interference with biofilm integrity and result in biofilm eradication. Also, Hashem and Abd El-Baky (2021), reported that *Lactobacilli* isolates formed strong biofilms and displayed potent anti-biofilm activities against MDR uropathogenic *E. coli*. Uropathogenic *E. coli* biofilms could be replaced by *Lactobacillus* biofilms.

The heat stability of *L. plantarum* Y3 CFS may be related to heat-stable bacteriocin-like substances. This was explained by Maria and Janakiraman (2012), they reported that bacteriocin of *Lactobacillus acidophilus* NCIM5426 was heat-stable (up to 100 °C) while the effect was missed after autoclaving for 15 min. The heat stability of the antimicrobial compounds of lactic acid bacteria is a property of bacteriocin-like substance.

The pH stability of *L. plantarum* Y3 CFS in a wide pH range. These outcomes are similar to Zhu et al. (2014) reported that plantaricin ZJ008 secreted by *L. plantarum* ZJ008 obtained from milk, was heat stable (121 °C, 30 min) and showed higher antimicrobial effect at pH values from 4 to 5. Also, Zamani et al. (2017) demonstrated that the CFS is stable at different pH values and showed a good anti-biofilm effect at low pH. Thus, anti-biofilm efficacy at low pH could result from increased solubility of anti-biofilm chemicals, improved protein folding, and/or increased binding and blockage of bacterial pathogen adhesions. Reda and Refaie (2019) reported that CFS of *Pediococcus pentosaceus* N33 was high ther-

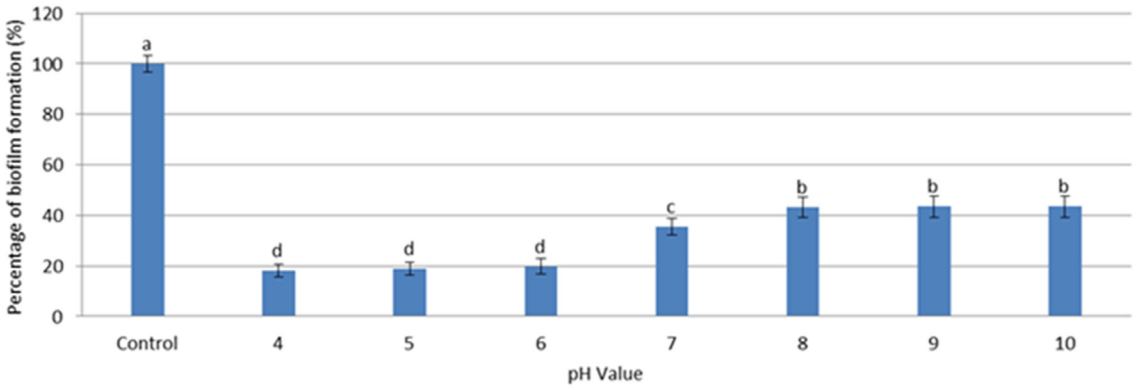

Fig. 4. Effect of different pH values on the anti-biofilm effect of CFS of LPY3 against ECU12. Statistical analysis was performed by One- way ANOVA and L.S.D. Values represent the mean of three replicates. Error bars are the standard deviation of the mean. Means with different letters within column are significant difference, P ≤ 0.05–0.01. Means with the same letters within column are non significant difference.

Table 3
Inhibition of biofilm formation of *E. coli* U12 on urinary catheter.

Days of incubation	Log No. of cells (CFU/cm^2) Control (*E. coli* U12 only)	Pre-coated with LPY3 culture	(T-Test)Mean Difference/control	Pre-coated with CFS	(T-Test)Mean Difference/control
1	7.5 ± 0.84^{bc}	3.6 ± 0.81^{a}	3.9**	4 ± 0.28^{a}	3.5**
2	7.9 ± 0.81^{bc}	2.7 ± 0.74^{a}	5.2**	3.6 ± 0.24^{a}	4.3**
3	8.7 ± 0.95^{bc}	2.5 ± 0.90^{a}	6.2**	3.5 ± 0.31^{a}	5.2**
4	8.8 ± 0.74^{bc}	1.7 ± 0.45^{ac}	7.1**	2.7 ± 0.35^{ab}	6.1**
5	9.5 ± 0.92^{bc}	1.6 ± 0.24^{ac}	7.9**	3.7 ± 0.42^{ab}	5.8**
6	9.8 ± 0.87^{bc}	1.5 ± 0.21^{ac}	8.3**	3.9 ± 0.51^{ab}	5.9**
7	9.9 ± 0.87^{bc}	0 ± 0.0^{ac}	–	4.5 ± 1.2^{ab}	5.4**

T-Test : ** significant difference at p < 0.05–0.01.
One- way ANOVA and L.S.D. Values are the mean of three replicates ± standard deviation.
Means with different letters within column are significant difference, P ≤ 0.05–0.01.

