Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(8):464-467

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Anti-biofilm activities of *Lactobacillus acidophilus* against *Staphylococcus aureus* ATCC 25923

Dung Nguyen[1], Trang Huynh[2] and Tu Nguyen[2*]

[1]Faculty of Resources and Environment, Thu Dau Mot University, Vietnam
[2]School of Biotechnology, HoChiMinh City International University, Vietnam National University - HoChiMinh City, Vietnam

ABSTRACT

Staphylococcus aureus ATCC 25923 could form biofilm in the medium containing glucose (1%) and K_2HPO_4 (0.1 %). When Lactobacillus was incubated with planktonic S. aureus at the same time, Lactobacillus formed biofilm together with S. aureus and reduce biofilm formation about 15% on polystyrene surface of 96 well plates, consequently. However, if Lactobacillus was added into S. aureus biofilm, Lactobacillus could reduce approximately 83% S. aureus biofilm. Therefore, the study warned us to be careful when use Lactobacillus in inhibition of S. aureus biofilm in order to eliminate biofilm effectively.

Keywords: anti-biofilm activity, *Staphylococcus aureus*, *Lactobacillus acidophilus*

INTRODUCTION

Staphylococcus aureus (*S. aureus*) belongs to a gram-positive group with a cocci shape. It was found in grape-like clusters. Frequently, *S. aureus* is mostly found in the anterior nares in humans which are the favorable conditions for maintenance and infection. Especially, *S.aureus* has characteristic of biofilm formation. When *S. aureus* enters into the circulatory system, it avoids the detection by immune system, binds to a specific surface including infection area and forms biofilm to survive in host [1]. Moreover, its biofilm can be created on both biotic and abiotic surface; so, *S. aureus* shows resistance to antibiotics that becomes a problem in treatment [2-7]. Some infectious diseases related to the *S .aureus*' biofilm formation were arthritis, endocarditis, and cystic fibrosis [8-10].

Bacteria could have characteristics to form biofilm. The extracellular polymeric matrix is made from the combination of exopolysaccharides, proteins, teichoic acids, enzymes, and extracellular DNA [11-12]. Regarding to previous studies, the matrix's structure is changed from strains to strains due to the environment and the conditions [13-14]. Biofilm may have a 3-dimension structure, but sometimes a single layer only [15-16]. By living in community, biofilms have various benefits and advantages from its parts and one of them is resistant to the immune system and antibiotics.

Nowadays, *Lactobacillus acidophilus* belongs to GRAS group showing antimicrobial activities. *Lactobacillus acidophilus* is gram-positive bacteria naturally living in human and animal digestive system. *Lactobacillus* is also used in dairy products including milk, yogurt… in combination with other microbes. *Lactobacillus* has potential to be used as an antibiotic medicine and a drug deliver [17].

As the above statement, the aim of the study of *Lactobacillus* was to know whether *Lactobacillus* inhibit *Staphylococcus* biofilm or not.

EXPERIMENTAL SECTION

Bacterial strains and growth

Lactobacillus acidophilus VTCC-B-871 was obtained from Vietnam type culture collection (Vietnam). *Lactobacillus acidophilus* (*L. acidophilus*) was grown in *Lactobacilli* MRS broth medium [18] at 37°C in 24 hours. *Staphylococcus aureus* ATCC 25923 was grown in LB broth and LB agar at 37°C in 24 hours under aerobic condition.

Preparation of *Staphylococcus aureus* biofilm

Bacterium was cultured in sterile flat-bottomed 96-well polystyrene microliter plates to form biofilm with five different types of medium: (1) LB, (2) LB with 1% of glucose, (3) LB with 1% glucose and 0.1% of K_2HPO_4, (4) LB with 0.1% of of K_2HPO_4, (5) LB with 0.1% of Na_2HPO_4.

