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Inhibition characteristics of biofilm structure of *Staphylococcus aureus*

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Abstract: Different extracts have different effects on the biofilm structure of *Staphylococcus aureus*, and the biofilm structure of *Staphylococcus aureus* will produce different inhibition reactions. In this study, different experimental reagent extracts were used to analyze the inhibition characteristics of *Staphylococcus aureus* biofilm structure. The inhibition characteristics of bacterial biofilm structure were obtained by using the same bacteria species and the same experimental environment. The results showed that the chloroform extract had a good inhibitory effect on the biofilm structure, which could effectively inhibit the formation of biofilm; the acetic acid extract had an impact on the formation of biofilm, which was destructive to the biofilm; the petroleum ether extract had no effect on the formation of biofilm, that is, it had no inhibitory effect.

Key words: *Staphylococcus Aureus*; Biofilm Structure; Inhibition Characteristics; Lead Compound.

Introduction

Staphylococcus aureus is ubiquitous in nature and can secrete a variety of exotoxins and enzymes, such as enterotoxin, leukocyte hemolysin and plasma coagulase, which seriously endangers human and animal health. *S. aureus* is about 0.8 nm in diameter. It is observed under a microscope as a single or cluster of grapes (1-3). The most suitable growth condition for aerobic or facultative anaerobic bacteria is pH 7.4, which does not require high nutrition. The hemolytic ring can be formed on the blood plate. It can grow well in 7.5% - 10% sodium chloride broth medium and can use a variety of sugar substances to complete the growth process, such as glucose, maltose, sucrose, lactose, etc.

S. aureus is widely distributed and can adapt to various environments, which can lead to a variety of infectious diseases. *S. aureus* can be divided into three types as community, hospital and breeding *S. aureus* (4-5). *S. aureus* strains from the community are genetically different from those from the hospital (6), but they can cross propagate, which makes the difference between CA-SA and ha-sa blurred (7). In the veterinary clinic, *S. aureus* often causes arthritis, mastitis, omphalitis, local abscess and other diseases in animals, causing huge economic losses in production and other aspects (8). *S. aureus* is one of the main pathogens causing food poisoning. According to the report of China Health Statistics Yearbook 2013-2015, from 20112014, there have been 1244 food poisoning cases caused by food borne pathogens in China, and the number of patients is 27479, among which the number of patients caused by *S. aureus* is more than 3000, accounting for 11.9% of the total (9).

S. aureus has become one of the main pathogens of mastitis in dairy cattle due to its strong pathogenicity, persistent presence in the environment, colonization in animal skin or mucous epithelium and low cure rate (10, 11). During the transportation and processing of milk and dairy products contaminated by *S. aureus*, *S. aureus* biofilm will be formed on the surface of milk can and packaging. *S. aureus* in the form of biofilm is 1000 times stronger than isolated *S. aureus*, and it is easy to cause the secondary infection (10, 11).

In addition, *S. aureus* can produce heat-stable toxins, alpha-toxin is one of the main virulence factors. The alpha-toxin can polymerize into heptatolymer, which forms a transmembrane channel on the cell membrane and leads to cell lysis (12).

Biofilm formation is one of the important pathogenic factors of *S. aureus*. The formation of biofilm is helpful for the growth and maturity of *S. aureus*. It usually forms organized membrane structure on the surface of inert objects, such as food processing machinery, packaging machinery, medical machinery, etc (13). After the formation of biofilm, the drug resistance and bactericidal resistance of *S. aureus* are significantly improved, which is difficult to be removed, and its reproductive and infection ability is enhanced, which makes it difficult to cure the disease of the infected (14, 15). The formation of biofilm can be divided into three stages: bacterial adhesion to the surface of the object and the formation of biofilm, the maturation of biofilm and bacterial shedding to pollute the surrounding environment. Each stage needs specific factors (13). *S. aureus* plays an important role in the formation of biofilm through the recognition of the cell surface and the ligand of the tissue surface. After bacterial attachment, the biofilm proliferates and synthesizes extracellular polysaccharide (14). Biofilm has many kinds of extracellular poly-

