Antagonistic and antiadhesive effects of two *Lactobacillus* probiotics against *Pseudomonas aeruginosa* isolated from burn patients

Mohammad Abootaleb¹, Nazila Arbab Soleimani^{2*}, Mohammad Reza Zolfaghari¹, Nassim Ghorbanmehr³ and Mohammad Reza Yazdian¹

¹Department of Biology, Qom Branch, Islamic Azad University, Qom, Iran.

²Department of Biology, Damghan Branch, Islamic Azad University, Damghan, Iran.

³Department of Biotechnology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran.

Email: nazilaarbab@yahoo.co.uk

Received 21 September 2019; Received in revised form 18 December 2019; Accepted 23 January 2020

ABSTRACT

Aims: Today, probiotic bacteria can be used as safe and suitable candidates for the prevention of pathogenic bacterial infections. They produce antimicrobial components and compete with pathogens for host cell receptors. The present study aimed at evaluating probiotic lactobacilli antagonistic and antiadhesive activities against multi-antibiotic resistant *Pseudomonas aeruginosa* isolated from burned patients.

Methodology and results: This study examined 100 strains of multidrug-resistant (MDR) *P. aeruginosa* isolated from patients, who were admitted to the burn units of hospitals. Antagonistic activity of *Lactobacillus casei* and *Lactobacillus plantarum* against *P. aeruginosa* was determined by a modified double layer and well diffusion methods. The anti-adhesive effects of two probiotic lactobacilli and co-aggregation assay were determined by the microtiter plate method. Finally, the expression of two main adhesion genes, *algD*, and *pelA* was investigated in 100 MDR *P. aeruginosa* isolates via real-time polymerase chain reaction (RT-PCR) assay. Based on the results, the *algD* gene was detected in 97 out of 100 *P. aeruginosa* isolates, and the *pelA* gene was found in all of the isolates. Based on our findings, lactobacilli bacteria exerted good inhibitory effects on *P. aeruginosa*, especially on *algD* and *pelA* expression. Also, co-aggregation and antiadhesive results showed the positive effects of lactobacilli on *P. aeruginosa*.

Conclusion, significance and impact of study: Probiotic lactobacilli can be a new suitable candidate for controlling *P. aeruginosa* infections.

Keywords: Anti-adhesive, antimicrobial, *Pseudomonas aeruginosa*, probiotic lactobacilli, glass slide

INTRODUCTION

Pseudomonas aeruginosa is a bacterium, responsible for many infections in burn patients (Moradali *et al.*, 2017). Researchers believe that adhesion of this bacterium to different target cells and its resistance to a variety of antibiotics are the main factors, which make it hazardous to human health (Ahmadi *et al.*, 2016; Kamaria *et al.*, 2016; Rodesney *et al.*, 2017). *Pseudomonas aeruginosa* has some ligands, known as adhesins, which interact with the host cell receptors and initiate infection (Crouzet *et al.*, 2017; Chang, 2018). This bacterium has two main adhesion genes, known as *pelA* and *algD*, which encode two proteins required for cell-to-cell interactions and resistance to a variety of antibiotics (Jahromi *et al.*, 2018; Goltermann and Tolker-Nielsen, 2017). Evidence shows that mutations in *pelA* and *algD* genes affect the initial adhesion and colonization of this bacterium, as evidenced by its inability to cause an infection (Goltermann and

Tolker-Nielsen, 2017). From a scientific perspective, by preventing the initial adhesion of pathogenic bacteria to target cells, infections can be controlled (Moradali *et al.*, 2017; Tallawi *et al.*, 2017).

Currently, the importance of probiotic lactobacilli and their products has been highlighted (Alexandre *et al.*, 2014). They can promote human health through several important mechanisms (Alexandre *et al.*, 2014; Vuotto *et al.*, 2014). They antagonize pathogenic bacteria by producing antimicrobial components and competing for host cell receptors. Recently, the antiadhesive effects of these beneficial bacteria have been the subject of debate among researchers. Generally, probiotic lactobacilli inhibit the adhesion of pathogens to target cells in two ways. First, they inhibit infections by blocking the adherence of pathogenic bacteria to host cell receptors, resulting in competition for receptors or coaggregation. Coaggregation is the recognition of cell-to-cell attachment between probiotic bacteria and pathogens, which enables

probiotics to form a barrier for inhibiting the growth and binding of pathogenic bacteria. Second, they secrete some inhibitory molecules, which decompose the main regulatory genes in adhesion and colonization of pathogenic bacteria (Vuotto *et al.*, 2014; Satpute *et al.*, 2016; Sornplang and Piyadeatsoontorn, 2016). The present study aimed at evaluating the antagonistic and antiadhesive activities of probiotic lactobacilli against multi-antibiotic resistant *P. aeruginosa* which isolated from burned patients.

