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Antibacterial and Anti virulence factors of Purified Dextran from *Lactobacillus gasseri* against *Pseudomonas aeruginosa*

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Abstract

The objective of the current study is to purify and characterize dextran from *Lactobacillus gasseri* and to detect its anti-virulence factors against hemolysin, pyocyanin, and biofilm formation of clinical *Pseudomonas aeruginosa* isolated from wounds and burns. Purification and characterization of dextran were carried out by a Thin Layer Chromatography (TLC) and a Fourier Trans Infrared Spectroscopy (FTIR). Purified dextran obtained was white, granular, and easily soluble in water. TLC results showed that the purified dextran was composed of glucose only; while FTIR showed that dextran polysaccharide contained both (1-6) ∝- D glucan and (1-3) ∝- glucan. Antibacterial effect of purified dextran was determined against *P.aeruginosa* isolates using Minimum Inhibitory Concentration (MIC) with concentrations ranging between 0.39 to 200 mg/ml; the MIC was 50 mg/ ml for all isolates. On the other hand, the effect of dextran on the virulence factors of *P.aeruginosa* was evaluated. Dextran inhibited hemolysin production of *P.aeruginosa* and the highest inhibition recorded was 29.03%. Pyocyanin production by *P.aeruginosa* clinical isolates was inhibited by dextran and the concentration was recorded between 2.28-2.35 µg/ml compared with 3.31- 3.39 µg/ml for control. The effect of purified dextran on biofilm formation was studied at different incubation periods (24, 48 and 72 h), the highest biofilm inhibition was observed after 72 h was 71.42 %, while the lowest inhibition after 24 h was 37.66% compared to control, which recorded 0% in the absence of dextran. In conclusion, the purified and characterized dextran from local *L. gasseri* had an inhibitory effect on the growth and virulence factors of clinical *P. aeruginosa* isolated from wounds and burns.

Keywords: *Lactobacillus*, Dextran, Antibacterial, Antivirulence factors

1. Introduction

Dextran has a chemical formula of H- $(C_6H_{10}O_5)_n$-OH and is produced by the action of the microorganism's extracellular dextransucrase(Guzman *et al*.,2018). Dextran, such as that produced by *Leuconostoc* species and other dextran-producing lactic acid bacteria including *Streptococcus*, *Lactobacillus*, *Pediococcus* and *Weissella*, typically varied in their relative molecular mass, degree of branching, type of branching, and length of branched chains (Kothari *et al*., 2015). It is a hydrophilic, neutral, biodegradable and biocompatible polysaccharide (El-Meliegy *et al*., 2018). Dextran is used in food, chemical and pharmaceutical industries as emulsifier, stabilizer, adjuvant and carrier; also it is used in the field of drugs as blood plasma volume expander (Bhavani and Nisha, 2010) and as drug delivery with no toxic side effects (Huang and Huang, 2019).

Pseudomonas aeruginosa is a non-fermenting, motile, rod-shaped, Gram-negative bacterium belonging to the Pseudomonadaceae family (Alhazmi, 2015). It is responsible for acute infections commonly associated with burn wounds, colonization of host tissue. Host tissue damage facilitates adherence and colonization (Lovewell *et al*., 2014). This bacterial species has many virulence factors as a strategy for survival in the host (Feng *et al*., 2016).

