Antibacterial and Antibiofilm Effects of Synbiotics against Multidrug-Resistant bacteria: Acinetobacter baumannii and Enterococcus faecalis

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Research

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Abstract

Background: Acinetobacter baumannii and Enterocoocus faecalis increase their resistance against antibiotic by producing biofilm. Antibiotic resistance has become a massive public health threat that require novel effective antibacterial and antibiofilm alternatives. The use of probiotics is interested to prevent and control certain infections. The objective of this study was to investigate the antibacterial and antibiofilm property of probiotics and synbiotics against multidrug-resistant A. baumannii and E. faecalis.

Methods: The antimicrobial and the antibiofilm activities of cell- free supernatants of four strains of Lactobacillus against 20 clinical multi-drug resistant (MDR) isolates of Acinetobacter baumannii and Enterocoocus faecalis were determined in the presence of 0.3% of sorbitol, raffinose, citrate, trehalose, inulin, and riboflavin using well diffusion agar and micro-dilution method.

Results: The cell- free supernatant of L. rhamnosus with citrate and trehalose showed the best antibacterial activity against MDR A. baumannii (28.8±2.1mm, 1.128 µL/mL), and L. rhamnosus with all of prebiotics against MDR E. faecalis (29.8±0 mm, 1.128 µL/mL) compare to probiotic alone. The prebiotics could improve the inhibitory effect of probiotics against the Gram-negative A. baumannii higher than Gram-positive E. faecalis. Biofilm formation was reduced in both pathogens in presence of synbiotics. L. plantarum with riboflavin and L. rhamnosus with or without inulin potently inhibits E. faecalis (50±0.86%) and A. baumannii (75±6.5%) biofilm formation, respectively.

Conclusions: The results of current study support the antibiofilm activity of metabolites produced by synbiotics, and suggest their use as suitable adjuvants as well as biocontrol agents for treatment.

Introduction

Healthcare-associated infections are caused by pathogens than can be transmitted from person to person, environment or contaminated healthcare personnel [1]. The multi-drug-resistant (MDR) organisms such as Acinetobacter baumannii and Enterococcus faecalis are among the important hospital acquired pathogens. Infections caused by A. baumannii include pneumonia, urinary tract infections, meningitis, endocarditis, peritonitis, skin and soft tissue infections [2, 3]. Enterococci can cause infections in the urinary tract, blood stream, and biliary tract frequently. It also causes, meningitis in neonates and endocarditis in adults [4]. Both of the organisms are therapeutic challenges since the emergence of MDR strains has threatened the lives of many patients at different countries [3]. Moreover, biofilms formation by these bacterial at the site of infection either on the indwelling catheters or on the tissues triggers the severity of the disease [5–7]. Biofilms are bacterial communities protected by extracellular polysaccharide matrix (EPS) that protect them from desiccation or nutritional stress and facilitate the environmental survival [2, 8, 9]. Furthermore, the presence of biofilms causes many problems in medicine, interfering with wound infections therapy as well as persistent infections on various medical devices [10]. Many strategies have been established and used to inhibit biofilm formation and now researchers have focused on natural, effective and novel antibiofilm agents [11].

Probiotic bacteria such as Lactobacilli are living microorganisms that are beneficial to human health [8]. They form the most important part of intestinal microflora in human and animals [12]. The probiotic bacteria are able to suppress the growth and pathogenicity of pathogenic organisms. The short-chain carbohydrates “prebiotics”, can selectively stimulate the growth and activity of specific bacterial species, especially lactobacilli. Compounds that simultaneously contain probiotic bacteria and the growth promoting prebiotic ingredients that exhibit synergistic effects are called “synbiotic”. Various studies have shown that use of synbiotic products have more beneficial effects on human health than solely probiotic [13, 14]. The probiotic lactobacilli, also produce various antimicrobial peptides, organic acids (acetic and lactic acids), bacteriocins and other substance that accumulate in the culture supernatant of Lactobacillus spp. [15, 16, 17].The results of a study by Valdez and colleagues revealed that metabolites of L. plantarum could be considered as potential therapeutic substances for the local treatment of burn infections caused by P. aeruginosa [18].Walencka et al. demonstrated

that surfactant obtained from L. acidophilus could inhibit biofilm formation of S. aureus and S. epidermidis [19]. Following these results, only a few studies focused on in vitro investigating of the bacterial pathogens mainly involved in biofilm-based infections, i.e., A. baumannii and E. faecalis.