MATERIALS AND METHODS

Study period and sample collection

This cross-sectional study was conducted between March-December 2018. One hundred *P. aeruginosa* clinical samples were collected from hospitalized burn patients in Iranian hospitals, from the blood, burn wounds and sterile body fluids.

Probiotic bacterial strains lactobacilli (*Lactobacillus casei* subsp. *casei* PTCC 1608, *Lactobacillus plantarum* PTCC 1058) were purchased from Persian Type Culture Collection.

Bacterial isolation

The *P. aeruginosa* colonies were identified via Gram staining, as well as standard biochemical tests including oxidase test, catalase test, Indol test, Methyl Red and Voges Proskauer (MR-VP) test, motility test, citrate test, fermentation of sugar types (TSI) and color production were done.

Antibiotic susceptibility testing: Disk diffusion method

Antibiotic susceptibility test was done by the Kirby-Bauer disk diffusion method. Briefly, standard 0.5 McFarland of each *P. aeruginosa* was prepared and cultured by sterile swab on Muller Hinton agar (MHA). After putting disk of antibiotics such as ciprofloxacin (5 µg), imipenem (10 µg), amikacin (30 µg), piperacillin (30 µg), gentamicin (30 µg), colistin (10 µg), ceftazidime (30 µg) on mentioned plates, they incubated for 24 h and the inhibition zone of bacteria in the present of each antibiotic was measures and the results (sensitive, resistance and intermediate) was reported according to CLSI guidelines. *Pseudomonas aeruginosa* ATCC 27853 strain was a positive control (CLSI, 2016).

Observation of *P. aeruginosa* adhesion on the glass slide

In order to investigate *P. aeruginosa* adhesion on the glass slide, 1 mL of *P. aeruginosa* overnight cultured (108 CFU/mL) was inoculated into a flask containing 100 mL of sterile Brain Heart Infusion (BHI) broth supplemented with 1% sucrose and two slides with and without *L. plantarum* and *L. casei* supernatants. The glass slides were washed

in distilled water twice after cleaning detergent solution, then dried and transferred to flasks then autoclaved at 121 °C for 15 min. After that, the flasks were transferred in a rotational incubator (100 rpm) at 35 °C for 18-20 h. Then, the glass slides took off from the flasks and rinsed twice with 10 mL of phosphate buffered saline (PBS) solution in order to eliminate unattached cells. The glass slides were stained with 2% crystal violet for 5 min, rinsed, exposed to the air and took snapshot by an optical microscope with a digital camera (Kos *et al.*, 2003; Tahmourespour *et al.*, 2011).

Antagonistic activities

Modified double-layer method

The antagonistic activities of probiotic lactobacilli were carried out according to Halder *et al.* (2017) with some modification. An overnight culture of each probiotic lactobacilli and *P. aeruginosa* were prepared in de Man Rogosa and Sharpe (MRS) broth and Muller Hinton broth (MHB) at 37 °C, respectively. A 100 µL of each probiotic lactobacilli culture was added on MRS agar and incubated for 30 min at 37 °C. The plate of MRS agar containing lactobacilli spot was overlaid with melted MHA and allowed to solidify and 100 µL of each *P. aeruginosa* (0.5 McFarland) individually inoculated by streaking the swab over the entire agar surface. The plates were incubated for 24 h at 37 °C. By measuring the clear zone around the spot, the antagonistic activity of probiotic lactobacilli was determined.

Well diffusion method

The cell-free supernatant (CFS) inhibitory activities of probiotic bacteria were investigated by the well diffusion method (Presti *et al.*, 2015). First, CFS was obtained by centrifuging an overnight culture of probiotic bacteria (10000 rpm, 10 min) and filtered with a 0.2 µm pore size filter. Wells sized (5 mm) were cut into the agar plate. Then, streak culture of each *P. aeruginosa* was prepared and 100 µL of each CFS was added to the wells. To evaluate the growth inhibition, plates were incubated at 37 °C for 24 h and clear zones around each well were measured.