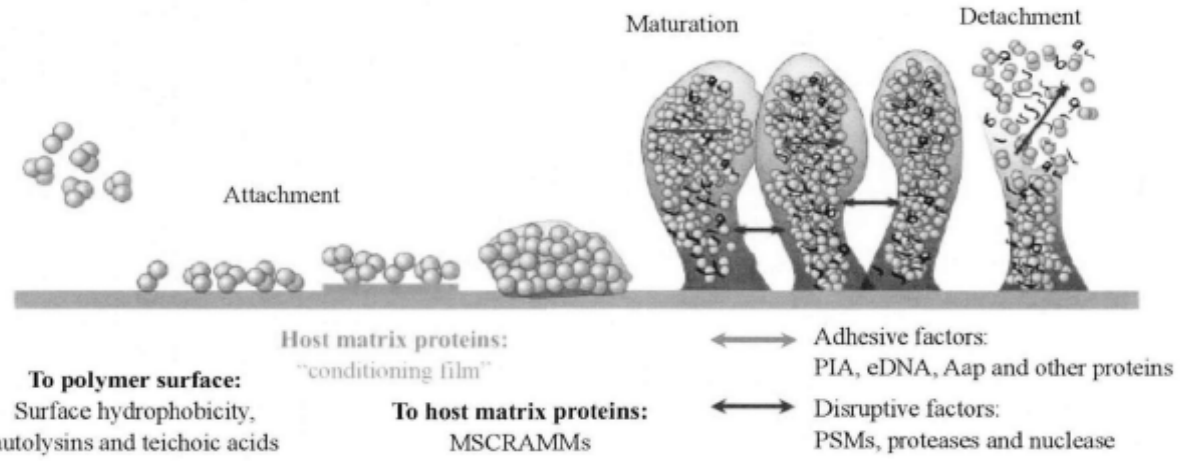

Figure 1. The biofilm formation process of *S. aureus* (22).

mers, such as a polysaccharide. Through these extracellular polymers, bacteria in biofilm adhere and gather, thus forming a unique and stable scaffold structure. The mature biofilm has high water content, up to 97070, and will form a mushroom-like cell structure. Finally, single cells and cell clusters will separate from the mature biofilm and form new infection sites (13, 15).

Aiming at the harmfulness of *S. aureus* to food and the human body, in the daily research, the form of inhibiting its biofilm growth was used to complete its control. The common inhibitors of *S. aureus* biofilm structure are planted essential oil, chemical agents, etc. in this study, the experimental form and common experimental reagents were used to complete the research on the inhibition characteristics of *S. aureus* biofilm structure (16).

S. aureus can form biofilm structure on the surface of a variety of materials, and its biofilm formation process can be divided into two main stages: the initial attachment of a single bacteria and the mutual exclusion between bacteria, and finally form a mature multicellular biofilm structure (17-19).

PIA is synthesized by the protein encoded by the ICa operon, and almost no biofilm is formed in the mutation strain of ICa. It has been found that the ICa operon exists in clinical strains of *S.* epidermidis. In the infected animal model, the virulence of the ICa mutant strain was significantly lower than that of wild strain, which indicated that the expression and synthesis of PIA were very important for the pathogenicity of *S.* epidermidis. However, some recent reports have found that some strains are negative for ICa but can still form biofilm in *S.* clinical strains, and molecular level studies have also revealed some biofilm formation mechanisms that are not dependent on PIA (20). This experiment will study the ability of biofilm formation, structure and biological characteristics of *S. aureus* in different culture systems, the tolerance of biofilm to antibiotics and other chemicals, and obtain the development direction of inhibition characteristics of *S. aureus*. In order to ensure the reliability of this experiment, according to the formation process of *S. aureus* biofilm, the experimental equipment and reagents are selected, and the experimental method is developed (21). The specific biofilm formation process has been shown in Figure 1.