The nosocomial and toxicogenic *P. aeruginosa* is a highly adaptable opportunistic bacteria that prevalent in patients with immuno-compromised cystic fibrosis causing invasive infections (Kany *et al*., 2018); it is responsible of 10% of total infections in the hospitals (Fazeli *et al.*, 2012). In burned patients *P. aeruginosa* is the causative agents of invasive infections, it is known as a resistant bacteria to a wide range of antimicrobial agents and the host immune system due to their ability in forming biofilms, causing difficulties in medical treatments (Alhazmi,2015). *P. aeruginosa* has been isolated from patient care equipment such as catheters, blood gas analyzers, breastfeeding bottles, from health care workers, and the environment such as sinks (Harnaen *et al*., 2015). The growth of *P. aeruginosa* could be strongly inhibited in the presence of dextran and the inhibition of growth reached 55.41% (Wang *et al*., 2010). A study by Wang *et al*. (2013) showed an inhibitory effect of dextran-coated by ceria nanoparticles (CeO_2) on the growth of *P.aeruginosa*; the dextran-coated may have promoted absorption of protein that inhibited bacterial growth. Therefore, this study aimed to examine the ability of dextran, purified from locally *L.gasseri* to inhibit growth and some virulence factors of *P.aeruginosa* isolated from burns and wounds.

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2. Materials and Methods

2.1. Microorganisms

2.1.1. Lactobacillus gasseri

Lactobacillus gasseri was isolated from healthy women's vagina; vaginal swabs were placed in MRS medium, incubated anaerobically at 37 ^{o}C for (24-48) h. The isolate was identified using cultural, cellular, and biochemical tests as well as the Vitek 2 system.

2.1.2. Pseudomonas aeruginosa

A total of 20 clinical *Pseudomonas aeruginosa* were isolated from burns and wounds from Baghdad hospitals. All isolates were subjected to cultural, microscopic, and biochemical tests as well as to the Vitek 2 system. The virulence factors (hemolysin**,** pyocyanin**,** and biofilm formation) of all isolates were detected and 4 isolates that had the same virulence factors were chosen for further work (data not shown).

2.2. Dextran Production

This process was done according to the procedure described by Onilude *et al.* (2013) with minor modifications. Briefly, 100 ml of autoclaved dextran production medium (sucrose 150 g. peptone 5.0 g, K_2HPO_4 15.0 g, $MnCl_2.4H_2O$ 0.01 g, yeast extract 5.0 g, NaCl 0.01 g, $CaCl_2$ 0.05 g, added to 1 liter of distilled water, pH 7.0), was inoculated with 2% of the *L.gasseri* suspension (from MRS broth) at a concentration of 9 x 10^8 CFU/ml and incubated at 37 ˚C for 24 h.

2.3. Precipitation of Dextran

The total culture medium of dextran production was precipitated by using an equal volume of chilled ethanol, shaken, centrifuged for 15 min at 10,000 rpm and the supernatant was decanted. Precipitated dextran was dissolved in distilled water for the removal of impurities. The dextran slurry was again precipitated with an equal volume of chilled ethanol (Sarwat *et al*., 2008). This step was repeated two times. The precipitated dextran was dried in the oven (40C°) for 45 minutes.

2.4. Purification of Dextran Produced by L .gasseri

The precipitated dextran was dissolved in distilled water, then the suspension of dextran was precipitated with an equal volume of chilled ethanol. Re-dissolving, precipitation, and washing were repeated three times for cell debris elimination (Abedin *et al*., 2013). Purified dextran was dried using the oven (40C° for 45 min) then calculated on a dry weight basis.

2.5. Characterization of Dextran Purified from L. gasseri

2.5.1. Thin-Layer Chromatography (TLC)

The purified dextran from *L. gasseri* was analyzed and characterized by Thin-Layer Chromatography (TLC) to confirm its components. Dextran (0.01 gm.) was hydrolyzed in 5 % HCl (v/v) and heated for an hour in a water bath at 100ºC. Equal weights (0.01gm) of glucose, galactose, and fructose were dissolved in 1mL of 1% ethanol. TLC was performed using silica. The position and distance of the spots were determined, and the relative flow (Rf) was calculated according to the equation described by Radhi *et al*. (2013):

$$RF = \frac{\text{Distance moved by substance}}{\text{Distance moved by the solvent front}}$$

2.5.2. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) has been carried out in the Department of Chemistry / College of Science/ Mustansiriyah University / Baghdad, Iraq. The instrument operates in the wavelength range of 400 – 4000 cm^{-1} that measures the amount of IR radiation reflected or transmitted through a sample. The results were obtained in the form of a graphical chart, in which the X-axis represents the wavelength, while the Y-axis represents the transmittance %.