Anti-adhesion activity

The effect of lactobacilli CSF on the *P. aeruginosa* adherence was determined based on Wu *et al.* (2013) and Bulgasem *et al.* (2015) with a few modifications. Briefly, MRS broth was used as a medium to culture probiotic bacteria following 16 h incubation at 37 °C to prepare a fresh overnight culture of *P. aeruginosa*. Equal volumes (75 µL) of probiotics supernatant and *P. aeruginosa* (0.5 McFarland) were inoculated into the microtiter plate wells. The control well contained *P. aeruginosa* standard strain and culture media; and negative control contained only media culture. The wells were then incubated for 24 h at 37 °C. Next, the wells

Table 1: Primer sequences of studied biofilm genes in *P. aeruginosa* isolates and reaction setup for 2 steps real-time PCR.

Primer sequences	Step 1		Step 2	
	Conditions	Volume reactions	Conditions	Volume reactions
<i>algD</i> F-GCTATGTCGGTGCAGTATGT R-ATCAACCAGGGCAAGTCG			1 cycle 95 °C.....5 min	Real Q Plus 2x Master Mix, green: 12.5 µL
<i>pelA</i> F-GCACCTGTACTACCACTTCTATTC R- GCATGTAATCGCTCATCCACA	1 cycle 25 °C.....10 min 47 °C.....60 min 70 °C.....10 min	Total RNA: 5 µL Random hexamer: 2 µL H ₂ O up to 10 µL	40 cycle 95 °C.....30 s 58 °C.....30 s 72 °C.....45 s	Primer F + R: 2 µL Template DNA: 5 µL Nuclease-free water: 5.5 µL
<i>proC</i> F: ATCAGTTGCTGCGGCTTCAG R: CCATCGACGTGGTCGAGTC			1 cycle 95 °C.....10 min	Total reaction volume: 25 µL

were emptied and washed three times with PBS (pH 7.4). A 150 µL of ethanol (96% [v/v]) was added to wells for 15 min to fix the attached cells. A 200 µL of crystal violet (2% [v/v]) was added to wells for 10 min to stain cells. After washing wells, a 200 µL of acetic acid (33% [v/v]) was used to measure the absorbance of each well at 492 nm using ELISA reader (Biotek.827 pH lab, Germany). The following formula was applied to calculate the adherence reduction rate:

$$\text{Adherence reduction rate (\%)} = [(A-B) - (T-B)] / (A-B) \times 100$$

where A and B refer to control well and negative control, respectively, and T refers to the test well.

Co-aggregation assay

Co-aggregation of probiotic bacteria with *P. aeruginosa* was done with a few modifications (Tulumoglu *et al.*, 2013; Berne *et al.*, 2015). An overnight culture of probiotic lactobacilli and *P. aeruginosa* were prepared at 37 °C. Cells were harvested (5000 rpm, 10 min, 20 °C) and mixed in equal volumes (OD= 0.2, 600 nm); then, the suspensions were incubated at 37 °C for 4 h. In the next step, following centrifugation (1600 rpm, 5 min, 20 °C), the OD was determined at 600 nm. The following formula was used to calculate co-aggregation:

$$\text{Co-aggregation (\%)} = (A1 - A2) / A1 \times 100$$

where A1 represents the strains OD just after mixing and A2 represents the supernatants OD after four hours.

Molecular identification of attachment genes *algD* and *pelA* in *P. aeruginosa* by RT-PCR

Isolation of total RNA

After inoculating the *P. aeruginosa* from glycerol stocks in nutrient agar (2 mL), they were grown at 37 °C overnight. Next, the strains were subcultured in the nutrient medium (5 mL) and grown to the mid-exponential phase (OD₆₀₀= 1.5-2.0). Afterward, an aliquot of culture (0.25 mL) was added to 2 mL of RNX-plus (Sinaclon, Iran). Isolation of total RNA was performed, based on the manufacturer's instructions. DNase treatment was applied to remove the residual DNA with 20 U of RQ1 DNase I (Sinaclon, Iran).

cDNA synthesis

A reaction mixture, consisting of 5 µg of RNA, was incubated in DEPC-treated water and 1 µL of random hexamers (Pars Tous, Iran). Next, it was incubated for five minutes at 65 °C and cooled down in the ice. After that, 10 µL of RT premixture (2x) was added and mixed by pipetting up and down gently in a total volume of 24 µL. Incubation was performed at 25 °C for 10 min and at 47 °C for 60 min. The reaction was terminated by heating at 70 °C for 10 min and chilling on ice. The collected cDNA was stored at -20 °C until further analysis.