This study was aimed to investigate the benefits of prebiotics (Raffinose, trehalose, riboflavin, citrate, inulin and sorbitol) on the antimicrobial and antibiofilm properties of four potential probiotics (Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus rhamnosus and Lactobacillus rutrei). Cell-free supernatants of probiotics and synbiotics (prebiotic-probiotics) cultures have been studied in in vitro model to evaluate the antimicrobial and antibiofilm activity against MDR A. baumannii and E. faecalis clinical isolates.

Materials And Methods

Bacterial strains and culture condition

Ten clinical strains of A. baumannii and 10 clinical isolates of E. faecalis, were collected from patients with nosocomial infections in Imam Reza and Ghaem hospitals, Mashhad, Iran. All the isolates were cultured in brain heart infusion (BHI) broth (Merck, Germany) and incubated at 37°C under aerobic condition. Then all the isolates were stored at − 80°C in broth media containing 20% glycerol.

Antibiotic Susceptibility Testing

Antibiotic resistance patterns of clinical isolates were determined using disk diffusion method according to the CLSI guidelines [20]. The following antibiotics were used: ceftriaxone (30 µg), ceftazidime (30 µg), imipenem (10 µg), amikacin (10 µg), gentamicin (10 µg), ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), tetracycline (30 µg), colistin (10 µg), cefazolin (30 µg), chloramphenicol (30 µg), ampicillin (10 µg) vancomycine (30 µg) and cephalexin (30 µg) (Mast, UK). A. baumannii PTCC 1855 (Persian Type Culture Collection) and E. faecalis PTCC 1774 were used as reference strains.

Biofilm Formation Assay

Biofilm production was determined using 96-well microtiter plate method and crystal violet method [21]; The optical density (OD) of each well was measured at 650 nm using ELISA reader. The percent of biofilm-forming capabilities was assessed in compare with control wells.

Probiotics

All probiotic strains were purchased from the Iranian Research Organization for Science and Technology (IROST) as lyophilized preparations. The probiotic strains were Lactobacillus plantarum PTCC 1058, Lactobacillus fermentum PTCC 1744, Lactobacillus rhamnosus PTCC 1637 and Lactobacillus rutrei PTCC 1655. The culture media used for Lactobacillii were Man-Rogosa-Sharpe (MRS) broth and MRS agar medium (Merck, Germany). The probiotic characterization of Lactobacilli was already approved based on tolerance to pH, NaCl, bile salt concentration and the level of producing organic acid [22, 23].

Prebiotics

Raffinose, trehalose, riboflavin, citrate, inulin and sorbitol (Sigma, USA) were used as Prebiotics.

Preparation Of Cell-free Supernatants Of Probiotic And Synbiotic

To prepare cell-free supernatants, 10^6 CFU/mL of one probiotic strain were inoculated in a volume of 15 mL of simple MRS broth media and MRS containing 0.3% of each prebiotic alone or in combination and incubated for 48 h in 37^{o}C under anaerobic condition with periodic mixing. After centrifugation at 7000 rpm for 20 min at 4^{o}C, the cultures were filtered by sterile Millipore filter. Two probiotic synergisms were also considered.

Evaluation Of Antagonistic Activity Of Probiotics And Synbiotics

To evaluate the antimicrobial activity of Lactobacillus strains with prebiotics, well diffusion agar and microdilution methods were used.

Well Diffusion Agar

The cell-free supernatant of synbiotic cultures were collected and used in well diffusion agar method was determined as previously described [24]; Plates were placed in 4^{o}C for one hour and then were incubated at 37 °C. After incubation period, the growth inhibition zone was measured and compared with that of the control group.