2.6. Antibacterial Activity of Purified Dextran against Pseudomonas aeruginosa Isolates

The antibacterial activity of dextran purified from *L. gasseri* against *P.aeruginosa* isolates was determined by the microdilution method in 96 – well flat-bottom microtiter plate based on minimum inhibitory concentration (MIC) values. The experiment was performed according to the procedures described by Salman *et al*. (2018). A stock solution of purified dextran in sterilized distilled water was diluted to a concentration ranging from 200 to 0.39 mg/ml. 125 µl of sterile Muller Hinton Broth was added in the first column of the 96 – well microplate, then 125 µl of dextran solution with a concentration of 200 mg/ml was added and mixed with the medium in the first column. Serially, 125 µl were transferred to subsequent wells and discarding 125 µl of the mixture in the last column, so that the final volume for each well was 125 µl. Control well contained 125 µl of Muller Hinton Broth only without dextran. The wells were inoculated with 2.5 µl of an overnight culture of *P.aeruginosa* isolates compared with the McFarland 0.5 ml. Microplates were covered and incubated at 37˚C for 24 h. The MIC was determined at a concentration in which no visible growth could be observed after subculturing on nutrient agar plates at 37C˚ for 24 h.

2.7. Effect of Dextran Purified from L. gasseri on P.aeruginosa Virulence Factors

2.7.1. Inhibition of Hemolysin Production

The inhibition of hemolysin production by dextran was detected using the procedure described by Lee *et al*. (2014), four *P.aeruginosa* isolates were grown in the absence and presence of dextran at sub MIC concentration, 20 µl of *P. aeruginosa* suspension compared to 0.5 McFarland was added to 80µl Nutrient broth mixed with 100 µl of dextran, while control contained only 180 µl of Nutrient broth and 20 µl of *P.aeruginosa* without dextran, incubated at 37˚C for 24 h.

Human red blood cells were prepared by centrifugation at 3000 rpm for 5 min and washed with PBS three times. The pellet diluted in PBS (330 µl red blood cells were added to 10 ml PBS).100 µl of bacterial (treated with dextran and control) was added to 10 ml red blood cells previously prepared and incubated at 37˚C for 1 hour. Centrifugation was applied at 12,000 rpm for 10 min to collect the supernatants, and then the optical densities were measured at 543 nm. Inhibition of hemolysin production percentage was calculated as the equation according to Lalitha *et al.* (2013) with some modification.

$$\% \text{ Inhibition of hemolysin production} = \frac{\text{O.D control} - \text{O.D treatment}}{\text{O.D control}} \times 100$$

2.7.2. Inhibition of Pyocyanin Production

The effect of purified dextran on pyocyanin production of *P.aeruginosa* isolates was evaluated using a pyocyanin quantitative assay described by Essar *et al*. (1990). The supernatant of *P.aeruginosa* grown in T-broth medium with dextran (sub MIC) at 37C° for 24 h was prepared by centrifugation at 1000rpm/10min. Control included supernatant of bacterial isolates grown in T-broth only without dextran. A volume of 7.5 ml of supernatant was extracted by 4.5 ml of chloroform, then vortexed for 10 sec (until the lower layer changed to blue color). Then two volumes of the blue layer were extracted by one volume of (0.2N) HCl and were mixed well for 10 sec to produced pink to deep red color. The absorbance of the acidic solution was measured at 520nm, using chloroform as blank. The concentrations expressed as micrograms of pyocyanin produced per milliliter of culture supernatant were calculated by the equation below,

O.D520 ×17.072= Conc. of Pyocyanin (µg/ml)