The aim of the current study is the investigation of inhibition characteristics of the biofilm structure of *S. aureus*.

Materials and Methods

Samples and strains

The standard strain CMCC (B) 26003 of *S. aureus* was used. The strain was stored in TSB medium containing 30% glycerol and stored at - 80 ℃.

Experimental equipment and chemicals

The selected experimental equipment is shown in Table 1.

Sodium chloride, anhydrous disodium hydrogen phosphate, sodium dihydrogen phosphate, agar powder and fish meal protein were all purchased from Sinopharm Group Chemical Reagent Co., Ltd. Beef extract was purchased from Beijing double rotation microbial culture medium products factory. DAPI nuclear dye was purchased from Shanghai Jinmai Biotechnology Co., Ltd.

Common nutrient broth medium (NB) included Fish meal protein 10g, Beef extract 5g, Sodium chloride 5g, DI water 1000 ml.

The pH value was adjusted to 7.2-7.4. The sample was autoclaved at 121 ℃ for 30 min, then ready for the cultivation of *S. aureus*, *Escherichia coli* and other pathogenic bacteria.

General nutrient agar medium (NA) included fish meal protein 10g, beef extract 5g, sodium chloride 5g, DI water 1000 ml.

The pH value was adjusted to 7.2-7.4. The sample was autoclaved at 121 ℃ for 30 min, then ready for the cultivation of *S. aureus*, *Escherichia coli* and other pathogenic bacteria.

Phosphate buffer [PBS, 0.03 mol/L, pH (7.27.4)] included anhydrous disodium hydrogen phosphate 3.067 g, sodium dihydrogen phosphate 1.008 g, nonionic surfactant 1.0 g, DI water 1000 ml.

The sample was autoclaved at 121 ℃ for 30 min, then used for dilution of the bacterial solution and test samples. Then the culture process of *S. aureus* was completed.,

Experimental reagents

TSB medium (tryptic soy broth medium): weigh 30g of TSB powder, dissolved in 1000ml of deionized

Table 1. Main experimental instruments and equipment.

Equipment	Model
Experimental bench (11-12)	SW-CJ-2fddouble single face purification bench
Gel imager	UVR-800 Uitra-Uiolet Procducts Limted Cambhdge UK
Vertical electrophoresis	JY-SPCT of Beijing Junyi Oriental electrophoresis Co., Ltd
Low-temperature table centrifuge	Beckman avantitm 30 low-temperature desktop high-speed centrifuge Beckman Corp
Table centrifuge	Eppendorf Centrifuge 5804 R31163Eppendorf Corp.
Constant temperature oscillation incubator	
Electric constant temperature incubator	HH.BLL.500-s electric constant temperature incubator
PCR amplification instrument (13-15)	Eppendorf Mustercycler Gradient PCR instrument
PH meter	PB-10 ph meter sartorius AG
Spectrophotometer	Eppendorf Biophotometer
Projection electron microscope	H-7650
Constant temperature water bath	
Glassware air dryer	C-20
Acidity meter	FE20K
Electronic balance	JA2003
Vortex Mixers	XH-C
Inverted fluorescence Microscope	DMIL
Portable ATP fluorescence detector	I-Genie
Ultrasonic cleaning machine	KQ---300DE
Gas chromatography-mass spectrometer	6890GC/5973NMSD

water, completely dissolved, autoclaved at 121 ℃ for 20min, and stored at 4 ℃ for standby.

TSA medium (tryptic soy broth agar): weigh 30g of TSB powder, dissolved in 1000 ml of deionized water, added 15g of agar powder after complete dissolution, shake well, autoclaved at 121 ℃ for 20 min, made a culture plate, and stored at 4 ℃ for standby.