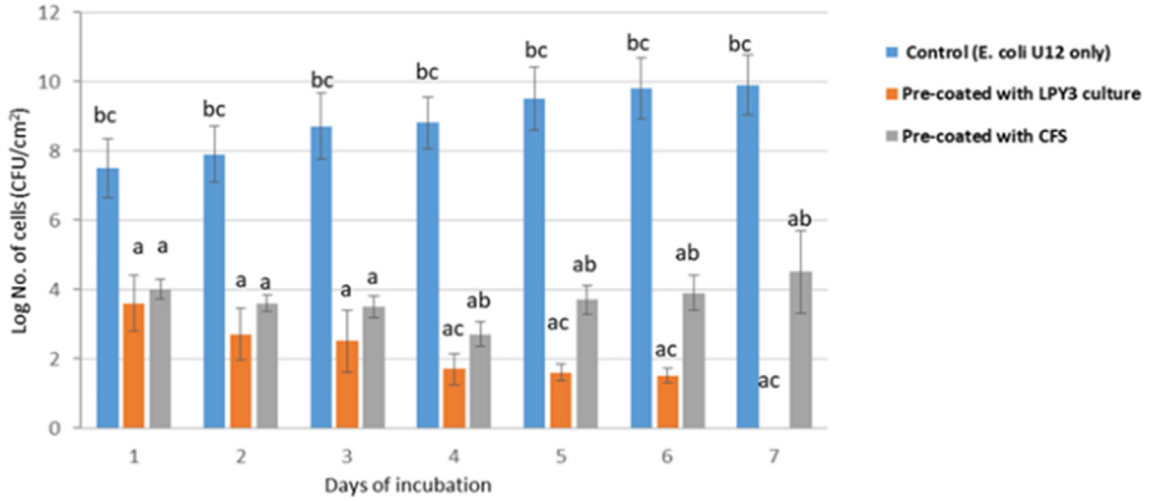

Fig. 5. Inhibition of biofilm formation of *E. coli* U12 on urinary catheter. Statistical analysis was performed by One- way ANOVA and L.S.D. T-Test : ** significant difference at p < 0.05–0.01 Values are the mean of three replicates ± standard deviation. Means with different letters within column are significant difference, P ≤ 0.05–0.01.

mostable for 20 min at temperature from 60 °C to 100 °C and also at 121 °C for 15 min. Its effect was high at pH from 2 to 6. Hassan et al. (2020a) recorded that, the bacteriocins extracted by *L. plantarum* and *L. helveticus* obtained from yogurt were active at a wide pH values, with maximum effect at pH 5. Also, the bacteriocin secreted by *L. plantarum* was a heat-stable and active at high temperatures. Also, Hassan et al. (2020b) noticed that, *L. plantarum* isolates grew slowly at pH 4 but quickly at pH from 5 to 6 and the growth rate declined at pH over 6. Barbosa et al. (2021) demonstrated that the R23 bacteriocins extracted by *Lactiplantibacillus plantarum* were active at a wide pH values from 2 to 8 and temperatures from 4 °C to 100 °C.

Pre-coating urinary catheter pieces with LPY3 culture or its CFS significantly reduced ECU12 biofilm as was obvious by the decreased viable cells number in biofilm for seven days for LPY3 culture, four days for CFS, and the reduction of viable cells number