Micro-broth Dilution Method

Antibacterial activity (MIC and MBC) of cell-free supernatants of probiotics growth in absence or in presence of prebiotics against clinical isolates of MDR A. baumannii were shown in Table 3. The MIC was recorded as µl of bacterial culture supernatants at a concentration of 10^8 bacteria/ml [29]. All probiotics exhibited an antibacterial effect against MDR A. baumannii. The addition of tested prebiotics, to the culture of L. plantarum, L. ruteri, L. fermentum and L. rhamnosus reduced the MIC value significantly when compared with the probiotics alone. MIC values of probiotics and synbiotics against MDR E. faecalis showed (Table 4) that prebiotics could not decrease the MIC of L. plantarum, and L. ruteri. The best inhibitory results against MDR E. faecalis were showed in presence of all prebiotics with L. rhamnosus culture and L. fermentum in presence of inulin. The prebiotics could improve the inhibitory activity of probiotics against the Gram-negative A. baumannii higher than Gram-positive E. faecalis. Synergistic effect of citrate and trehalose with L. ruteri was better than separately against MDR A. baumannii. The addition of trehalose and citrat (in combination) to L. fermentum and L. rhamnosus cultures showed the best inhibitory effects against MDR A. baumannii.

Table 3
The antimicrobial activity (average MIC and MBC) of synbiotics and probiotics cultures supernatant against ten MDR A. baumannii

Probiotics	µl /mL	Sorbitol	Raffinose	Citrate	Trehalose	Inulin	Riboflavin	Trehalose + Citrat	All	Non
L. fermentum	MIC	1.4	1.8	1.16	1.16	1.8	1.8	1.128	1.32	1.4
	MBC	1.8	1.8	1.64	1.64	1.8	1.8	1.128	1.64	1.8
L. rhamnosus	MIC	1.64	1.4	1.32	1.32	1.4	1.4	1.128	1.4	1.4
	MBC	1.64	1.8	1.32	1.32	1.8	1.4	1.64	1.32	1.8
L. plantarum	MIC	1.4	1.4	1.32	1.32	1.16	1.4	1.64	1.16	1.4
	MBC	1.4	1.8	1.64	1.64	1.32	1.8	1.64	1.16	1.8
L. ruteri	MIC	1.64	1.32	1.32	1.32	1.32	1.4	1.16	1.32	1.4
	MBC	1.64	1.8	1.64	1.32	1.8	1.4	1.32	1.8	1.4
Abbreviation: All: all of prebiotics, Non: probiotic alone in absence of prebiotics										

Table 4
Antimicrobial activity (average MIC and MBC) of synbiotics and probiotics cultures supernatant against ten MDR E. faecalis

Probiotics	µl /mL	Sorbitol	Raffinose	Citrate	Trehalose	Inulin	Riboflavin	Trehalose + Citrat	All	Non
L. fermentum	MIC	1.64	1.4	1.32	1.64	1.128	1.32	1.32	1.64	1.32
	MBC	1.64	1.64	1.8	1.8	1.64	1.4	1.64	1.8	1.4
L. rhamnosus	MIC	1.32	1.4	1.32	1.4	1.32	1.4	1.32	1.128	1.32
	MBC	1.64	1.32	1.32	1.4	1.64	1.32	1.8	1.32	1.64
L. plantarum	MIC	1.32	1.4	1.32	1.4	1.32	1.4	1.4	1.64	1.32
	MBC	1.64	1.4	1.64	1.64	1.32	1.4	1.64	1.8	1.4
L. ruteri	MIC	1.32	1.64	1.4	1.32	1.32	1.4	1.64	1.32	1.32
	MBC	1.8	1.8	1.4	1.4	1.32	1.64	1.64	1.64	1.8
Abbreviation: All: all of prebiotics, Non: probiotic alone in absence of prebiotics										

Determination Of Minimum Bactericidal Concentration (mbc)

Ten micro-liter of the concentrations above the MIC value were added into the nutrient agar, and incubated at 37 °C for 18–24 hours. The concentration which showed no growth was considered as MBC.

Determination Of Antibiofilm Activity Of Probiotics And Synbiotics

The microtiter plate assay was used to determine the antibiofilm activity of probiotics and synbiotics of cell-free supernatants [26]. 100 µL of each MDR isolates of both species of A. baumannii and E. faecalis (about 10^6 CFU/mL) were

added to each well. Then 100 µL of each supernatant (1.9–1000 µL/mL) were added to individual wells. The final volume was adjusted to 200 µl per well using MRS broth. Wells without free-cell supernatant were considered as control. After incubation, entire contents of the plates were poured off, and washing, staining processes were performed as discussed above.