TSBg medium (TSB medium containing 0.25% glucose): weighed TSB powder 3G, glucose particles 0.0075g, dissolve in 100ml deionized water, after complete dissolution, autoclaved at 121 ℃ for 20 min, store at 4 ℃ for standby.

Congo red agar plate: weighed 0.8 g Congo red powder, dissolved it in 1000ml deionized water, sterilized it with high-pressure steam for 20 min; weigh 37g brain heart infusion powder, 50 g sucrose, 10g agar powder, dissolved it in 1000ml deionized water, sterilize it with high-pressure steam for 20min. Cool the agar and sucrose to about 55 ℃, mix Congo red sterilized by high-pressure steam into the agar and sucrose, mixed them well, pour them into a sterile culture dish, cool and solidify them, and stored them at 4 ℃ for future use. The materials and concentrations of the experimental reagents are shown in Table 2.

Experimental Materials

In this experiment, in addition to the experimental equipment and reagents, the corresponding experimental materials were also needed in the experiment. The experimental materials were selected in Table 3.

Table 2. Materials and concentrations of the experimental reagents used in this experiment. Where AR: Analytical reagent and BR: Biological reagent.

Material name	Material purity
Crystal violet	AR
Congo red	AR
Trypsin medium	
Glucose	AR
Glutaraldehyde	BR
Silver nitrate	AR
Hydroquinone	AR
Azure	BR

Experimental methods

According to the previous studies (2-5) (Figure 2) on the biofilm structure of S. aureus, the experimental route was done.

Establishment of biofilm model of S. aureus

The glass cover was put into the liquid culture medium of the experimental reagent, sterilized for 0.5 h

Table 3. The experimental materials. Where AR: Analytical reagent and BR: Biological reagent.

Material name	Material purity
Beef extract	AR
sodium chloride	AR
AGAR	AR
Fish meal peptone	BR
Potassium dihydrogen phosphate	AR
Sodium dihydrogen phosphate	AR
Petri dish	
Magnesium sulfate	AR
lactose	BR
O-nitrobenzene-p-d-pyranogalactopyranoside	AR
Ethylenediaminetetraacetic acid	AR
Trimethylaminomethane	AR
Iodacetic acid	AR
Malonic acid	AR
Sodium phosphate	AR
glycine	
Sodium dodecyl sulfate	AR
Glycerol	AR
Glucose-6-phosphate dehydrogenase Kit	
BCA micro protein detection kit	
ATP content determination kit	
AKP test kit	
Galactopyrase Kit	

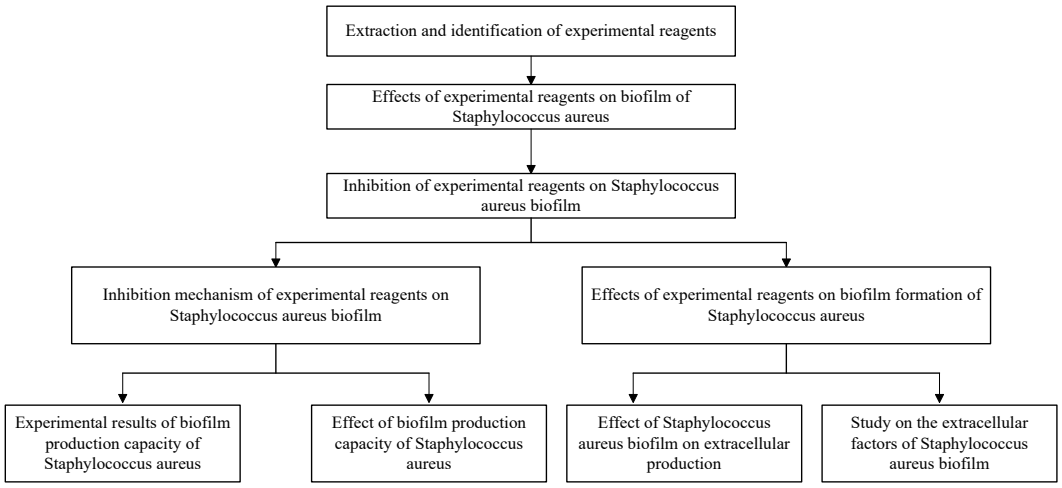

Figure 1. Experimental route for the biofilm structure of *S. aureus*.