Inhibition of pyocyanin production percentage was calculated as the equation below,

$$\% \text{ Inhibition of pyocyanin production} = \frac{\text{Conc.of pyocyanin}\left(\frac{\mu g}{ml}\right)\text{control} - \text{Conc.of pyocyanin}\left(\frac{\mu g}{ml}\right)\text{treatment}}{\text{Conc.of pyocyanin}\left(\frac{\mu g}{ml}\right)\text{control}} \times 100$$

2.7.3 Inhibition of Biofilm Formation

The effect of dextran on the biofilm formation of *P.aeruginosa* was studied using 96 flat-bottom well microtiter plates according to the procedure described by Ali (2012). *P.aeruginosa* isolates were grown at 37 ºC for 24, 48, and 72 hours in the presence and absence of dextran at the sub MIC concentration. The inhibition percentage of the biofilm formation was calculated according to the equation described by Namasivayam *et al*. (2012).

$$\% \text{ Inhibition of biofilm formation} = \frac{\text{O.D control} - \text{O.D treatment}}{\text{O.D control}} \times 100$$

3. Results

3.1. Identification of Bacterial Isolates:

3.1.1. Lactobacillus gasseri

White to yellow, smooth, and round colonies of *L.gasseri* were observed on the surface of MRS *Lactobacillus* agar. Microscopical examination showed that the cells of *L.gasseri* were gram-positive, non–spore forming and short rods single or in pairs. Biochemical tests showed that *L.gasseri* isolate was catalase and oxidase negative. For Vitek 2 ANC ID card results, isolate showed positive results of D-Cellobiose, D-Glucose, D-Fructose, D-Galactose, D-Sucrose, Maltose, D-Mannose, Ala-Phe-Pro-arylamidase, L-pyrrolydonyl arylamidase, L-Proline arylamidase, Tyrosine arylamidase, Maltotriose, Leucine arylamidase, Phenylalanine arylamidase, N-acetyl –D-glucosamine, Arbutin and Esculin hydrolysis, while it gave negative results for remaining tests in the card.

3.1.2. Pseudomonas aeruginosa Isolates

Colonies of *P.aeruginosa* on MacConkey agar appeared as pale yellow, smooth round colonies, and gave grape-like odor, while on *pseudomonas* agar appeared green colony. Microscopically, *P. aeruginosa* isolates appeared as gram-negative very small straight rods that occurred as single or in pairs. All isolates gave positive results for catalase and oxidase tests. Identification of isolates by Vitek 2 GN ID card showed positive results of L-pyrrolydonyl arylamidase, Glutamyl arylamidase, D-Gamma glutamyl transferase, D-Glucose, D-Mannose,Beta-alanine arylamidase, Tyrosine arylamidase, Lipase, Citrate(sodium), Malonate, L-lactate alkalipisation, Succinate alkalipisation, Cormarate, O/129 Resistance (Comp.*Vibirio*), L-malate assimilation and L-lactate assimilation, while the isolates gave negative results for remaining tests in the card(L-Arabitol, D-Mannitol, D-Sorbitol, Saccharose/Sucrose, D-Trehalose, Urease, Phosphatase, Oriniythine Decarboxylase, and Lysine Decarboxylase).

3.2. Purification of dextran Produced by L .gasseri

Dextran was purified after production under optimum conditions (30 °C for 24 h. with 15% sucrose, 4% of inoculum size at pH 7.0 under aerobic conditions). The total dry weight of dextran produced from *L.gasseri* after purification was 1.12 g/L, which represents the yield of dextran. Purified dextran was characterized as whitish, granular, and highly soluble in water.