Real-time PCR assay

Changes in alginate-specific (*algD*) gene and Pellicle polysaccharide (*pelA*) gene expression in *P. aeruginosa* were measured using RT-PCR assay in the presence and absence of *Lactobacillus* supernatants. Quantitative PCR (qPCR) assay was performed according to the manufacturer's instructions, using SYBR Green and 2x RT-PCR Master Mix and special primers were designed

by authors. Also, the Green No-Rox kit was used for RT-PCR detection (Corbett, Australia). The primer sequences and reaction setup are shown in Table 1. Based on the melt curve analysis, the presence of the RT-PCR product was examined after 40 cycles. It should be noted that the reference housekeeping gene was the pyrroline-5-carboxylate reductase (*proC*) gene. The comparative C_t ($\Delta\Delta C_t$) method was finally applied to determine C_t values and fold differences were measured as $2^{-\Delta\Delta C_t}$.

Statistical analysis

The obtained data were analyzed using SPSS version 23.0 (SPSS, Chicago, IL). The t-test was employed with a significance level < 0.05 .

RESULTS

Antagonistic activity

Generally, probiotic bacteria, especially *Lactobacillus*, can antagonize pathogenic bacteria and prevent their growth and infection by producing inhibitory substances. Since most of *P. aeruginosa* isolated from different samples shown different results to antibiotic susceptibility tests (Table 2), the antagonistic activity of probiotic lactobacillus (*L. casei* and *L. plantarum*) were done using a modified double layer and well diffusion (*Lactobacillus* CFS) methods to find a new safe way to control this bacterium. The average inhibition zone of *P. aeruginosa* in the presence of *L. casei* and *L. plantarum* cell cultures (Figure 1a) and CFS of these probiotic bacteria (Figure 1b) has shown in Table 3.

Figure 1: (a) Antagonistic activity of probiotic lactobacilli (*L. casei* and *L. plantarum*) against multi-antibiotic resistant *P. aeruginosa*. (b) The inhibitory effect of CFS probiotic lactobacilli (*L. casei* and *L. plantarum*) against multi-antibiotic resistant *P. aeruginosa*.

Table 2: Antibiotics resistance profile of *P. aeruginosa* in the different clinical specimens.

		Ciprofloxacin	Colistin	Amikacin	Ceftazidime	Ciprofloxacin	Gentamicin	Imipenem
Sample								
Blood and Sterile body fluids	R	16	17	12	15	18	15	18
	S	17	14	21	17	12	17	13
Burn wounds	R	20	8	18	24	31	30	27
	S	47	61	48	44	39	38	41
	I			1				1
Total	R	36	25	30	39	49	45	45
	S	64	75	69	61	51	55	54
	I			1				1

R: Resistant; S: Sensitive; I: intermediate

Anti-adhesion activity

One of the best characteristics of probiotic bacteria is their anti-adhesion effect against pathogenic microorganisms which makes them proper candidates to prevent infection. In the present study, the average of the anti-adhesive effect of *L. casei* and *L. plantarum* supernatants on *P. aeruginosa* was shown in Table 3. So, probiotic lactobacilli (*L. casei* and *L. plantarum*) have anti-adhesive activities against multi-antibiotic resistant *P. aeruginosa* (Figure 2).

Co-aggregation

Co-aggregation is a process where probiotic bacteria attach to pathogenic bacteria and block their attachment to the host cells and also by the production of antagonistic components, they can inhibit the growth of pathogens. By the finding of this study, co-aggregation between *L. casei* and *L. plantarum* with *P. aeruginosa* was achieved in accordance with the results in Table 3.

Molecular identification of attachment genes *algD* and *pelA* in *P. aeruginosa*

After checking the ability of *P. aeruginosa* attachment to glass slide (Figure 2), the presence of *pelA* and *algD* genes in MDR *P. aeruginosa* was investigated. The

Figure 2: *P. aeruginosa* adhesion on the glass slide. 1: control group (*P. aeruginosa* biofilm formation). 2, 3: experimental group (in the presence of *L. plantarum* and *L. casei* supernatants).

results showed that 97 *P. aeruginosa* isolates had the *algD* gene, and all of them contained the *pelA* gene. Based on the findings, it was hypothesized that these two genes play an important role in cell-to-cell interactions of this bacterium (Figure 3). In addition, gene expression of biofilm components, *pelA*, and *algD*, in *P. aeruginosa* were examined. It was found that after *L. casei* and *L. plantarum* supernatants treatment, it caused reductions in *pelA* and *algD* gene expression in *P. aeruginosa* with comparison to the absence of supernatants (Table 3).

