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Original article

Evaluation the antibacterial and antibiofilm effect of purified dextran from *Lactococcus lactis* ssp *cremoris* against some pathogenic bacteria

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ABSTRACT

Background and Objectives: The development of innovation methods to treat and stop the formation of biofilm has drawn a lot of attention since biofilms are a major health issue due to their multidrug resistance, host defense and other challenges. The goal of our study was to create biopolymer dextran from *Lactococcus lactis* ssp *cremoris* and confirm its antimicrobial activity, antibiofilm properties and ability to be included in the preparation of dressings that create an environment that is conductive to wound healing.
Methods: The *dsrLL* gene, which is responsible for dextran production, was examined using an RT-qPCR assay. Sequencing phylogenetic analysis was also achieved. The efficacy of purified dextran was also studied for its antibacterial and antibiofilm activity and in the preparation of wound dressings. **Results:** The growth reduction of up to 87 % against *K. pneumoniae*$_{(1)}$, the purified dextran seemed the most effective. For *A. baumannii*$_{(1)}$ and *P.aeruginosa*$_{(1)}$, the anti-biofilm impact of purified dextran reached maximum values of 55 % and 44 %. Additionally , 50 , 46 and 257 % of the various polymers : Dextran , PVA and the combination of Dextran and PVA , were added and coated the cotton gauze , respectively. **Conclusion:** *Lactococcus lactis* ssp *cremoris*$_{S4}$ with the highest gene expression level of *dsrLL* gene produced dextran. The purified dextran had antibacterial and antibiofilm effect against pathogenic bacteria. Also, dextran can be used in wound dressing, especially with PVA.

Introduction

Microorganisms, including viruses, bacteria, parasites or fungi, have a significant impact on wound healing because they affect infection risk, healing time, patient recovery time, and health–care expenses [1]. Due to the clinical concern of anti-infectious biomaterials, many researchers have conducted studies to improve implant surfaces through coatings that could effectively prevent bacterial adhesion, kill bacteria on contact, or enable controlled elution of antimicrobial compounds. In particular, infectious diseases caused by pathogenic microorganisms have become major public health threats as they can prolong or impair the wound healing process, leading to tissue morbidity, sepsis, and implant failure [2].

The creation of biofilm is a serious issue in many fields such as industry, public health and

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medicine since it raises morbidity and mortality. It adds a significant financial cost to the healthcare industry, Bacterial biofilm formation is a serious and ongoing issue [3], Bacterial species have evolved the ability to form biofilm to withstand and survive in harsh environments such as mechanical stress and antibiotic treatments. A biofilm is a collection of microorganisms that are permanently bonded to a substrate and encased in an extracellular polymeric material that the microorganism manufacture on their own and is made up of proteins, lipids, extracellular polymeric substances (EPS) and environmental DNA (e-DNA) [4]. It was claimed that natural metabolites could stop the formation of biofilms in variety of ways, such as by blocking the synthesis of polymer matrix and peptidoglycans ,stopping the extracellular matrix from being produced , suppressing cell adhesion and attachment , breaking down the structure of microbial membranes, and lowering the synthesis of virulence factors. This would stop the formation of biofilm and QS network [5, 6].

In actuality, a large number of LAB from genera *Weissella*, *Leuconostoc*, and *Lactobacillus* are capable of producing EPS , more especially dextrans . Dextransucrases (Dsr), which members of glycosyl hydrolases (GH) family 70 , a microbial metabolite of various molecular configurations that secretes as an extracellular polysaccharide in the culture medium of the bacterial fermentation system [7] , and hydrolyse sucrose to produce fructose and glucose molecules. They can also catalyze the transfer of a glucose molecule onto an expanding chain of α-glycosidically linked polymers [8]. The primary explanation is the product specificity of glucansucrases, some of which also form other connections besides α-(1→6) linkages [9] . The α-glucans that make up these homopolysaccharides have a linear backbone of d-glucopyranosyl units , α-(1,6) links in the main chain and varying proportions of α-(1,4), α-(1,3), or α-(1,2) ramifications [10] . They are widely used in the food sectors since they are safe food additives that also have the ability to gel , emulsify, stabilize , and viscosify , which they have important effects on the organoleptic characteristics of food [11] . and the use of natural polysaccharides in wound healing [12] ,which its biodegradability and biocompatibility, are still the focus of extensive research . Consequently, the goal of our study was to create biopolymer dextran from *Lactococcus lactis* ssp *cremoris* , which was isolated from sausages , and confirm its antimicrobial activity against both Gram-positive and Gram-negative bacteria, we also tested its antibiofilm properties and its ability to be used in preparation of dressings to create an environment that is conductive to wound healing.

Materials and Methods

Microorganisms

Lactococcus lactis ssp *cremoris* isolation and identification

Vacuum packed fermented meat products (sausage) were purchased from local market for isolation homo lactic acid cocci bacteria. After puncturing each bag of sausage with sterile needle and collecting, drops were obtained from the liquid in the packet [13]. Then, a series of 10-fold dilutions ranging from 10^1 to 10^4 in phosphate buffer saline was prepared from the tested samples, with inoculation in M17 broth at 1% and cultivation at 30°C for 24 h under anaerobic conditions. Based on the fourth dilution, 0.1 ml was added to M17 agar plates containing 0.004% (w/v) bromocresol purple, 1% (w/v) calcium carbonate and incubated at 30°C for 48 h [14]. The isolates were identified using cultural, cellular, and biochemical tests as well as the Vitek 2 system.