3.3. Characterization of Dextran Purified from L. gasseri

3.3.1. Thin-Layer Chromatography (TLC)

Purified dextran contents were analyzed by TLC to determine their components of monosaccharides to confirm that the purified polysaccharide is dextran. The Rf values of hydrolyzed dextran were identical or so close to the glucose. The Rf value of dextran was 0.33, while the Rf values of glucose, fructose, galactose were (0.33, 0.24, 0.53) respectively. This result indicated that purified dextran from *L.gasseri* was composed only of glucose which confirmed that purified polysaccharide was dextran (Figure 1).

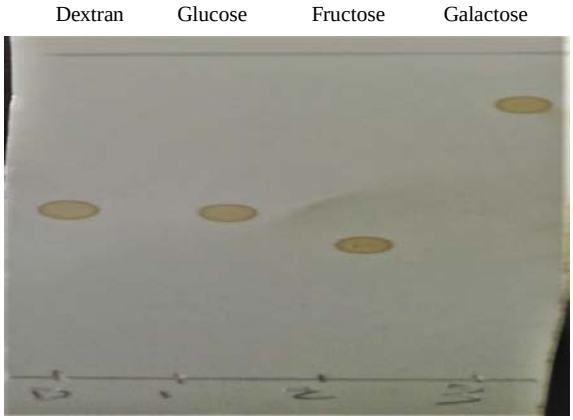

Figure 1. Thin – Layer Chromatography Analysis of Dextran Purified from *Lactobacillus gasseri*. Lane D: Dextran; Lane 1: Glucose; Lane 2: Fructose; Lane 3: Galactose

3.3.2. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR- spectra analysis was applied to detect the functional groups of purified dextran from *L. gasseri* (Figures 2 and 3). The FTIR results of purified dextran showed that the bands in the region of 3275 and 3390.97 ^{cm} $^{-1}$ were due to the hydroxyl (O −H) stretching, while the band in region 2931.90 ^{cm} $^{-1}$ was due to (C −H) stretching vibration, the band region of 1633.78 and 1639.55 ^{cm} $^{-1}$ were due to bending vibration band of the (OH) group, while the band region found in the 1456.30 $^{cm-1}$ and 1375.29 $^{cm-1}$ were due to symmetrical stretching of carboxylic groups(confirming the polysaccharide nature of the compound), the band region of 1219.05^{cm} $^{-1}$ and 1043.52 ^{cm} $^{-1}$ were due to the (C −O) and (C −C) bonds, while the band region of 1028.09^{cm} $^{-1}$ was for ∝ (1,6) glycosidic acid bands (1-6)- ∝-D glucan, the band region 995 ^{cm} $^{-1}$ was the (1-3) ∝- glucan.