at 121 ℃, and stored it in the refrigerator at 4 ℃ for standby. The activated TSB medium was inoculated on TSA medium and cultured overnight at 37 ℃. Take single colony with d > 1mm and inoculate it in TSB culture medium, culture it overnight at 37 ℃ for 12h, then dilute it to OD600 = 0.4 (equivalent to 5x108cfu / ml, and further dilute it to 106cfu / ml; take 2 ml of the bacterial solution and inoculate it in the 24 well plates with cover glass, the hole containing only TSB culture medium was blank control, mix it well and cover it, sealed it with sealing film, incubated it in 37 ℃, 50 rmp shaker for 72h.

The colony of *S. aureus* (d > 1 mm) was selected from TSA solid medium and cultured in TSB. After being blown evenly, the samples were positioned in Congo red solid medium. After 24 hours of aerobic incubation at 37 ℃ and then 24 hours at room temperature, the results were observed. The positive strains are dry and bright black colonies, while the negative strains are smooth and red colonies.

Pretreatment

Colony observation

The *S. aureus* colonies were grown on the Colombian blood agar plate to the slides evenly; then the flame of the smear fixed, it dyed with crystal violet solution for 5s, and then dried it with filter paper after washing with clear water; it dyed with iodine solution for 5S, and then dried it with filter paper after washing with clear water; the decolorizing solution added, it shakes from time to time until there was no purple shedding, about 10-30s, and then filtered it with filter paper after washing with clear water. The paper dried, it dyed with dye solution for 5S, washed it with water, dried the slide naturally, and then observed it under the biological microscope.

Determination of MBIC and MBFC of experimental reagents

200 ml of sample inoculate in 48 well plates, 2 ml of sterile TSB culture medium added, and corresponding experimental reagents added so that the final concentrations were 0.25, 0.5, 1.0, 2.0 and 4.0mg/ml, and cultured at 37 ℃ for 2d, sterile TSB well as the control. After culture, 20 ml of the experimental solution into each well added and continued to incubate for 4h. After carefully removing the supernatant, the 48-well plate cleaned with sterilized water to remove the floating bacteria. After decolonization, the OD value measured at 570 nm with the enzyme labeling instrument. The mature biofilm in the well plate cultured, the floating bacteria washed with sterile water and then the experimental reagent of gradient concentration added. After incubation at 37 ℃ for 24h, the MTT solution was used to dye and decolorize, then the OD value was determined.

Strain Activation and Suspension Configuration

S. aureus preserved in glycerin at - 20 ℃ was inoculated on the TSA plate and placed in a 37 ℃ constant temperature incubator for 20 h; a single colony was picked up on the TSA plate and placed in a 37 ℃ constant temperature incubator for 20 h; one of the colonies with a diameter greater than 1mm was picked up and cultured in 5ml of bacteria-free TSB medium at 37 ℃, 220r / min for about 4 h (i.e. entering the logarithmic growth period) to obtain a bacterial suspension. The activated *S. aureus* suspension was washed with sterilized PBS for three times at 4 ℃, 5000r and 10min, and then the concentration of bacteria suspension was adjusted to OD600 0.1 with PBS.

Cleaning of the glass cover

Immerse the cover slides in acetone to remove the grease on their surfaces, then immersed the cover slides in 5mol/L hydrochloric acid solution for 15 min, then washed them with detergent, and finally wash them with deionized water. Sterilize the washed cover slides at 121 ℃ for 15 min, and then use them for standby. Then the style of the glass covers selected. After cleaning, the cover plate was used to complete the acquisition process of experimental bacteria.