Figure 3: (A) PCR for detection *pelA*. Lane 1: Gene ruler 25 bp DNA ladder, Lane 2: PCR product of *pelA* (120 bp). (B) PCR product of *algD*, Lane 1: Gene ruler 50 bp DNA ladder, Lane 2: PCR product of *algD* (97 bp). (C) Lane 1: Total RNA extracted from *P. aeruginosa* after *L. casei* supernatant treatment. Lane 2: Total RNA extracted from *P. aeruginosa* after *L. plantarum* supernatant treatment.

Table 3: Antagonistic activities of *L. casei* and *L. plantarum* against *P. aeruginosa*.

Samples	Average inhibition zone (mm)		Adherence reduction rate (%)	Co-aggregation (%)	Gene expression (fold change)	
	Modified double-layer method	Well diffusion method			<i>pelA</i>	<i>algD</i>
<i>L. casei</i>	23.0 ± 1.3	21.0 ± 1.1	51.9	44.8	3.8	2.4
<i>L. plantarum</i>	21.0 ± 0.8	12.0 ± 0.6	42.1	37.3	5.8	3.1

DISCUSSION

Bacterial adhesion falls into two major categories, i.e., nonspecific and specific (Formosa-Dague *et al.*, 2018). Nonspecific adhesion is reversible and occurs by

physiochemical forces (Fijan, 2014; Tallawi *et al.*, 2017). In this type of adhesion, mobile bacteria move toward chemo attractants, such as glucose and oligopeptides, and grow on surfaces; chemotaxis regulates and

prepares bacteria-host cell interactions (Formosa-Dague *et al.*, 2018). Specific adhesions are irreversible and occur when specific bacterial adhesions (capsule, pili, some exopolysaccharides), interact with host cell receptors which are important for initial bacterial infections. Nowadays, researchers believe that by preventing bacterial-host cell interaction, the infection can be avoided (Fijan, 2014; Moradali *et al.*, 2017; Tallawi *et al.*, 2017). Due to a lack of knowledge about the consumption of antibiotics, the prevalence of MDR bacteria is on the rise, which can be threatening for human health; therefore, researchers are seeking new safe alternative treatments (Goltermann and Tolker-Nielsen, 2017). According to many studies, probiotic bacteria, especially lactobacilli and their secondary metabolic products, can be proper candidates for controlling and preventing infection (Lavery *et al.*, 2014; Harimawan and Ting, 2016). In the present study, antimicrobial and antiadhesive effects of these bacteria and their supernatants against *P. aeruginosa* were investigated.

In the present study, 100 multi-antibiotic resistant *P. aeruginosa* isolated from burned patients were investigated, with regard to the presence of two main genes (*algD*, *pelA*) involved in their attachment and antimicrobial resistance. *algD* gene, one of the gene products of 12 genes *alg* operon, has an essential role in the initial alginate biosynthesis and its polymerization (Al Dawodeyah *et al.*, 2018). Alginate, a capsular polysaccharide is a protective structure of *P. aeruginosa* against antimicrobial substances, immune system and helps bacteria to interact with host cell receptors (Al Dawodeyah *et al.*, 2018; Heba *et al.*, 2018). According to the present findings, 97 (97%) *P. aeruginosa* isolates had *algD* gene. This finding is in line with the results of a study by Heba *et al.* (2018) which showed that among 61 *P. aeruginosa* isolates from respiratory tract infections, 98% contained *algD* gene. Their results showed that this gene is very important for adhesion, especially in this antibiotic-resistant bacterium (Heba *et al.*, 2018). Jahromi and colleagues reported that multi-drug resistant *P. aeruginosa* had a high percentage of *algD* (88.6%-100%) depending on the geographical regions which are supported by the present study (Jahromi *et al.*, 2018). Moreover, another key gene, *pelA*, which is particularly important in *P. aeruginosa* adhesion to host cell receptors, was investigated in the current study. *PelA* is the most important gene among seven genes involved in the biosynthesis of PEL polysaccharide, which is important for *P. aeruginosa*-host cell interactions and biofilm formation (Colvin *et al.*, 2013). In this study, all isolated *P. aeruginosa* (100%) contained the *pelA* gene. In another study, *pelA* stimulates bacterium attachment and resistant to a variety of antibiotics (Sharma *et al.*, 2017). Jennings *et al.* (2015) demonstrated that PEL polysaccharide enhances resistance to aminoglycoside antibiotics when *P. aeruginosa* attaches to host cells and produces biofilm. These findings, to a great extent, corroborate the present study (Jennings *et al.*, 2015).