Pathogenic bacteria collection and identification

Ten isolates of pathogenic bacteria that were isolated from wound infection were obtained from the Department of Biology / College of Science / Mustansiriyah University, including 2 isolates of *Klebsiella pneumoniae*, 2 isolates of *Acinetobacter baumannii*, 2 isolates of *Pseudomonas aeruginosa*, 2 isolates of *Escherichia coli* and 2 isolates of *Staphylococcus aureus*. The isolates were re-identified by cultural, microscopical and biochemical tests, using the Vitek 2 system.

Screening of *L. lactis* ssp *cremoris* isolates for dextran production

Mucoidy Method

Dextran production was determined via a screening test for mucoid colonies and ropiness [15]. Following incubation, the mucoidy of colonies was determined by examining the visual appearance, and the ropiness was determined by touching the colonies with a sterile inoculation loop. The isolates that produced mucoid colonies and had a ropy phenotype were recorded as dextran-producing.

Ethanol precipitation method

Lactococcus lactis ssp *cremoris* isolates were inoculated in dextran screening agar at 2% of the isolate suspension at a concentration of 9 × 10^8 CFU / ml (absorbance at a wavelength of 600 nm about 0.134). Following 24 h. incubation at 30 ° C, the mucoid substance was mixed with 2 mL of absolute ethanol. Dextran precipitation indicates that the biopolymer was produced [16].

Phenol sulfuric acid method for total carbohydrate content estimation

According to the reported work [17], the Phenol sulfuric acid method was used, and dextran was applied as a standard carbohydrate. A working standard of dextran (0.1 mg/ 100 ml Distilled water) was prepared and diluted to obtain series concentrations, involving 0.2, 0.4, 0.6, 0.8 and 1 ml to plot the standard curve of dextran, subsequently, 0.1 ml and 0.2 ml of the dextran sample were prepared, complete the volume to 1 ml by adding distilled water. Then, about 1 ml of 5 % phenol and 5 ml of 96 % sulfuric acid were added. An EMC-11-UV Spectrophotometer at 490 nm was employed to measure the absorbance.

Determination of the expression level of *dsrLL* gene

The total RNA from four *L. lactis* ssp *cremoris* isolates that were growing on M17 broth for 24 h at 30 °C, was extracted using the *TransZol* Up Plus RNA Kit (TransGen Biotech, ER501-01, China) to detect **the expression levels of the *dsrLL* gene**. The cDNA template was obtained by reverse transcription (EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix Kit) according to the manufacturer's instructions. Taken 2 µL cDNA template and 6 µL of the *TransStart*® Top Green qPCR Super Mix components and mixed well, 1 µL of upstream and downstream primers, and 6 µL of nuclease-free water were mixed. The relative expression of mRNA in the bacterial cells using QIAGEN Rotor Gene Q Real-time PCR System (Germany) was performed, and *16S rRNA* was selected as an internal standard. The reaction conditions of the whole process were as follows: 94˚C for 30 s; then 35 cycles at 94˚C for 10 s; 56/64˚C for 15 s for the target gene and *16S rRNA*, respectively; 72˚C for 20 s. Ultimately tested at 55-95˚C for 30-35 s, and calculated the relative gene expression by the $2^{-\Delta\Delta Ct}$ method. The sequence of oligonucleotide primers that were used to detect *dsrll* and *16sRNA* genes is listed in Table 1.

Extraction and sequencing DNA

DNA extraction

Following the Kit's instruction, the *EasyPure*® Genomic DNA Kit (TransGen, biotech. EE101-01) was used to extract the DNA from *L. lactis* ssp *cremoris*$_{S4}$.

Polymerase chain reaction (PCR)

By the manufacturer's instructions for the *2 xEasyTaq*® PCR SuperMix, a polymerase chain reaction was conducted in a thermal cycler to amplify the extracted DNA. The 516 bp product of the designed *dsrll* gene was amplified using: F(AATACGGTGGTGCCTTCTTG) and R (AGACTTGTTGCCCTCGCTTA), while the 1465 bp product of the *16S r*RNA gene was amplified using: F(AGAGTTTGATCMTGGCTCAG) and R (GGTTACCTTGTTACGACTTACTT) [18]. The outcomes of the PCR product are checked using agarose gel electrophoresis on a 1 % (w/v) agarose gel at 70 volts for 60 min.

Phylogenetic analysis of Sequencing

The automated DNA sequencer ABI3730XL, manufactured by Macrogen Corporation – Korea, was used to perform Sanger sequencing of PCR products for *L. lactis* ssp *cremoris*$_{S4}$ and *16S r*RNA genes. Using BLAST (Basic Local Alignment Search Tool), DNA sequences were examined and compared to reference sequences already available on the National Center for Biotechnology Information (NCBI) website. A phylogenetic tree was then created.

Dextran production from *L. lactis* ssp *cremoris*

According to the procedure described by Onilude *et al* . [19], with some modifications, 100 ml of autoclaved dextran production medium (150 g sucrose, 5.0 g peptone, 15.0 g K_2HPO_4, 0.01 g $MnCl_2.4H_2O$, 5.0 g yeast extract, 0.01 g NaCl, and 0.05 g $CaCl_2$ added to 1 liter distilled water, pH 7.0) were inoculated with 2% (9 × 10^8 CFU / ml) of bacterial suspension and incubated at 30 ˚C for 24 h.