Identification of biofilm production ability of bacteria

Identification by Congo red plate method

The activated bacterial solution was scribbled on Congo red plate with inoculation ring, at 37 ℃ for 24 h, and the colony growth was observed.

Judgment criteria

If the colony turns black with metallic luster and transparent circle, it has the ability to produce membrane; otherwise, it is negative and cannot produce membrane.

Identification by crystal violet staining

The prepared standard bacterial suspension with 200ml per well inoculated into the 96 well plate, 6 multiple holes for each kind of bacteria, TSB take as blank control, 37 ℃, 24h; carefully suck and discarded the bacterial solution in each hole with a pipette, then PBS washed for 3 times, after natural drying, 100ml 2.5% glutaraldehyde added into each hole for 90min; then the fixed solution discard, and it washed with PBS for 2 times; finally, 100ml 1% crystal violet Dye used for 15 min, the dye solution washed with distilled water, at room temperature dried, and decolorized with 35% ethanol for 30 min.

Microscopic morphology of biofilm

Observation of biofilm by silver staining

After adding 500ml of staining reagent to the 24-well plate, 5ml of TSB medium was added to the hole, and a sterile cover glass (20 mm x 20 mm) was added to the hole. The cover glass was cultured for 2 d at 37 ℃, washed to remove free bacteria, and then treated with LC-EO (1 mg/ml, 2 mg/ml and 4 mg/ml) for 2 h.

Observation of biofilm by laser confocal microscope

After adding 500 ml of experimental reagent into the 24-well plate, 5ml of TSB medium was added into each hole, 1 sterile cover glass (20 mm x 20 mm) was added, and the biofilm was cultured at 37 ℃ for 3 days at a constant temperature. After cleaning the cover glass to remove the free bacteria, the test reagents (1 mg/ml, 2 mg/ml and 4 mg/ml) were used for 2 hours. Finally, dye in the dark for 15 min, and observe CLSM. The maximum excitation wavelength and the maximum emission wavelength are 364nm and 454 nm, respectively.

Observation of biofilm by scanning electron microscope

The 2 cm * 2cm nylon piece prepared for cleaning and sterilization, and it used for biofilm culture. The culture process was the same as that of strain culture. The free bacteria and culture medium were removed by gently cleaning the nylon surface with sterilized water, and treated with 2mg / ml experimental reagent for 2h. After treatment, 2.5% glutaraldehyde was added and fixed at 4 ℃ for 12 h, and finally washed with sterilized water for 3 times. The fixed samples were dehydrated with an ethanol gradient for 15 min and dried, and then used for SEM observation to complete the experimental control process.

Data processing and analysis

All experiments were repeated three times to get the average value. The results of the experiment were analyzed by one-way ANOVA in SPSS22.0 software, $P < 0.05$ was regarded as a significant difference level.

Results

Identification of the ability of *S. aureus* to produce membrane

As shown in Figure 3, most colonies were dark red or dark brown, and a few are black. After 72 hours, the strain was black, dry and bright. A transparent circle was produced around the colony and the color became stronger, indicating that it was positive, that is to say, the experimental strain was positive. After crystal violet staining, the value of the enzyme marker at the test sample was 0.851, which was between 0.5-1.5. According to the judgment standard of biofilm-forming positive strain, the test strain used in this test was the middle membrane strain.

Figure 3. Formation of black colonies in this experiment.

Through the test results, we can know that the strains selected in the experiment can be used as the samples of this experiment to complete the inhibition characteristics of *S. aureus* biofilm structure.