Biofilm formation assay

Biofilm was formed in sterile flat-bottomed 96-well polystyrene microlitter plates based on the previous studies. One colony was inoculated in 5 mL of LB broth and cultured at 37°C for 18 to 20 hours with agitation at 180 rpm to obtain a culture with 10^9 CFU/mL. The bacteria were diluted at 1:100 with appropriate medium: (1) LB, (2) LB with 1% of glucose, (3) LB with 1% glucose and 0.1% of K_2HPO_4, (4) LB with 0.1% of K_2HPO_4, (5) LB with 0.1% of Na_2HPO_4 and added 200 µL of diluted bacteria into the wells of 96-well plate. 200 µL of bacteria in NaCl 0.9% was put into one well to be used as a control. The plates were incubated at 37°C without agitation in 24, 48 and 72 hours [19]. After incubation, the broth in each well of the plate was discarded; then, the wells were washed three times with 200µl of sterile phosphate buffer saline, pH 7.4 (PBS). The wells were heated at 60°C for 60 minutes [19]. The wells were stained with 50µl of 0.1% crystal violet for 10 to 15 minutes at room temperature. The crystal violet was removed by micropipette; then, the wells were washed with 200 µL of sterile distilled water for three times to remove the excess crystal violet solution. The plate was dried at room temperature before adding 200 µL of 95% ethanol. The plate was incubated for 15 to 20 minutes at room temperature with gently agitation and measured the OD with the wavelength at 630nm. The assay was performed three times.

Evaluation of *Lactobacillus acidophilus* in *S. aureus* biofilm preformation

Culturing S. aureus together with Lactobacillus acidophilus

One colony of *S. aureus* was inoculated in 5 mL of LB broth and cultured at 37^{o}C for 18 to 20 hours to obtain a culture with 10^9 CFU/mL. The bacterial culture was diluted according to a ratio (1:100) with LB containing glucose (1%) and K_2HPO_4 (0.1%). 180 µL of diluted bacterial culture was added into the wells of 96-well plate. Add 20 µL (10^9 CFU/mL) of *L. acidophilus* to obtain 10^8 CFU/mL in each well containing *S. aureus*. 200 µL of bacteria in NaCl (0.9%) was put into other wells to be used as a control. Incubate the microliter plates at 37^{o}C in 24 hours.

After incubation, the plate was inverted and the remaining medium in the wells was removed. Wash the wells with PBS pH 7.4 for 3 times. Put the 96-well plate in the incubator at 60^{o}C for 60 minutes to remain the attachment of the bacteria by being heat-fixed. After incubation, add 50µL of crystal violet solution (0.1%) to each well and keep it for 10 to 15 minutes at room temperature. Then, the wells were washed with distilled water for two or three times to remove the excessive crystal violet solution. Add 200 µL of absolute ethanol to each well, and incubate for 15 to 20 minutes at room temperature with gently agitation. Measure OD of the 96-well plate with the wavelength at 630 nm.

Adding Lactobacillus acidophilus into S. aureus biofilm

The *S. aureus* biofilm was formed in 96-well plate at 37°C in LB medium with glucose (1%) and K_2HPO_4 (0.1%) for 24 hours with the method above. The bacterial biofilm attached at the bottom of the plate was treated with (10^7, 10^8 CFU/mL) *L. acidophilus* at 37°C for addition 24 hours. Measure the absorbance of biofilm before and after treated. The percentage of biofilm disruption was calculated.

Analysis

SPSS 16 version and Excel 2007 were used to analyze collected data. Results were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

***Staphylococcus aureus* biofilm formation**

S. aureus was cultured in five different growth media (Table 1) and time of incubation (24, 48 and 72 hours) to determine these influences on biofilm formation on polypropylene 96-well plate. The absorbance of biofilm formation was observed by spectrophotometer at wavelength 630 nm at different times and media (Table 1).

Table 1 represented the formation of biofilm of two *S. aureus* strains in 24, 48 and 72 hours in different medium. From table 1, bacterium produced the highest amount of biofilm at 24 hours and in LB containing glucose and

K_2HPO_4. This supplemented medium stimulated the biofilm formation while LB medium didn't support for biofilm formation due to the less substrate for biofilm formation, probably. Actually, LB medium is only a nitrogen source. Based on the general characteristics relating to biofilm producing capacity [20, 21], the environmental factors including temperature, pH, nutrient and glucose affect the biofilm formation [22]. From table 1, K_2HPO_4 and Na_2HPO_4 didn't strongly influence on biofilm production but glucose showed the biofilm production, suggesting that the plastic device used to contain glucose for *S. aureus* infecting patients should be careful. In addition, LB containing glucose (1%) and K_2HPO_4 (0.1%) caused *S. aureus* to form the highest biofilm. It was meant that biofilm was formed in the existence of glucose and K_2HPO_4 that modified reaction together. With these results, LB containing glucose and K_2HPO_4 was selected to produce biofilm for study.