Determination Of Biofilm Killing Activity Of Probiotics And Synbiotics

The biofilm of A. baumannii and E. faecalis was established as discussed before. After that, the well content was removed and the wells were washed to remove the planktonic cells. 100 µL of the supernatant of each synbiotic dilution (1.9–1000 µL/mL) were added to wells and the plates were incubated at 37 ºC for 24 h, again. The biofilm was stained with crystal violet assay (discussed above). The biofilm killing effects of each synbiotic were estimated by determining the OD_{650} of each well in comparison with control wells (bacterial wells without supernatant) [27]. Gentamicin was used as positive control for both Gram-positive A. baumannii and Gram-negative E. faecalis.

Statistical analysis

Each experiment was performed in triplicates. Statistical analysis was performed using SPSS (Version 21) software and One-way analysis of variance (ANOVA). Significance level was set at $p \leq 0.05$.

Results

Antibiotic resistance pattern

With exception of colistin and tetracycline, all isolates of A. baumannii in this study were (100%) resistant to the tested antibiotics (Table 1 and Fig. 1). Isolates of E. faecalis were also resistant to the majority of the antibiotics (Table 2 and Fig. 2). The results indicated that these strains were MDR. MDR bacteria which was defined as an acquired non-susceptibility to at least 1 antibiotic in 3 or more antimicrobial classes [28].

Table 1
Antibiotic resistance patterns of ten A. baumannii isolated from patients

Antibiotic	Sensitive(%)	Intermediate(%)	Resistant(%)
Amikacin	10	0	90
Ampicillin	0	0	100
Cefazolin	10	0	90
Cefotaxime	0	0	100
Ceftazidim	10	0	90
Ceftriaxone	10	20	70
Chloramphenicol	0	10	90
Ciprofloxacin	0	0	100
Colistin	100	0	0
Gentamicin	0	0	100
Imipenem	0	20	80
Meropenem	10	30	60
Solfametaxazol	0	0	100
Tetracycline	100	0	0

Table 2
Antibiotic resistance patterns of ten E. faecalis isolated from patients

Antibiotic	Sensitive(%)	Intermediate(%)	Resistant(%)
Amikacin	10	10	80
Ampicillin	0	0	100
Cefazolin	10	10	80
Cephalexin	0	0	100
Chloramphenicol	0	10	90
Ciprofloxacin	20	20	60
Clindamycin	30	30	40
Erythromycin	60	30	10
Gentamicin	0	10	90
Imipenem	0	40	60
Streptomycin	70	10	20
Tetracycline	30	0	70
Vancomycin	0	0	100

Potential of Biofilm formation in investigated isolates

All isolates of A. baumannii and E. faecalis were able to produce biofilm. The potency of biofilm formation in the studied isolates was moderately positive.

Evaluation of the antagonistic effects of probiotics and synbiotics on planktonic cells

Well-plate Method

The results of antimicrobial activities of Lactobacillus strains against A. baumannii isolates are shown in Fig. 3. Probiotics L. rhamnosus and L. fermentum showed the potent inhibitory effects. L. rhamnosus with citrate and trehalose (separately) was reported to have the most potent inhibitory effects against MDR A. baumannii clinical isolates with an inhibition zone diameter of 28.8 ± 2.1 mm followed by L. fermentum with citrate (26.5 ± 1 mm), L. ruteri with citrate (26.3 ± 0 mm), and L. plantarum with trehalose (26 ± 1 mm) ($p \leq 0.05$). The synergistic results of two probiotic showed that, among them, L. fermentum and L. ruteri had the highest synergistic inhibitory effect on A. baumannii strains with an inhibitory zone diameter of 22 ± 1.2 mm.

The results of antibacterial effects against 10 MDR E. faecalis isolates are shown in Fig. 4. L. rhamnosus with all of prebiotics had the highest inhibitory effect against E. faecalis (29.8 ± 0 mm). L. fermentum with inulin showed the inhibitory activity with an inhibition zone of 29.3 ± 2.2 mm diameter followed by L. rhamnosus with sorbitol (27 ± 2 mm) ($p \leq 0.05$). Insertion of prebiotic to L. ruteri and L. plantarum cultures has no effect on growth of clinical strains in compared with probiotic when used alone.