Precipitation of Dextran

This process was accomplished by precipitation a total culture medium of dextran production by utilizing the same volume of chilled ethanol [20]. The precipitated dextran was dried in the oven (40˚C) for 45 minutes [21], to evaluate the dextran dry weight.

Purification of dextran by using gel filtration Chromatography

Purification of dextran using Sephadex G-100 gel (Pharmacia Fine Chemicals, Sweden) was carried out in a column of 1.5 × 60 cm with 0.1 M phosphate buffer pH 7.0 at a flow rate of 18 ml / h, then the separated elution fractions were collected and detected at a wavelength of 220 nm using a spectrophotometer. The dextran concentration using the phenol sulfuric acid method, as previously described [17] of the separated peaks was estimated and the activity was evaluated.

Antibacterial effect of purified dextran

Overnight cultured bacterial isolates were grown in the nutrient broth containing purified dextran

(1:1), and without dextran as a control after inoculating with 1% bacterial isolates individually, with comparing them with MacFarland. The tubes were incubated at 37 ° C for 24 h. After the incubation period, a serial dilution of the culture was performed, 0.1 ml from each dilution was transferred and cultured on nutrient agar, the plates were then incubated at 37 ° C for 48 h [22]. The numbers of colonies were calculated, and after comparing it with the control, the inhibition percentage was calculated according to the following equation:

$$\textbf{Reduction of growth } \% = \frac{\textbf{Number of control colonies} - \textbf{Number of treated colonies}}{\textbf{Number of control colonies}} \times \textbf{100}$$

Antibiofilm effect of purified dextran

A microtiter plate was used to examine the effect of dextran on biofilm formation of clinical bacteria as described by Salman and Kareem [23]. Isolates were grown on flat – bottom 96 - well microtiter plates for 24 h at 37 °C. Compared to 0.5 MacFarland, 20µl of bacterial suspension was added in to each well containing 80 µl sterilized Brain Heart Infusion broth with 2% sucrose and mixing with 100 µl of purified dextran, while control contained only 180 µl BHI broth with 2% sucrose and 20µl of bacterial suspension, after incubation the medium was removed from the wells, washed three times with sterile PBS to remove the unattached bacterial cells and allowed to dry for 15 min at room temperature, the wells were then filled with 200 µl of crystal violet (0.1%) and left for 20 min. The stained wells were rinsed three times with PBS (PH 7.2) to remove unbound dye and left to dry at room temperature for 15 min 200 µl of 95% ethanol was added to each well and an ELISA reader at 630 nm was used to read optical density. The biofilm formation inhibition percentage was calculated according to the equation shown below:

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

Examination of the antibiofilm of purified dextran by Field Emission Scanning Electron Microscopy (FE-SEM)

To examine the antibiofilm activity of purified dextran by scanning electron microscopy (SEM) , bacterial biofilm was developed on cover slips, followed by the addition of purified dextran. Cover slips with purified dextran and without dextran as control were incubated at 37 °C for 24 h [24], harvested and washed three times with PBS, then the cells were fixed with 2.5% glutaraldehyde. Finally, the samples were examined by FE - SEM [25] .

Degradation of biofilm by purified dextran

A microtiter plate with purified dextran was used to test the effect of biofilm degradation. Brain Heart Infusion broth supplemented with 2% sucrose, based on the procedure reported by Jaffar et al. [26], the plate was inoculated with bacterial suspension compared with 0.5 MacFarland.The final liquid volume in each well was 200 µl, each well containing 180 µl sterilized BHI broth with 2% sucrose and 20 µl of bacterial suspension. After incubation at 37°C for 72 h, the broth was removed from the wells and then added 200 µl of dextran to each well, the control well contained only 200 µl BHI broth with 2% sucrose, after incubation at 37°C for 24 h, dextran was removed from the well and washed three times with sterile PBS, allowed to dry at room temperature for 15 min, then the wells were filled with 200 µl of crystal violet (0.1%) and left for 20 min. The stained wells were rinsed 3 times with PBS saline (pH 7.2) to remove unbound stain and left to dry at room temperature for 15 min. Finally, 200 µl of 95% ethanol was added to each well and an ELISA reader at 630 nm was used to read the optical density. The degradation ratio of biofilm formation was calculated by the equation shown below:

$$\textbf{Degradation biofilm } \% = \frac{\text{O.D control - O.D treatment}}{\text{O.D control}} \times 100$$

Preparation of wound dressing using purified dextran

Square pieces of gauze with a length of 4 cm was sterilized , then place them in sterile dishes , immerse them in 20 ml of each of : 4 % purified dextran , 6 % of Poly vinyl alcohol (PVA), (PVA dissolve well in sterilized distilled water at 90 °C) , purified dextran - PVA as 1 : 1 (mixed well with stirrer for 10 min) , and sterile distilled water as a control , then dried at room temperature for 2 days [27] .

Measuring the ability of the gauze to carry therapeutic polymers

The gauze is weighed after drying for all the above treatments, and the following equation is applied:

$$Drug\ add\ on\ \% = \frac{W1 - W2}{W2} \times 100$$

Where W1 is the weight of the coated sample and W2 is the weight of an uncoated sample.