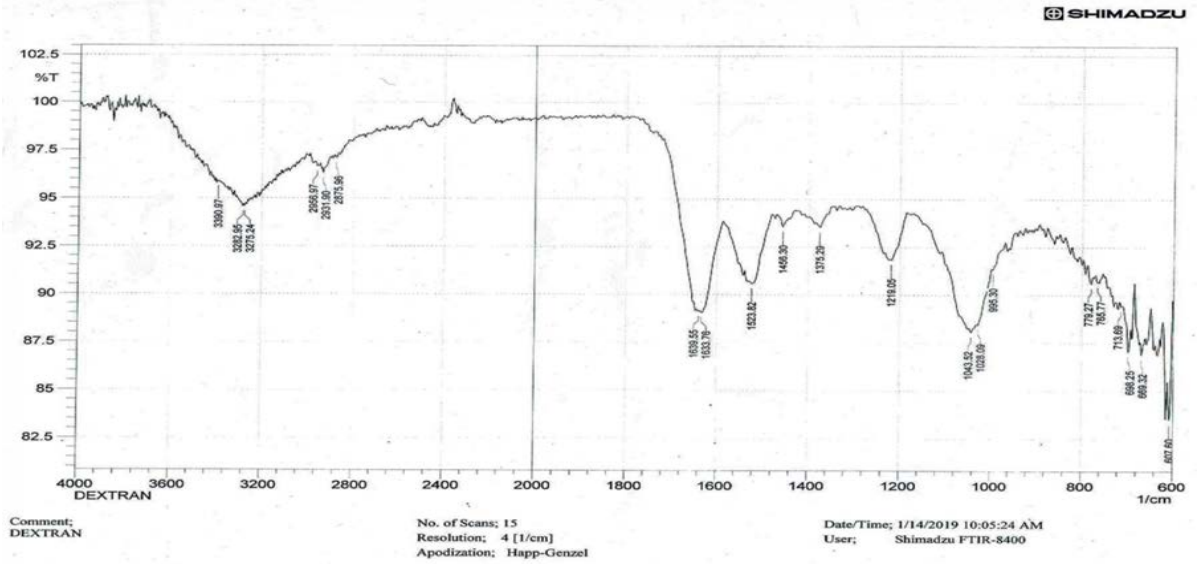

Figure 2. FTIR Spectra of Dextran Purified from *Lactobacillus gasseri*. T%: transmittance %; Wavelength range of 600 – 4000 cm^{-1}

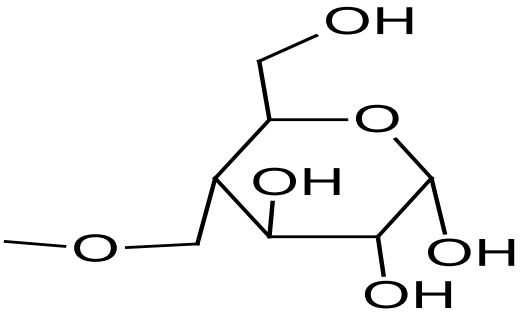

Figure 3. Suggested structure of purified dextran from *Lactobacillus gasseri* in the current study according to the functional groups of FTIR results

3.4. Antibacterial Activity of Purified Dextran against P.aeruginosa Isolates

The antibacterial activity of purified dextran from *L.gasseri* was examined against *P.aeruginosa* isolates by determination of the MIC of dextran at a concentration ranging from (200- 0.39) mg/ml. Results indicated that the MIC of dextran was 50 mg/ml against all twenty tested *P.aeruginosa* isolates compared to control, among them the four isolates included *P.aeruginosa*(Pb_2), *P.aeruginosa*(Pb_5), *P.aeruginosa*(Pw_{15}) and *P.aeruginosa*(Pw_{18}) that were chosen for subsequent experiences according to their virulence factors (Table 1).

Table 1. Minimum Inhibitory Concentration (MIC) of Dextran against *Pseudomonas aeruginosa* Isolates

Bacterial isolates	Dextran Concentration (mg/ml)										
	200	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	Control
P.aeruginosa(Pb_2)	-	-	- MIC	+	+	+	+	+	+	+	+
P.aeruginosa(Pb_5)	-	-	- MIC	+	+	+	+	+	+	+	+
P.aeruginosa(Pw_{15})	-	-	- MIC	+	+	+	+	+	+	+	+
P.aeruginosa(Pw_{18})	-	-	- MIC	+	+	+	+	+	+	+	+

"-" no growth of bacteria; "+" growth of bacteria; "Control" Muller Hinton broth only; "Pb" isolated from burns; "Pw" isolated from wounds; " MIC " Minimum Inhibitory Concentration

3.5. Effect of purified Dextran on virulence factors of P.aeruginosa isolates

3.5.1. Inhibition of Hemolysin Production

Purified dextran from *L.gasseri* had an inhibitory effect on hemolysin production of all *P.aeruginosa* isolated from both burns and wounds. Hemolysin production decreased in all *P.aeruginosa* isolates with no significant differences between isolates. The optical density of hemoglobin released from lysed erythrocyte ranged between 0.65 and 0.67 for treatment with dextran compared to 0.88 and 0.93 of control. Dextran inhibited hemolysin production with the inhibition of 27.17 % and 23.86 % for *P.aeruginosa* isolated from burns, and 26.96 %, and 29.03 % for *P.aeruginosa* isolated from wounds (Table 2). No

significant differences between *P. aeruginosa* isolates were observed.