Table 1: Absorbance of *Staphylococcus aureus* ATCC 25329 at wavelength 630nm after incubated 24, 48 and 72 hours in different medium

Time (hours)	LB	LB + glucose	LB + glucose + K_2HPO_4	LB + K_2HPO_4	LB + Na_2HPO_4	Control
24	1.87 ± 0.18	2.17 ± 0.05	3.13 ± 0.10	1.79 ± 0.09	1.76 ± 0.24	0.17 ± 0.02
48	1.80 ± 0.15	3.7 ± 0.05	4.58 ± 0.12	1.52 ± 0.10	2.14 ± 0.11	0.22 ± 0.06
72	2.3 ± 0.10	4.85 ± 0.05	6.8 ± 0.12	1.80 ± 0.04	2.4 ± 0.05	0.20 ± 0.03

Biofilm morphology study

After incubated in 96-well plate, the biofilm of both *S. aureus* strains were observed under might microscope (Fig 2) to check the bacterial morphology. *S. aureus* illustrated the *S. aureus* biofilm under scanning electron microscope (SEM) after fixation and dehydration. As shown in fig. 2, the cells were covered by a layer that was thought as extra-polysaccharide produced by *S. aureus*. Glucose and K_2HPO_4 involved in extra- polysaccharide via some genes.

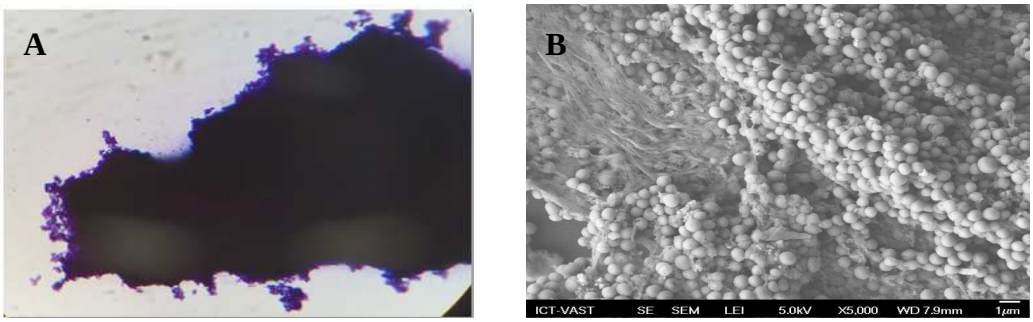

Figure 2: Representative *Staphylococcus aureus* biofilm observed under light microscope (A); Scanning Electron Microscope (B) x 5000 times

Evaluation of *Lactobacillus acidophilus* in *S. aureus* biofilm preformation

In order to clearly understand the effects of *Lactobacillus acidophilus* in *S. aureus* biofilm preformation, *Lactobacillus acidophilus* was incubated together with *S. aureus*. After measuring the absorbance, *L. acidophilus* reduced biofilm formation insignificantly (Table 2).

Table 2: Absorbance of *Staphylococcus aureus* biofilm containing *L. acidophilus*

Amount of L. acidophilus (CFU/mL)	*OD of biofilm before treatment*	OD biofilm after treatment
10^7	3.08±0.09	2.70±0.36
10^8	3.14±0.11	2.85±0.6

The percentage of untreated biofilm was measured approximately 86-88%. Probably, *L. acidophilus* could inhibit *S. aureus* at the first stage and then produce biofilm in mixture of *Lactobacillus acidophilus* and *S. aureus* by microscope analysis (Fig. 2). The results warned us it will be carefully considered to use *Lactobacillus acidophilus* to preserve food contained in plastic bags as well as medical plastic tools and so on.