Bacterial reduction of prepared wound dressing

The effect of prepared wound dressing was studied against P. *aeruginosa* and *S. aureus*, isolated from the wound using the procedure described by Venkatrajah *et al*. [27]. The prepared dressing of purified dextran, PVA and dextran-PVA mixture was transferred separately into the flask (250 ml) and the control flask contained only nutrient broth. Flasks inoculated with 1 ml of bacterial isolates compared with MacFarland, approximately $1.5x10^8$ CFU/ml. After incubation at 37 °C for 24 h, 100 ml of sterilized distilled water was added to each flask and mixed for 1 min and then decimal serial dilutions. Transfer 0.1 ml of the dilutions, separately and spread on nutrient agar and incubate at 37 °C for 48 h. After incubation, the number of colonies was counted, and the bacterial reduction percentage was calculated using the following equation:

$$\boldsymbol{R\ \% = \frac{(B-A)}{B} \times 100}$$

Where A is the total number of bacterial colonies from each treatment (coated cotton gauze), and B is the total number of bacterial colonies from the control (uncoated cotton gauze).

Results and Discussion

***Lactococcus lactis* ssp *cremoris* isolation and identification**

Four isolates were Gram-positive, facultative anaerobic and non-motile cocci that are shown singly, in pairs and chains, usually forming yellowish-white small, shiny colonies belonging to Lactococci on M17 agar and yellow colonies on M17 plus bromocresol purple and $CaCo_3$, isolates of LAB was confirmed as homofermentative cocci lactic acid bacteria, catalase and oxidase was negative, Vitek 2 system analysis was the final identification for confirming *L. lactis* ssp *cremoris*.

Screening of *Lactococcus lactis* ssp *cremoris* isolates for dextran production

Screening test for mucoidy and ropiness

Lactococcus lactis ssp *cremoris* isolates were examined for dextran production by screening for the presence of mucoid colonies and ropiness on the surface of the dextran screening medium. Isolates with mucoid colonies and ropiness were recorded as dextran-producing. Results showed that *L. lactis* ssp *cremoris* isolates were observed to have mucoid colonies and a large degree of ropiness and were therefore positive for dextran production Table 2 in the Supplement.

Ethanol precipitation method

Dextran production from *L.lactis* ssp *cremoris* isolates was confirmed by using the ethanol precipitation method. The results are slightly different from those of the mucoid screening test, in which easier collection and separation of dextran was observed to produce high levels of dextran when using the ethanol precipitation method. The isolates varied in their ability to produce dextran Table 2 in the Supplement.

Phenol sulfuric acid method for total carbohydrate content estimation

Dextran concentration was determined by using the phenol-sulfuric acid method. *L.lactis* ssp *cremoris*$_{S4}$ with the highest dextran concentration of about 2.660 mg/ml was selected as observed in Table 2 (see Supplement).

Determination of the expression level of *dsrLL* gene

RT-qPCR was used to determine gene expression fold change for *dsrLL* gene responsible for the production of dextransucrase, in charge of dextran production. The results showed that there is an increase in the gene expression level of the *dsrLL* gene in *L. lactis* ssp *cremoris*$_{S4}$ encoding dextran production, while *L. lactis* ssp *cremoris*$_{S3}$, which had the lowest gene expression, compared to the other isolates. Based on this, *L.lactis* ssp *cremoris*$_{S4}$ was chosen for the dextran production in large quantities for use in subsequent experiments Table3.

Phylogenetic analysis of Sequencing

The high expression level of the *dsrll* gene of *L. lactis* ssp *cremoris*$_{S4}$ led to the selection of the PCR product, which was then sequenced in comparison to an NCBI reference sequence of *16S r*RNA. One technique to quantify the differences between two sequences is to use sequence identity. Regarding *16S r*RNA PCR product, the results demonstrated 96 % compatibility of *dsrll* gene of *L. lactis* ssp *cremoris*$_{S4}$, and the phylogenetic tree based on the sequence of *dsrll* gene PCR products was displayed in Figure 1.

Dextran production and precipitation by the Ethanol precipitation method

Precipitation of a total culture medium of dextran production was done using chilled ethanol six times, and then the precipitated dextran was dried to obtain the dry weight of dextran from the fermented culture medium.

Purification of dextran

Gel Filtration Chromatography using a Sephadex G-100 column to purify biopolymer dextran produced by *L. lactis* ssp *cremoris*$_{S4}$ isolate. Eighty-two dextran fractions were collected, and 3 ml of effluent in each tube was analyzed for the quantity of dextran. Two peaks appeared, and the highest amount of dextran was recorded in fractions 17 and 50. The quantity of total carbohydrates in the biopolymer dextran was evaluated by the phenol-sulfuric acid method [17], and it was found to be 0.055 mg/ml and 0.101 mg/ml, respectively.

Antibacterial effect of purified dextran

Purified dextran effects on pathogenic bacterial growth were studied using the Co-culture method. The purified dextran appeared to be more active against *K. pneumoniae*$_{(1)}$, with a reduction of growth reaching 87 %, followed by 81 %, 77 % against *S. aureus*$_{(2)}$, *A. baumannii*$_{(1)}$, respectively. For *K. pneumoniae*$_{(2)}$, *S. aureus*$_{(1)}$, *A. baumannii*$_{(2)}$ and *P. aeruginosa*$_{(1)}$, the antibacterial effect of purified dextran amount to 45, 44, 42 and 41 %, sequentially, while, the percentage of bacterial growth reduction was 34 and 32 % respectively for each *P. aeruginosa*$_{(2)}$ and *E. coli*$_{(1)}$. Finally, the lowest effect of purified dextran was against *E. coli* $_{(2)}$ with 22%, see Table 4 in the Supplement.