In the current study, the antagonistic effects of probiotic lactobacilli against *P. aeruginosa* were evaluated. The result showed that the complete culture of both probiotic lactobacilli and their supernatant inhibited *P. aeruginosa* growth, but the inhibitory effect of *L. casei* was more effective. Vuotto *et al.* (2014) reported that probiotic culture and their supernatant were able to inhibit *P. aeruginosa* burn infection (Vuotto *et al.*, 2014). One of the main properties of probiotic lactobacilli which makes them so potent to antagonize pathogenic bacteria is the production of antimicrobial substances (Bulgasem *et al.*, 2015; Harimawan and Ting, 2016). Some research studies demonstrated that antibacterial compounds of probiotic lactobacilli can interact with the bacterial cell membrane by reducing the pH of the environment and denaturing proteins and finally by changing the physiology and morphology of cytoplasmic membrane of bacteria and leakage of its contents, eradicate pathogens (Lavery *et al.*, 2014; Sivaraj *et al.*, 2018).

In the present study, both probiotic lactobacilli were coaggregated with *P. aeruginosa*. Optimal results were achieved with *L. casei*. Based on the findings, to estimate compounds by probiotics (lactobacilli) against pathogenic bacteria, the coaggregation assay can be a reliable method, as it facilitates close contact between cells (Presti *et al.*, 2015; Sivaraj *et al.*, 2018). Today, several distinct surface lactobacilli proteins are predicted to enhance binding to the surface of pathogenic bacteria, which inhibits pathogen attachment and colonization (Monteagudo-Mera *et al.*, 2019). As described in other studies, probiotic bacteria through coaggregation with pathogenic bacteria can prevent their growth and attachment to host cells, as confirmed in the present study (Sivaraj *et al.*, 2018). Since the antiadhesive effects of probiotic bacteria are very important for preventing the first stage of infection, the antiadhesive activity of *L. casei* and *L. plantarum* was investigated in the current study. Both lactobacilli could inhibit *P. aeruginosa* adhesion to the microtiter plate, although it was observed that the antiadhesive effect of *L. casei* was more significant than that of *L. plantarum*. Researchers have highlighted the antiadhesive role of probiotic bacteria, but the mechanisms of their action remain undetermined (Liu *et al.*, 2011; Vuotto *et al.*, 2014). Monteagudo-Mera *et al.* (2019) demonstrated that *P. aeruginosa* colonization and adhesion were inhibited by *L. plantarum* supernatant. In addition, Bienenstock *et al.* (2013) demonstrated that probiotic bacteria prevent pathogenic bacteria attachment due to competition for specific host cell receptors. These findings are in line with the results of the current study, although further investigation about the exact mechanisms of antiadhesive activity of probiotic bacteria is needed.

CONCLUSION

In general, the initial step for most bacterial infection is the interaction between bacteria and host cells and *P. aeruginosa* is not an exception. Overall, our findings showed that the supernatant of probiotics *L. casei* and *L.*

plantarum could inhibit the bacterial infection and biofilm formation of *P. aeruginosa*. Therefore, it is proposed that these probiotic bacteria can be used as therapeutic approaches in preventing and/or dealing with hospital-acquired infections.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Hossein Keyvani, a full professor of Iran University of Medical Sciences who contributed to this research.