In this experiment, through ethanol ultrasonic reflux extraction and liquid-liquid extraction, the experimental reagents were extracted and separated to obtain chloroform phase extract component, petroleum ether phase extract component and acetic acid extract component. Taking the result of experimental bacteria selection as the test bacteria, three kinds of extracts were tested for bacteriostasis,

Chloroform phase extract

The chloroform phase extract was dripped into the culture dish of the experimental sample, and the experimental results were observed by a high power microscope after standing for a period of time. Through the experimental steps, the experimental results are as follows (Figure 4).

The results showed that the chloroform extract had a high bacteriostatic activity and the average diameter of the bacteriostatic circle was 19 mm. Through the observation of the microscope, the effect of this kind of experimental reagent on *S. aureus* biofilm. The extract significantly changed the morphology of *S. aureus* biofilm, decreased the number of bacteria and no anvil liquid was found, which indicated that the extract had an inhi-

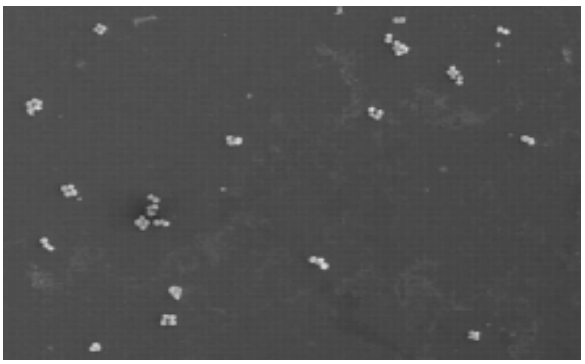

Figure 4. The results of the chloroform phase extract observed by a high power microscope.

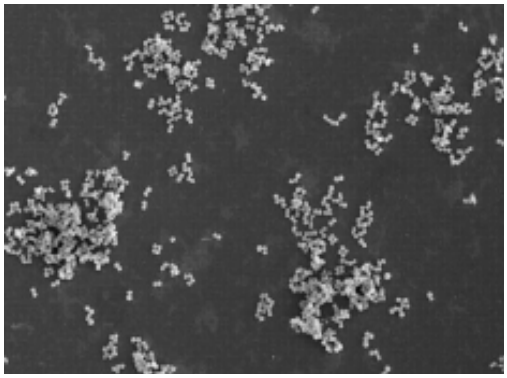

Figure 5. Bacteriostatic circle of the acetic acid extract

bitory effect on the biofilm of *S. aureus* and eliminated the mature biofilm.

Acetic acid extract

The average diameter of the bacteriostatic circle of the acetic acid extract was 9 mm. It can be seen that the production of bacterial biofilm was obviously reduced, and the emulsion layer is also thinner. Although it was still covered by biofilm, its morphology was still obvious compared with that of *S. aureus*. According to the experimental results, this kind of substance can inhibit the formation of *S. aureus* biofilm, and the higher the concentration, the smaller the amount of biofilm formation (Figure 5).

Petroleum ether extract

It can be seen that bacteria are surrounded by thick bacterial biofilm, and almost no *S. aureus* should present the shape of grape string. At a higher magnification, it can be clearly seen that this group of experimental results bacteria loses, rough surface, attached with a layer of polymer (Figure 6).

Discussion

S. aureus widely exists in nature and is an important pathogen next to *Escherichia coli*. The formation of biofilm is a common state of bacterial survival. After the formation of biofilm, the morphological structure, metabolic function and physiological characteristics of bacteria become very complex, which is an important reason for bacterial resistance. The results showed that the antibiotic dosage needed to kill biofilm was more than 5000 times that needed to kill planktonic bacteria. The enhancement of drug resistance increases the difficulty of removing biofilm, which often leads to the occurrence and recurrence of clinical chronic multiple infections (23-29).

At present, the mechanism of bacterial biofilm resistance to antibiotics is not very clear. Researchers speculate that it may be caused by the following possible mechanisms: the barrier effect of biomass; special microenvironment (nutrient composition, the concentration of metabolites, osmotic pressure and oxygen concentration in biofilm, etc., which decline gradient from the outside to the inside); the role of bacterial density sensing system; the generation of resistance genes in biofilm; and Secretion of antibiotic hydrolase; activation of the stress response: activation of the antibiotic pump system, etc (28, 30).