Polysaccharides generated from bacteria that exhibit antimicrobial activity can be divided into antiviral, antifungal, and antibacterial. strong antibacterial activities against both gram-positive and gram-negative pathogenic bacteria, including *S. aureus, Listeria monocytogenes, Enterococcus faecalis, E. coli, P. aeruginosa, Shigella flexneri* and *Salmonella typhimurium* and has been demonstrated by EPSs from a range of bacterial strains belonging to the genera *Lactococcus, Lactobacillus,* and *Streptococcus*. Furthermore, a range of gram-positive and gram-negative pathogenic bacteria are susceptible to the antibiofilm properties of polysaccharides originating from the *Lactobacillus* and *Enterococcus* genera. Biofilm development in *E. Faecalis, L. monocytogenes, S. aureus, P. aeruginosa, S. typhimurium, E.coli*

and other bacteria were impacted by these polysaccharides [28, 29].

Purified dextran isolated from *L. gasseri* had a suppressive impact as antibacterial and antivirulence factors against *P. aeruginosa* isolated from wounds and burns [23]. Polysaccharides, Antibacterial activity depends on several aspects, including solubility, molar ratio, chemical bonding, molecular weight, chemical modification and extraction [30].

Antibiofilm effect of purified dextran

Dextran exhibited antibiofilm effects against pathogenic bacteria, so the current study investigated its effect on biofilm formation. Results revealed that dextran reduced biofilm formation with an inhibition percentage. A record of 19 % and 22 % for *K.pneumoniae*$_{(1)}$ and *K. pneumoniae*$_{(2)}$, respectively. Moreover, this inhibitory effect reached a maximum value of 55 % and 44 % for both *A. baumannii*$_{(1)}$ and *P. aeruginosa*$_{(1)}$, indicating that its anti-biofilm activity against *A. baumannii*$_{(1)}$ and *P.aeruginosa*$_{(1)}$ was better than its activity against *K. pneumoniae*$_{(1)}$ and *K. pneumoniae*$_{(2)}$. While the ratio of biofilm formation inhibition of dextran against *A. baumannii*$_{(2)}$ *E. coli*$_{(2)}$ was 30 % and 23 %, respectively, as shown in Table 5 in the Supplement.

Adhesion inhibitors, efflux pump inhibitors, polymeric substance synthesis inhibitors, quorum sensing inhibitors, cyclic diguanylate inhibitors, and polymer surface modification are among the

techniques and developments that are in charge of identifying antibiofilm agents and their mechanism of biofilm suppression [31].

The structural analog of the polysaccharide secreted by the bacteria is generally thought to be an exogenous polysaccharide that inhibits the formation of biofilm by other bacteria. These exogenous polysaccharides can also block the

bacterial cell surface binding sites for their polysaccharide [32]. The external polysaccharides may function as signal molecules to control the expression of genes linked to the formation of biofilm [33]. One of the best ways to reduce biofilms is to inhibit adhesion, the manufacture of fimbriae, surface proteins, virulence factor genes, and other bacterial structures involved in this process is inhibited by some compounds, adhesion and biofilm formation are ultimately disrupted [34], therefore, the idea that different EPS extracted from LAB can reduce or inhibit microbial biofilms is becoming more supported by scientific evidence, as a result, these EPS may be used to develop new approaches to address food safety concern and infections linked to bacterial biofilm [29] . Rheama and Ibrahim in their studies showed that among of 24 isolates of *Acinetobacter baumannii* 16 isolates (66.7%) appeared as biofilm forming [35]. While AL-Kadmy *et al* showed in their work that all of the clinical isolates (21/21) formed strong biofilms on abiotic surfaces. There were no weak biofilm producers [36].

Klebsiella pneumoniae is a healthcare-associated pathogen, and the risk of infections is increased in the presence of medical devices. it is known for its ability to form biofilm on biotic and abiotic surfaces. *K. pneumoniae* biofilm helps protect the bacteria from host immune responses and antibiotics. Biofilm formation in these isolates was tested using Congo red and Tissue Culture Plate methods. In the Congo red method, 33% of the isolates were biofilm producers and 63% could form biofilm using the TCP method, divided as: 14% strong, 15% moderate, 34% weak, and 37% non-biofilm-producing for the reported works [37,38]. It showed that each isolate had a different ability to form biofilm under the same test conditions. *Staphylococcus aureus* isolates showed that 8 of the 14 were moderate producers, with 2 being weak and 4 being strong biofilm producers.

Examination of the antibiofilm of purified dextran by Field Emission Scanning Electron Microscopy (FE-SEM)

A field emission scanning electron microscope (FE-SEM), is one of the most popularly used instrumental methods for examining and analyzing the microstructure and morphology of solid materials. It operates using the same principles as a light microscope, with one main difference: It uses focused electron beams instead of photons to magnify an object [39]. SEM is also used for the assessment of the shape, size, and location of microorganisms in biofilm as well as the stages of biofilm formation, i.e., bacterial interactions and the production of extracellular polymer substances [40].

The FE-SEM image of *A. baumannii*$_{(1)}$ isolate treated with purified dextran showed high reductions in biofilm formation, while the image of the control (without purified dextran) showed a biofilm composed of multiple layers with a large number of attached bacteria (see Fig. 2).