Table 2. Inhibition of Hemolysin Production for *Pseudomonas aeruginosa* by Purified Dextran from *Lactobacillus gasseri*

Bacterial Isolates	O.D(543nm) of Hemoglobin		Hemolysin Inhibition %
	Control (without dextran)	Treatment (with dextran)	
Pseudomonas aeruginosa (Pb_2)	0.92	0.67	27.17
Pseudomonas aeruginosa (Pb_5)	0.88	0.67	23.86
Pseudomonas aeruginosa (Pw_{15})	0.89	0.65	26.96
Pseudomonas aeruginosa (Pw_{18})	0.93	0.66	29.03

Pb: isolated from burns; Pw: isolated from wounds; O.D: Optical density

3.5.2. Inhibition of Pyocyanin Production

In this study, purified dextran from *L. gasseri* showed an inhibitory effect on pyocyanin production against all tested *P.aeruginosa* isolates. The concentration of pyocyanin produced from *P.aeruginosa* isolates decreased after treatment of bacterial isolates with purified dextran (sub MIC) compared with control. The inhibition percentage recorded was 30.47% and 31.56% for *P.aeruginosa* isolated from burns, while 29.90 % and 31.32% for *P.aeruginosa* isolated from wounds (Table 3). No significant differences between *P. aeruginosa* isolates were observed.

Table 3. Inhibition of Pyocyanin Production for *Pseudomonas aeruginosa* by Purified Dextran from *Lactobacillus gasseri*

Bacterial Isolates	Pyocyanin Concentration(μg/ml)		Pyocyanin Inhibition %
	Control (without dextran)	Treatment (with dextran)	
Pseudomonas aeruginosa (Pb2)	3.38	2.35	30.47
Pseudomonas aeruginosa (Pb_5)	3.39	2.32	31.56
Pseudomonas aeruginosa (Pw_{15})	3.31	2.32	29.90
Pseudomonas aeruginosa (Pw_{18})	3.32	2.28	31.32

Pb: isolated from burns; Pw: isolated from wounds

3.5.3. Inhibition of Biofilm Formation

The biofilm formation of clinical *P.aeruginosa* isolated from burns and wounds were investigated by using the microtiter plate technique. This assay involved quantitation of the biofilm biomass attached to the walls of the microtiter plate. Purified dextran from *L. gasseri* had an inhibitory effect on biofilm formation of all *P.aeruginosa* isolates isolated from wounds and burns. Biofilm formation decreased in all *P.aeruginosa* isolates at different incubation periods (24, 48, 72) h compared to the control. The highest inhibition percentage for biofilm formation (71.42)% was recorded at 72 h for *P.aeruginosa* (Pw_{18}) isolated from wounds, while the lowest inhibition percentage (37.66)% was recorded at 24 h for *P.aeruginosa* (Pb_5) isolated from burns (Table 4).

Table 4. Inhibition Percentage of Biofilm Formation by Purified Dextran from *Lactobacillus gasseri* at Different Incubation Periods.

Bacterial Isolates	Inhibition of Biofilm (%)		
	Incubation Periods		
	24 h	48 h	72 h
Pseudomonas aeruginosa (Pb_2)	43.37	57.14	67.64
Pseudomonas aeruginosa (Pb_5)	37.66	48.83	58.94
Pseudomonas aeruginosa (Pw_{15})	44.87	59.34	64.94
Pseudomonas aeruginosa (Pw_{18})	47.61	60.00	71.42