Antibiofilm Activities Of Probiotics And Synbiotics

The results of inhibition of biofilm formation suggested that, the addition of probiotics and synbiotics supernatant could successfully influence the biofilm formation of pathogenic strains. Comparison of biofilm formation levels among microtiter plate cultures showed that the biofilm inhibition effects of probiotics and synbiotics supernatant was dose dependent. L. plantarum with riboflavin and L. rhamnosus alone or in combination with inulin were found to have the most potent activity than the others and showed better antibiofilm effects against MDR isolates E. faecalis and A. baumannii. Biofilm formation of the experimental group of E. faecalis and A. baumannii was decrease by 75 ± 6.5% and 50 ± 0.86%, in compare to the control group, respectively. According to the results, antibiofilm activity of the probiotics and synbiotics was different ($p < 0.05$). (Table 5 and Fig. 5). Our findings revealed that L. rhamnosus exhibited better antibiofilm effects than the others, and prebiotics don't have any effect in antibiofilm activity of L. rutteri.

Biofilm Killing Activities Of Probiotics And Synbiotics

Probiotics and synbiotyics could not remove or disperse E. faecalis and A. baumannii biofilm after its formation.

Discussion

In recent years, inhibition of bacterial biofilm formation has been an attractive target for therapeutic intervention [26]. This strategy leads to the discovery and development of antibiofilm compounds for MDR bacteria including A. baumannii and E. faecalis. E. faecalis and A. baumannii are among the important pathogens found in many healthcare-related infections, and are difficult to eradicate because of their resistance to the broad spectrum antibiotics and production of biofilm [30]. In the current study, the clinical isolates of A. baumannii and E. faecalis were resistant to the most tested antibiotics and were

reported as MDR strains. Many researchers studied the antimicrobial effects of probiotics but few investigations reported the effects of synbiotics on pathogens. We observed that all tested probiotics in this study have good antimicrobial properties. Furthermore, cell-free supernatant of L. rhamnosus showed the most potent inhibitory effect against A. baumannii and E. faecalis, and L. plantarum and L. rutteri exhibited the lowest inhibitory activities. These results are in agreement with the opinion of Coconnier et al., which reported that probiotics could influence the growth and pathogenesis of Klebsiella [31]. Production of metabolites such as acetic acid and lactic acid by probiotic bacteria can alter the pH and inhibit adhesins and, invasins of pathogenic bacteria [31]. In a similar study, Grimoud et al. reported that Lactobacillus strains could produce the antimicrobial agents against intestinal pathogens in comparison with Bifidobacterium, and had the most antimicrobial activities [32]. Mamianas et al. stated that L. plantarum has good antimicrobial effects against S. aureus, E. coli and Bacillus subtilis [33]. To date, only a few carbohydrates have been defined and reported as prebiotics, including inulin, lactulose, β-galacto- oligosaccharides, and fructo-oligosaccharides [29]. In the present study, raffinose, trehalose, riboflavin, citrate, inulin, and sorbitol was used as prebiotic. These prebiotics were used in lower concentrations (0.3%) than previous studies (0.5–5%) [34, 35].The results show the efficiency of prebiotics at lower concentration. Our results showed that cell-free supernatant of L. rhamnosus with citrate or trehalose and L. fermentum with trehalose have the best antibacterial activity against Gram-negative A. baumannii. The findings also showed, the supernatant of L. rhamnosus followed by L. fermentum with inulin have potent inhibitory effects against Gram-positive E. faecalis. In the study by Mandadzieva et al., antimicrobial activity of different strains of Lactobacillus against Enterobacter aerogenes was increased in the presence of oligosaccharides [34, 35] which was similar with our findings. The study found that the adsorption of abnormal sugars could increase the production of antimicrobial agents on specific pathways [34, 35]. The mechanism of this stimulatory activity and how the lactic acid bacteria use oligosaccharides is still unclear due to the unique characteristics of the strain. The present study indicate that prebiotics can trigger the antimicrobial properties of probiotics, by increasing the production of antimicrobial metabolites and bacteriocins. In this study, the antimicrobial effect was studied at different conditions between probiotic and prebiotic, as well as in synergism with two probiotics. Likewise, the synergism of two probiotics had less antimicrobial activity compared to the synbiotic. This observation can be due to the competitive effects of two bacteria to uptake nutrients, etc. The increased antimicrobial activity is dependent on the type of probiotic strains, the strains of the pathogenic bacteria, and the presence of one or more prebiotic. In the current study, the prebiotics improve the antimicrobial activity of probiotics against the Gram-negative compare to the Gram-positive isolates suggesting that the metabolites involved in inhibitory effects are different or act differently.