Degradation of bacterial biofilm by purified dextran

Degradation of bacterial biofilm by purified dextran was detected; results showed that purified dextran appeared 20 % and 18 % for breakdown biofilm formation by *P.aeruginosa*$_{(1)}$ and *A. baumannii* $_{(1)}$, respectively. While noted, there is no activity against the preformed biofilm by other isolates (see Table 6 in the Supplement).

Enzymatic breakdown, physical disruption, chemical treatments, quorum sensing suppression, and competitive exclusion are some of the methods that can be employed to breakdown biofilms. Many therapeutic approaches are also designed to target the EPS matrix to eliminate biofilms [41]. By disrupting the cell surface and intercellular communication, antibacterial substances from several microbial species have also demonstrated possible anti-biofilm activity against many harmful bacteria [42]. Natural polymer-based surface coatings have gained popularity, including dextran, anti-adhesive coatings of algal polysaccharide ulvan, and dermatan sulfate [43].

Preparation of wound dressing

The ratio of polymer applied to cotton gauze is shown in Table 7. The add-on percentage of different polymers: Dextran , PVA, and the mixture of Dextran and PVA , that coated the cotton gauze, was 50 , 46 and 257 % respectively . The polymers added to the surface of the prepared cotton gauze are also presented in Figure 3.

Due to its good stability, biocompatibility, porous structure, low friction coefficient and the moisture content of similar to the body's tissues, the Polyvinyl alcohol hydrogel has been extensively investigated as promising applications in tissue replacement and repair materials, drug carrier, cartilage and skin substitutes, skin wound dressings and scaffold of cell culture, and others. Improving the mechanical properties of the PVA hydrogel by incorporating reinforce material into the PVA

hydrogel to form the composite hydrogel materials, maintaining its biocompatibility, PVA enhanced molecular interactions between the polymer and PVA because of forming the hydrogel junctions that are mainly produced by interaction of hydrogen bonding between - OH groups of PVA and other polymers [44,45]. The addition of dextran thickened the hydrogel and enlarged the pore size. It could be estimated that the addition of dextran would make the hydrogel fiber better organized and more homogeneous [46].

Bacterial reduction of prepared wound dressing

The bacterial reduction of the polymers coated gauze had been estimated against *P. aeruginosa* $_{(1)}$, *S. aureus*$_{(2)}$ isolates from a wound infection The results showed that the Dextran - PVA coated cotton gauze mixture led to areduction in the number of bacteria, and the percentage of bacterial reduction was close for both *P. aeruginosa*$_{(1)}$, *S. aureu*$_{(2)}$, where it was 88 % and 86 %, respectively, while *P. aeruginosa*$_{(1)}$, *S. aureus*$_{(2)}$ reduction percentage by dextran-coated cotton gauze was 90 % and 30 %, respectively. Also, the percentage of reduction in the number of bacteria using a PVA polymer-coated cotton gauze for each of *P. aeruginosa* $_{(1)}$, *S. aureus*$_{(2)}$ was 82 % and 78 %, respectively (see Fig. 4).

By absorbing exudate, preventing bacterial growth, maintaining fluid balance, and being simple for the the patient or health care professional to use, wound dressings have been demonstrated to improve the wound healing process, an ideal antibacterial wound dressing should be one that not only exerts bacterial killing but is also able to inhibit fouling (preventing adhesion of bacteria) of the surface and reduce eventual biofilm formation [47]. In hydrogel or nanoparticles form, dextran has been explored as a non-cytotoxic biomaterial that is capable of wound healing [48] . Also, these blends can interfere with biofilm formation, which is a crucial factor in microbial infections. The hydrogel matrix can disrupt the extracellular polymeric substance (EPS) that holds biofilms together [47].

Table 1. Screening for dextran production by both Mucoidy and Ethanol Precipitation Methods and dextran concentration.

Bacterial isolates	Result		Dextran concentration (mg/ml)
	Mucoidy method	Ethanol Preciptation Method	
1 – *Lactococcus lactis* ssp *cremoris*$_{S1}$	+	++	**1.989**
2 - *Lactococcus lactis* ssp *cremoris*$_{S2}$	+	++	**2.112**
3 - *Lactococcus lactis* ssp *cremoris*$_{S3}$	+	+	**1.154**
4 - *Lactococcus lactis* ssp *cremoris*$_{S4}$	+	+++	**2.660**

Table 2. Reduction of bacterial growth by purified dextran.

Bacterial isolates	Reduction of growth %
Klebsiella pneumoniae$_{(1)}$	87
Klebsiella pneumoniae$_{(2)}$	45
Acinetobacter baumannii$_{(1)}$	77
Acinetobacter baumannii$_{(2)}$	42
Pseudomonas aeruginosa$_{(1)}$	41
Pseudomonas aeruginosa$_{(2)}$	34
Escherichia coli$_{(1)}$	32
Escherichia coli$_{(2)}$	22
Staphylococcus aureus$_{(1)}$	44
Staphylococcus aureus$_{(2)}$	81

Table 3. Inhibition of biofilm formation by purified dextran at 24 h.