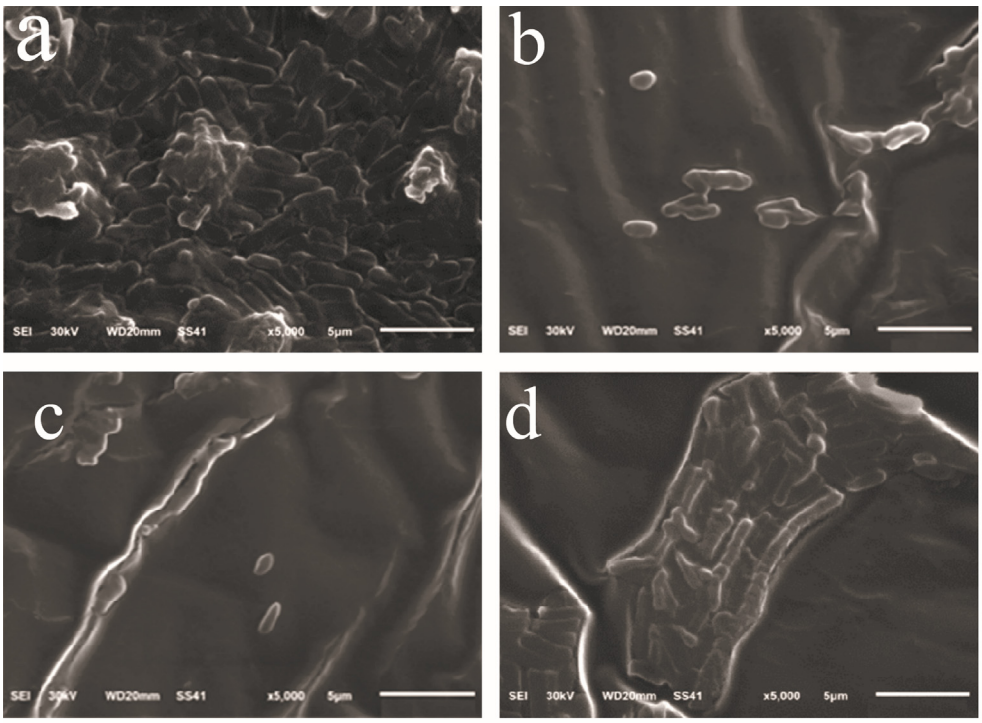

Fig. 6. Electron micrographs of ECU12 adhered on urinary catheter pieces after treatment with CFS of LPY3, **a:** positive control, **b:** first day, **c:** third day, **d:** sixth day.

of ECU12 after four days was not significant compared to the reduction at four days of exposure. This might be because of the higher amount of extracellular polymeric substances and lower bacterial metabolism in the 5-, 6- and 7-days biofilm comparing to the biofilm of 4 days (Babrud et al., 2019). Hence, LPY3 culture showed higher anti-biofilm effect than its CFS. Soleimani et al. (2010) showed that inhibition of biofilm formation might be associated with the antagonistic effect of LAB, production of inhibitory substances, and competition for nutrients. Present findings agree with findings of reports done by Ezeonu and Kanu (2016), they reported that pre-coating of the catheter with *L. acidophilus* inhibited the adherence of *Klebsiella, Escherichia coli*, and *Staphylococcus aureus*, to the catheter surfaces. Also, the present findings agree with Chen et al. (2017) stated that biofilms generated by probiotic *Escherichia coli* strain Nissle 1917 inhibited the adherence of pathogenic *Enterococcus faecalis* to urinary catheters and indwelling catheters for 11 days. Carvalho et al. (2021) showed a reduction in biofilms of *E. coli* after treatment by biofilms of *L. plantarum* by about 76% to 99% for 3 to12 h.

Scanning electron microscopy confirmed the reduction of adherence of ECU12 to pre-coated urinary catheter with CFS of LPY3 on the first and third day. Kıran et al. (2020) indicated a reduction in adherence of *Listeria monocytogenes* on different surfaces (stainless steel, polycarbonate, and polyvinyl chloride surface) after treatment with *L. plantarum* CFS by using SEM. Kim et al. (2020) found that *S. cerevisiae* CFS reduced *S. aureus* biofilms formation by using SEM. Also, Hashem and Abd El-Baky (2021) demonstrated by SEM a reduction in biofilms formation in the microtiter plates by uropathogenic MDR *E. coli* isolates when kept with *Lactobacilli* CFS for 48 h.

6. Conclusion

The findings from this preliminary study revealed that, *L. plantarum* has the ability to suppress biofilms formation on urinary catheter when applied to catheters, and that this inhibitory activity could be caused by both the elimination of adhesion surface for the bacterial uropathogen and production of antimicrobial compounds.

The results suggest a prospective intervention technique that needs to be further investigated before being implemented. Concerns of catheter occlusion by the coating, and the probability of becoming infected with *Lactobacillus*, may come to mind when thinking about practical implementation. Further trials involving small animals would provide answers to these questions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are thankful for Mansoura University for carrying out examination by scanning electron microscopy. The authors are thankful for Dr. Hend Magdy for carrying out statistical analysis of data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2022.01.014.

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