Comparison with the controls, we found that the pathogenic strains were moderate biofilm producer ($p < 0.05$). The inhibitory effects on biofilm formation by cell-free supernatant of probiotics and synbiotics revealed that, biofilm formation ability of A. baumannii was decreased by 75 ± 6.5% in presence of L. rhamnosus with inulin but this combination could not remove biofilm after its formation. L. plantarum in presence of riboflavin was found to be more potent than the others in inhibition of E. faecalis biofilm formation and had good antibiofilm effect (50 ± 0.86% decrease). We did not found any significant difference between antibiofilm effects of probiotics alone or in combined with prebiotics, revealed that, prebiotics had no significant effect on biofilm control. Probiotics also showed more activity in biofilm control against A. baumannii than E. faecalis. In the current study none of probiotics and synbiotics removed biofilm after formation. In another study, B. cereus displayed significant decrease in biofilm formation in the presence of L. plantarum or L. pentosus supernatants that is in parallel with our findings [17]. Besides, other sties stated that supernatant of both of these probiotics showed good antibiofilm effect against P. aeruginosa and K. pneumonia [36, 17]. In the same way, the antibiofilm property of L. acidophilus has been exhibited only in co-culture with S. aureus but not reduction of biofilm mass of E. coli [37]. Surface adhesion of bacteria is major factor of biofilm formation. The anti-adhesion property of probiotics is commonly associated with competitive adherence for binding sites or ability of metabolite production of synbiotics to inhibit the adhesion of pathogenic bacteria to a surface. In another study was described biosurfactants derived from probiotic lactobacilli showed both antibacterial and antifungal activities against several resistant pathogens, A. baumannii, E. coli and S. aureus [11, 5]. In addition, biosurfactants have been reported to remove stablished biofilms of Bordetella bronchiseptica and B. pumilus

[38, 39]. Probably, this means that antibiofilm effects involved to preventing adhesion of pathogenic bacteria to 96-well microtiter plate surface. Another possibility could be referred to the inhibition of quorum sensing [17].

There is an urgent need for developing novel agents to control biofilms in medical settings. A wide range of promising treatments have been evaluated in different biofilm-related infections.

Conclusion

The results of this work described a very promising antibacterial and antibiofilm activity of cell-free supernatant of probiotics and synbiotics against problematic nosocomial pathogens, A. baumannii and E. faecalis. However, they have more activity when inhibits pre-established biofilms than when they inhibit established biofilms. Therefore, these probiotics and synbiotics can produce metabolites that inhibit both growth and biofilm formation. These capabilities support possibilities for probiotics and synbiotics as an alternative therapeutic agent for the prevention and/or treatment of these nosocomial infections as the origin of several metabolites such as anti-adhesion agents (i.e. biosurfactant). However, further in vivo studies are recommended to investigate this hypothesis.

Declarations

Acknowledgement

This study was approved by Islamic Azad University, Neyshabur Branch, as a MS thesis.

Authors' Contributions

Samaneh Dolatabadi designed the study, wrote the protocol, and the draft of the manuscript. Niki Laal-Kargar contributed to sampling and performed the microbiological tests. Mahnaz Mohtashami performed the statistical analysis.

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Competing interests

The authors declare that they have no competing interests.

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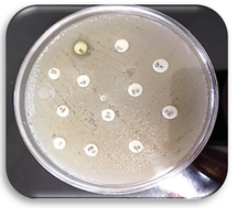
Figures

Figure 1

Results of disc diffusion method showing resistant A. baumannii isolates.

Fig. 2.