Bacterial isolates	Inhibition of biofilm formation %
Klebsiella pneumoniae$_{(1)}$	19
Klebsiella pneumoniae$_{(2)}$	22
Acinetobacter baumannii$_{(1)}$	55
Acinetobacter baumannii$_{(2)}$	30
Pseudomonas aeruginosa$_{(1)}$	44
Pseudomonas aeruginosa$_{(2)}$	-33
Escherichia coli$_{(1)}$	-51
Escherichia coli$_{(2)}$	23
Staphylococcus aureus$_{(1)}$	- 13
Staphylococcus aureus$_{(2)}$	- 17

***Negative results (-) : No inhibition of biofilm**

Table 4. Degradation of bacterial biofilm by purified dextran.

Bacterial isolates	Degradation of biofilm %
Klebsiella pneumoniae$_{(1)}$	- 17
Klebsiella pneumoniae$_{(2)}$	- 30
Acinetobacter baumannii $_{(1)}$	18
Acinetobacter baumannii$_{(2)}$	- 38
Pseudomonas aeruginosa$_{(1)}$	20
Pseudomonas aeruginosa$_{(2)}$	-167
Escherichia coli $_{(1)}$	-51
Escherichia coli$_{(2)}$	- 44
Staphylococcus aureus$_{(1)}$	- 15
Staphylococcus aureus$_{(2)}$	- 9

***Negative results (-) :** No Degradation of biofilm

Figure 1. Phylogenetic tree analysis of *L.lactis* ssp *cremoris*$_{S4}$

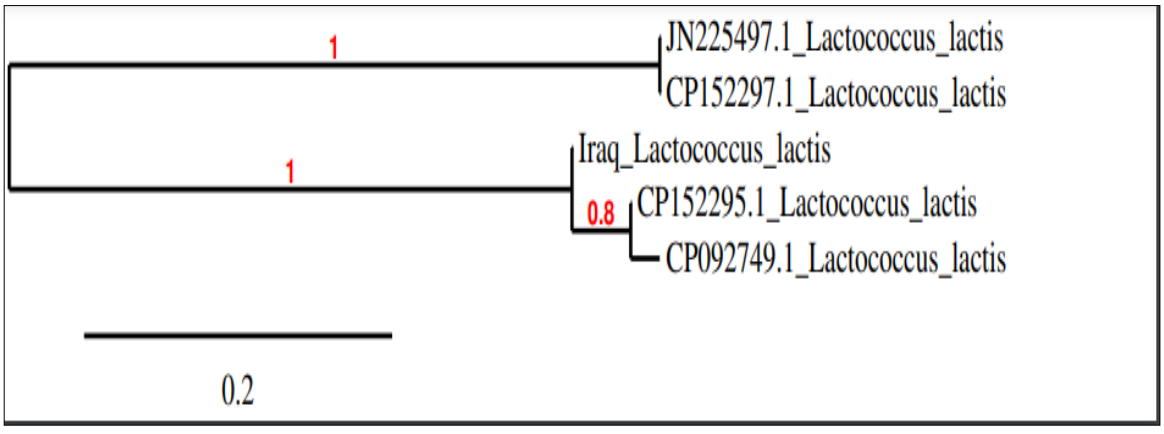


Figure 2. The SEM image of antibiofilm effect of purified dextran against *Acinetobacter baumannii*$_{(1)}$ isolate.
Fixed with 2.5% glutaraldehyde : A and B control (without purified dextran) C and D treated with purified dextran at 30000x and 8000x

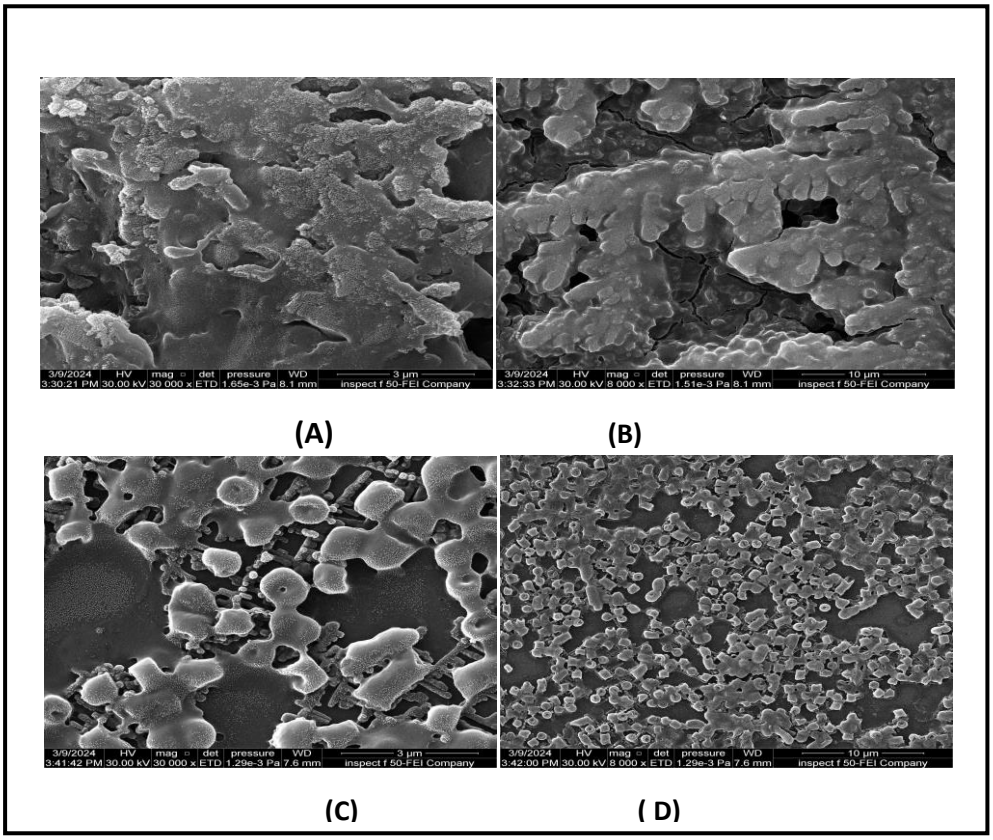


Figure 3. Coated cotton gauze with purified dextran and its blend with PVA.
A: Uncoated cotton gauze , B: PVA coated gauze , C: Dextran coated gauze , D: mixed Dextran and PVA coated gauze .