REFERENCES

- Ahmadi, K., Hashemian, A. M., Pouryaghobi, S. M., Akhavan, R., Rozmina, S. and Bolvardi, E. (2016). Antibiotic resistance properties of *Pseudomonas aeruginosa* isolated from cases of superficial infections at the emergency unit. *Jundishapur Journal of Microbiology* 9(1), e27646.
- Al Dawodeyah, H. Y., Obeidat, N., Abu-Qatouseh, L. F. and Shehabi, A. A. (2018). Antimicrobial resistance and putative virulence genes of *Pseudomonas aeruginosa* isolates from patients with respiratory tract infection. *Germs* 8(1), 31-40.
- Alexandre, Y., Le Blay, G., Boisramé-Gastrin, S., Le Gall, F., Héry-Arnaud, G., Gouriou, S. and Le Berre, R. (2014). Probiotics: A new way to fight bacterial pulmonary infections? *Médecine et Maladies Infectieuses* 44(1), 9-17.
- Berne, C., Ducret, A., Hardy, G. G. and Brun, Y. V. (2015). Adhesins involved in attachment to abiotic surfaces by Gram-negative bacteria. *Microbiology Spectrum* 3(4), 1-45.
- Bienenstock, J., Gibson, G., Klaenhammer, T. R., Walker, W. A. and Neish, A. S. (2013). New insights into probiotic mechanisms: A harvest from functional and metagenomic studies. *Gut Microbes* 4(2), 94-100.
- Bulgasem, B. Y., Hassan, Z., Abdalsadiq, N. K., Yusoff, W. M. and Lani, M. N. (2015). Anti-adhesion activity of lactic acid bacteria supernatant against human pathogenic *Candida* species biofilm. *Health Science Journal* 9(6), 1-9.
- Chang, C. Y. (2018). Surface sensing for biofilm formation in *Pseudomonas aeruginosa*. *Frontiers in Microbiology* 8, 2671-2678.
- CLSI, Clinical Laboratory Standards Institute (2016). Performance Standards for Antimicrobial Susceptibility Testing. 26th edn. CLSI supplement M100. Wayne, PA.
- Colvin, K. M., Alnabelseya, N., Baker, P., Whitney, J. C., Howell, P. L. and Parsek, M. R. (2013). PelA deacetylase activity is required for Pel polysaccharide synthesis in *Pseudomonas aeruginosa*. *Journal of Bacteriology* 195(10), 2329-2339.
- Crouzet, M., Claverol, S., Lomenech, A. M., Le Sénéchal, C., Costaglioli, P., Barthe, C. and Vilain, S. (2017). *Pseudomonas aeruginosa* cells attached to a surface display a typical proteome early as 20 minutes of incubation. *PLoS ONE* 12(7), e0180341.
- Fijan, S. (2014). Microorganisms with claimed probiotic properties: an overview of recent literature. *International Journal of Environmental Research and Public Health* 11(5), 4745-4767.
- Formosa-Dague, C., Castelain, M., Martin-Yken, H., Dunker, K., Dague, E. and Sletmoen, M. (2018). The role of glycans in bacterial adhesion to mucosal surfaces: how can single-molecule techniques advance our understanding? *Microorganisms* 6(2), 39-65.
- Goltermann, L. and Tolker-Nielsen, T. (2017). Importance of the exopolysaccharide matrix in antimicrobial tolerance of *Pseudomonas aeruginosa* aggregates. *Antimicrobial Agents and Chemotherapy* 61(4), e02696-16.
- Halder, D., Mandal, M., Chatterjee, S., Pal, N. and Mandal, S. (2017). Indigenous probiotic *Lactobacillus* isolates presenting antibiotic like activity against human pathogenic bacteria. *Biomedicines* 5(2), 31-41.
- Harimawan, A. and Ting, Y. P. (2016). Investigation of extracellular polymeric substances (EPS) properties of *P. aeruginosa* and *B. subtilis* and their role in bacterial adhesion. *Colloids and Surfaces B: Biointerfaces* 146, 459-467.
- Heba, Y. Al. D., Nathir, O., Luay, F. A. and Asem, A. S. (2018). Antimicrobial resistance and putative virulence genes of *Pseudomonas aeruginosa* isolates from patients with respiratory tract infection. *Germs* 8, 31-40.
- Jahromi, S. I. P., Mardaneh, J., Sharifi, A., Pezeshkpour, V., Behzad-Behbahani, A., Seyyedi, N. and Khosravani, S. A. (2018). Occurrence of a multidrug-resistant *Pseudomonas aeruginosa* strains in hospitalized patients in southwest of Iran: Characterization of resistance trends and virulence determinants. *Jundishapur Journal of Microbiology* 11(4), e57341.
- Jennings, L. K., Storek, K. M., Ledvina, H. E., Coulon, C., Marmont, L. S., Sadvovskaya, I., Secor, P. R., Tseng, B. S., Scian, M., Filloux, A. and Wozniak, D. J. (2015). Pel is a cationic exopolysaccharide that cross-links extracellular DNA in the *Pseudomonas aeruginosa* biofilm matrix. *Proceedings of the National Academy of Sciences of the United States of America* 112(36), 11353-11358.
- Kamaria, P. A., Binita, J., Aring, B. J. and Sinha, M. (2016). Incidence of multidrug-resistant *Pseudomonas aeruginosa* isolated from burn patients tertiary care hospital, Jamnagar, Gujarat, India. *Journal of Medical and Dental Sciences* 15(7), 31-34.
- Kos, B. V. Z. E., Šušković, J., Vuković, S., Šimpraga, M., Frece, J. and Matošić, S. (2003). Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. *Journal of Applied Microbiology* 94(6), 981-987.
- Lavery, G., Gorman, S. and Gilmore, B. (2014). Biomolecular mechanisms of *Pseudomonas aeruginosa* and *Escherichia coli* biofilm formation. *Pathogens* 3(3), 596-632.