Pb: isolated from burns; Pw: isolated from wounds

4. Discussion

Dextran is a homo-polysaccharide cationic polymer synthesized by dextransucrase enzyme; it consists of several α-glucans linked by α-(1-6) glycosidic bonds with branched linkages such as α-(1-3) linked as a single unit or lengthen side chain (Du *et al*., 2017). In this report, dextran was purified from local probiotic bacterium *L.gasseri* and characterized to detect the functional groups and the component of purified dextran. Indeed, we observed that *L.gasseri* produced dextran composed only of glucose from sucrose medium, which indicated that Lactobacillus isolate had dextransucrase. In FTIR spectra analysis we detected that dextran produced by *L.gasseri* contained both α (1, 6) and α (1-3) linkages indicating the glucan nature of purified dextran. The Rf value for dextran produced by *L. acidophilus* was identical to that of glucose which indicated that dextran was composed solely of glucose (Abedin *et al*., 2013). Salman and Salim (2016) reported that the dextran from *L.mesenteroides* ssp. *mesenteroides* was made of glucose and contained both α (1-6) and α (1 -3) linkages.

In the present study, dextran purified from *L.gasseri* showed antibacterial and anti-virulence factors such as biofilm, pyocyanin, and hemolysin of clinical *P.aeruginosa* isolated from burns and wounds; and we suggest that dextran may suppress the production of quorum sensing mediated virulence factors. The findings of the study suggested that dextran can be combined with antibiotics, and used as an effective skin sanitizer in both medical ointments and drugs used to treat burns and skin injuries. The inhibitory activity of the polysaccharide polymer may be due to the linking of the polysaccharide in the bacterial outer membrane (Aziz et al., 2012). Also, polysaccharide caused leakage of protein, DNA binding, and cytoplasmic membrane permeability (He *et al*., 2010). The modification of fluidity can increase membrane permeability (Wu et al., 2016). Protein leakage causes impairment of metabolic enzymes then leads to bacterial cell death (Shu *et al.*, 2019). The destruction of cell membrane permeability caused metabolic dysfunction, inhibited energy synthesis, and induction cell death (Zou *et al*., 2015). The other suggestion of the inhibitory effect of several polysaccharides is the trapping of cationic minerals or nutrients which can reduce the bioavailability of nutrients (Jun *et al.*, 2018).

The antibiofilm mode of action of polysaccharide hypotheses was reported. The most antibiofilm polysaccharides from probiotic bacteria act as surfactant molecules that modify the physical properties of bacterial cells. Also, polysaccharides may act as signaling molecules that modulate the gene expression of bacteria (Kim *et al*., 2009). *Lactobacillus* polysaccharide act as a signal that caused the down-regulation of several genes related to biofilm formation (Rendueles *et al*., 2013). The polysaccharides might block lectin dependent adhesion of *P. aeruginosa* or sugar-binding proteins present on the bacterial surface, or block tip adhesins of fimbriae and pili (Zinger-Yosovich and Gilboa-Garber, 2009). The competitive inhibition of multivalent carbohydrate-protein interactions is another possible mode of action of antibiofilm polysaccharide (Wittschier *et al*., 2007).

In the present study, dextran purified from *L.gasseri* showed an antibiofilm effect against *P. aeruginosa* isolates. Salman and Khudair (2019) showed that the purified polymer (homopolysaccharide) produced by *L. mesenteroides* had an inhibitory effect against several virulence factors of *Candida albicans*. Further, Li *et al*. (2014) showed that the exopolysaccharide purified from *L. helveticus* had inhibition activity against biofilm formation by pathogenic bacteria *S. aureus, P. aeruginosa,* and *E. coli*. The exopolysaccharide purified from *L. plantarum* exhibited strong inhibition against biofilm formation by pathogenic bacteria, including *P. aeruginosa*, *E. coli*, *S. typhimurium, and S.aureus* (Liu *et al*., 2017).

5. Conclusion

In conclusion, the purified and characterized dextran from local *Lactobacillus gasseri* had an inhibitory effect on the growth and some virulence factors of *P.aeruginosa* isolated from wounds and burns.

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