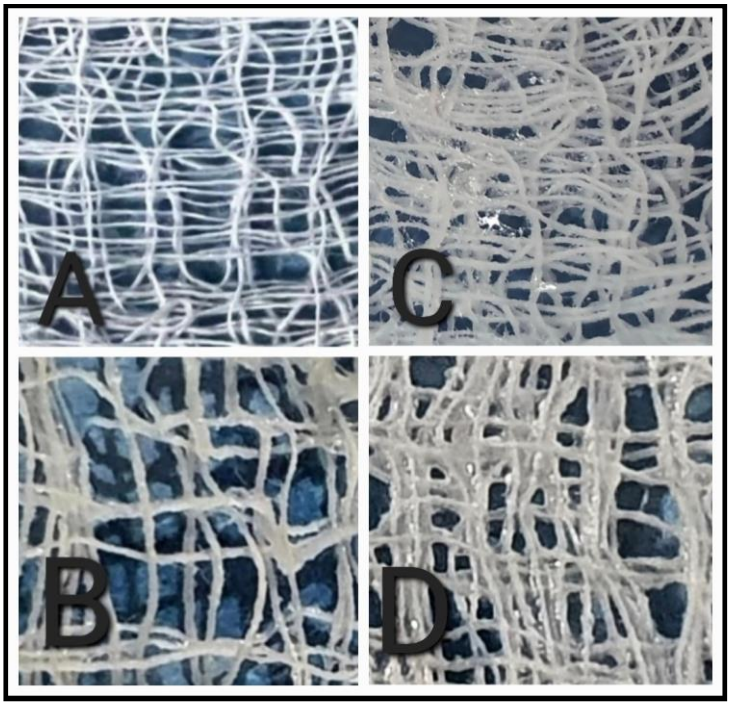
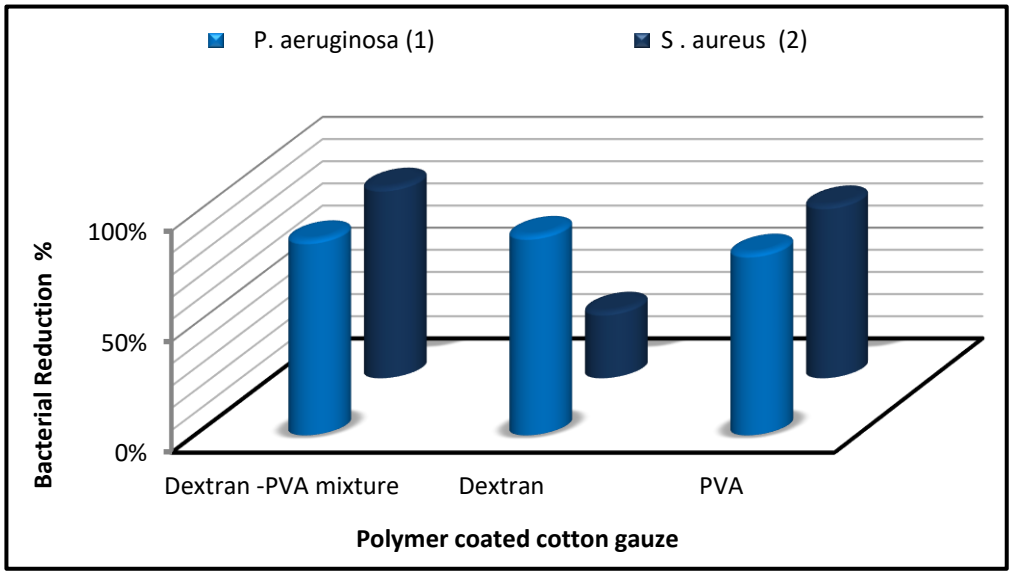


Figure 4. Reduction of *P. aeruginosa* (1) and *S. aureus* (2) growth in dextran and its blend wound dressing.



Conclusion

Lactococcus lactis ssp *cremoris* S_4 isolated from sausages with the highest gene expression level of *dsrLL* gene, and the *L. lactis* ssp *cremoris*$_4$ produced dextran. The purified dextran had antibacterial and antibiofilm effects against pathogenic bacteria. Additionally, dextran can be used in wound dressing, especially with PVA.

Ethics approval and consent to participate

The Ethics Committee of the Mustansiriyah University approved and oversaw this study

Consent for publication

All authors agree to publish this work

Availability of data and material

No Data associated with the manuscript

Competing interests

The authors have no conflicts of interest regarding the publication of this paper

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Authors' contributions

All authors contributed equally in writing—original draft preparation, all authors have read and agreed to the published version of the manuscript

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