As that stated situation, *Lactobacillus acidophilus* was used to treat *S. aureus* after *S. aureus* formed biofilm. The percentage of untreated biofilm was measured approximately 53-61% (Table 3). From results as showed in table 2, table 3, *Lactobacillus acidophilus* could form biofilm in *S. aureus* planktonic cells due to they could produce some extracellular compounds that communicated together leading *Lactobacillus acidophilus* produced biofilm more than *Lactobacillus acidophilus* only.

Table 3: Absorbance of treated *S. aureus* with *L. acidophilus* at different concentration in 1 day

Amount of L. acidophilus (CFU/mL)	OD of biofilm before treatment	OD of biofilm after treatment
10^7	3.23±0.41	1.98±0.14
10^8	3.72±0.17	2.15±0.6

CONCLUSION

Obviously, although *Lactobacillus acidophilus* belongs to general recognition of safety (GRAS) group that was used as probiotic, the dose of this bacterium and the way of use in biofilm treatment should be considered well to have the best treatment for human life.

REFERENCES

[1] FD Lowy. *N. Engl. J. Med.*, **1998**, 339, 520–532.
[2] I Fedtke; F Gotz; A Peschel. *Int. J. Med. Microbiol.*, **2004**, 294, 189-194.
[3] DP Greenberg; AS Bayer, AL Cheung, JI Ward. *Infect. Immun.*, **1989**, 57, 1113-1118.
[4] M Hussain; A Haggar; C Heilmann; G Peters; JI Flock, M Herrmann. *Infect. Immun.*, **2002**, 70, 2933-2940.
[5] D Joh; ER Wann; B Kreikemeyer; P Spezial. *Matrix Biol.*, **1999**, 18, 211-223.
[6] PW Park; J Rosenbloom, WR Abrams, RP Mecha. *J. Biol. Chem.*, **1996**, 271, 15803-15809.
[7] JM Patti; BL Allen; MJ McGavin; M Hook. *Annu. Rev. Microbiol.*, **1994**, 48, 585-617.
[8] GM Costa; C Pizzi; C Leone; A Borghi; E Cordioli; R. Bugiardini. *Cardiologia.*, **1999**, 44, 675-678.
[9] I Raad. *Lancet*, **1998**, 351, 893-898.
[10] S Rajan; L Saiman. *Semin. Respir. Infect.*, **2002**, 17, 47-56.
[11] MB Melchior; H Vaarkamp; J Fink-Gremmels, *Vet. J.*, **2006**, 171, 398-407.
[12] J Parra-Ruiz; C Vidaillac; MJ Rybak. *Rev. Esp. Quimioter.*, **2012**, 25, 10-16.
[13] H Rohde; JK Knobloch; MA Horstkotte; D Mack. *Med. Microbiol. Immunol. (Berl)*, **2001**, 190, 105-112.
[14] P Landini. *Res. Microbiol.*, **2009**, 160, 259-266.
[15] CG Kumar. *Int. J. Food Microbiol.*, **1998**, 42, 9-27.
[16] V Deibel. *J. Food Safety*, **2001**, 1, 6-7.
[17] TTV Doan; HKT Nguyen. *J. App. Pharm. Sci.*, **2013**, 3, 33-36.
[18] JD De Man; MA Rogosa; ME Sharpe. *J. Appl. Bact.*, **1960**, 23(1), 130-135.
[19] RK Agarwal; S Singh; KN Bhilegaonkar; VP Singh, *Int. Food Res. J.*, **2011**, 18, 1493–1498.
[20] KA Nathan; Mark JM; J William Costerton; GL Jeff; EP Mary; ES Mark, *Virulence*, **2011**, 2(5), 445-459.
[21] TJ Beveridge; SA Makin; JL Kadurugamuwa; Z Li, *FEMS Microbiol. Rev.*, **1997**, 20, 291-303.
[22] S Periasamy; HS Joo; AC Duong; THL Bach; VY Tan; SS Chatterjee et al,. *PNAS*, **2012**, 109, 1281-1286.