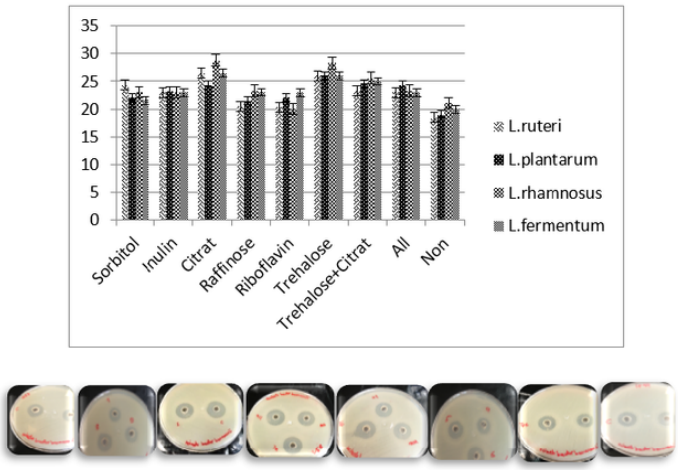


3

Figure 2

Disc diffusion method showing resistant isolates of E. faecalis.

Fig.3.

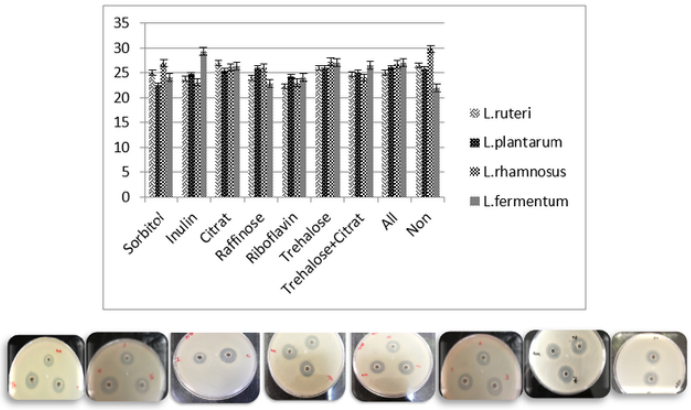


4

Figure 3

Inhibition zone (mm) of probiotic and synbiotic cell-free supernatants against A. baumannii. C: citrate, I: inoline, 2: Trehalose, 4: Riboflavin, 5: Raffinose, 6: Sorbitol, All: all probiotics, Non: non probiotics.

Fig. 4.

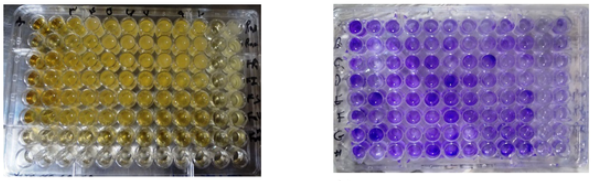


5

Figure 4

Inhibition zone (mm) of probiotic and synbiotic cell-free supernatants against E. faecalis. C: citrate, I: inoline, 2: Trehalose, 4: Riboflavin, 5: Raffinose, 6: Sorbitol, All: all probiotics, Non: non probiotics.

Fig. 5.



a) b)

6

Figure 5

Antibiofilm activity of probiotic and synbiotic cell-free supernatants against a) A. baumannii, b) E. faecalis using Chrystal violet method by determining the OD620 of each well in comparison with control wells.

Table 5

Average effect of synbiotics and probiotics culture supernatant on biofilm formation

	MBIC (µl/ml) of MDR A. baumannii				MBIC (µl/ml) of MDR E. faecalis			
	L. fermentum	L. rhamnosus	L. plantarum	L. ruteri	L. fermentum	L. rhamnosus	L. plantarum	L. ruteri
Raffinose	3.8	15.6	7.8	15.6	7.8	7.8	31.2	31.2
Citrate	7.8	7.8	31.2	15.6	3.8	31.2	15.6	62.5
Trehalose	7.8	3.8	15.6	7.8	7.8	31.2	7.8	15.6
Inulin	15.6	1.9	7.8	7.8	15.6	15.6	62.5	31.2
Riboflavin	3.8	3.8	3.8	31.2	3.8	7.8	1.9	7.8
Sorbitol	3.8	7.8	3.8	15.6	15.6	15.6	7.8	15.6
All	7.8	3.8	15.6	7.8	7.8	7.8	31.2	62.5
Non	15.6	1.9	7.8	7.8	3.8	7.8	7.8	7.8

Abbreviation: All: all of prebiotics, Non: probiotic alone in absence of prebiotics