- Liu, Y., Wang, L., Zhou, X., Hu, S., Zhang, S. and Wu, H. (2011).** Effect of the antimicrobial decapeptide KSL on the growth of oral pathogens and *Streptococcus mutans* biofilm. *International Journal of Antimicrobial Agents* **37(1)**, 33-38.
- Moradali, M. F., Ghods, S. and Rehm, B. H. (2017).** *Pseudomonas aeruginosa* lifestyle: A paradigm for adaptation, survival, and persistence. *Frontiers in Cellular and Infection Microbiology* **7**, 39-68.
- Monteagudo-Mera, A., Rastall, R. A., Gibson, G. R., Charalampopoulos, D. and Chatzifragkou, A. (2019).** Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. *Applied Microbiology and Biotechnology* **103(16)**, 6463-6472.
- Presti, I., D'Orazio, G., Labra, M., La Ferla, B., Mezzasalma, V., Bizzaro, G. and Di Gennaro, P. (2015).** Evaluation of the probiotic properties of new *Lactobacillus* and *Bifidobacterium* strains and their *in vitro* effect. *Applied Microbiology and Biotechnology* **99(13)**, 5613-5626.
- Rodesney, C. A., Roman, B., Dhamani, N., Cooley, B. J., Katira, P., Touhami, A. and Gordon, V. D. (2017).** Mechanosensing of shear by *Pseudomonas aeruginosa* leads to increased levels of the cyclic-di-GMP signal initiating biofilm development. *Proceedings of the National Academy of Sciences of the United States of America* **114(23)**, 5906-5911.
- Satpute, S. K., Kulkarni, G. R., Banpurkar, A. G., Banat, I. M., Mone, N. S., Patil, R. H. and Cameotra, S. S. (2016).** Biosurfactant/s from *Lactobacilli* species: Properties, challenges and potential biomedical applications. *Journal of Basic Microbiology* **56(11)**, 1140-1158.
- Sornplang, P. and Piyadeatsoontorn, S. (2016).** Probiotic isolates from unconventional sources: A review. *Journal of Animal Science and Technology* **58(1)**, 26-37.
- Sharma, C., Singh, B. P., Thakur, N., Gulati, S., Gupta, S., Mishra, S. K. and Panwar, H. (2017).** Antibacterial effects of *Lactobacillus* isolates of curd and human milk origin against food-borne and human pathogens. *3 Biotech* **7(1)**, 31-40.
- Sivaraj, A., Sundar, R., Manikkam, R., Parthasarathy, K., Rani, U. and Kumar, V. (2018).** Potential applications of lactic acid bacteria and bacteriocins in anti-mycobacterial therapy. *Asian Pacific Journal of Tropical Medicine* **11(8)**, 453-459.
- Tahmourespour, A., Salehi, R. and Kermanshahi, R. K. (2011).** *Lactobacillus acidophilus*-derived biosurfactant effect on *gtfB* and *gtfC* expression level in *Streptococcus mutans* biofilm cells. *Brazilian Journal of Microbiology* **42(1)**, 330-339.
- Tallawi, M., Opitz, M. and Lieleg, O. (2017).** Modulation of the mechanical properties of bacterial biofilms in response to environmental challenges. *Biomaterials Science* **5(5)**, 887-900.
- Tulumoglu, S., Yuksekdog, Z. N., Beyatli, Y., Simsek, O., Cinar, B. and Yaşar, E. (2013).** Probiotic properties of *Lactobacilli* species isolated from children's feces. *Anaerobe* **24**, 36-42.
- Vuotto, C., Longo, F. and Donelli, G. (2014).** Probiotics to counteract biofilm-associated infections: Promising and conflicting data. *International Journal of Oral Science* **6(4)**, 189-194.
- Wu, C., Labrie, J., Tremblay, Y. D. N., Haine, D., Mourez, M. and Jacques, M. (2013).** Zinc as an agent for the prevention of biofilm formation by pathogenic bacteria. *Journal of Applied Microbiology* **115(1)**, 30-40.