The formation of biofilm is a very complex process. Many studies have shown that the intercellular polysaccharide, a factor that mediates the adhesion between bacteria, is a necessary substance for bacteria to form a biofilm. The Congo red plate experiment adopted in this chapter is the PIA staining method, which is used to determine whether *S. aureus* can form a biofilm. Its operation is simple and fast, and it is a common biological method Qualitative detection method of membrane formation (30, 31).

In this experiment, the morphological observation was used to detect *S. aureus* biofilm. Silver staining showed that the amount of proteoglycan complex was the largest when biofilm was cultured to the third day, and the number of viable bacteria in biofilm did not increase continuously with the increase of culture time. The results of crystal violet staining showed that most of the bacteria in the biofilm died after 3 days. The reason for this phenomenon may be that various extracellular polymers secreted by the biofilm during the formation process will accumulate with the culture time, wrap the bacteria itself in it, affect the normal metabolism of bacteria, make some bacteria cannot reproduce normally and die. The results showed that the metabolism and the secretion of extracellular polymers were different at different times, which played an indispensable role in the formation of biofilm (32).

The bacteriostatic effect of the extract of the experimental reagent has been well confirmed. Different experimental reagents contain different bacteriostatic components and have different solubility. Therefore, even if it is the same experimental reagent, the extract obtained by different extraction methods may be different, and its antibacterial ability will also be different. Water and organic solvents are commonly used in the bacteriostatic substances in the experimental reagents, while organic solvents mainly include ethanol, acetone, acetic acid, etc (33).

In this experiment, it is found that the antimicrobial substances of the selected experimental reagents can be divided into three kinds of extracts, each of which has a significantly different antibacterial ability. On the whole, the chloroform extract has a stronger antibacterial ability, the other two are weaker.

Chloroform phase extract showed a strong antibacterial effect, and the antibacterial effect of the acetic acid extract was better than that of petroleum ether extract because whether it was *S. aureus* or Vibrio parahaemolyticus, the antibacterial circle of water extract was larger than that of alcohol extract (only in *E. coli*).

Crystal violet staining and colony count were used to studying the effect of experimental reagents on the removal of biofilm. The experimental results showed that the removal of biofilm was dose-dependent. With the increasing concentration of experimental reagents, the removal rate of biofilm increased. After 8 hours of treatment, the removal rate of 4 mg/ml reagent to the surface biofilm was over 99.99%. The results of confocal laser scanning electron microscopy and scanning electron microscopy showed that some experimental reagents could break down the complex three-dimensional structure of bacterial biofilm, reduce the thickness of the membrane, make the close biofilm become sparse, and the bacteria were released into free bacteria from

the membrane barrier and then inactivated by experimental reagents (34).

Through the content, we can determine the inhibition characteristics of *S. aureus* biofilm structure as follows: chloroform extract has a good inhibition on the biofilm structure of strains, the acetic acid extract has an impact on the formation of biofilm, petroleum ether extract has no impact on the formation of biofilm, that is, it has no inhibition.

Bacterial biofilm formation is a serious threat to food safety and human health, so inhibiting bacterial biofilm formation on food processing equipment or medical equipment has become the main way to prevent "biofilm-related infection" (35).

The anti-bacterial and anti-oxidation ability of the experimental reagents has been reported in a large number of studies, while the inhibition of the experimental reagents on the formation of biofilm is just in its infancy, and foreign studies are mainly concentrated on thyme, sage, oregano, etc. The antimicrobial activities of cinnamon and garlic have been widely recognized. The main antimicrobial components are cinnamaldehyde and Allicin, but their effects on biofilm are still unknown. There are a few kinds of literature about this research direction, and they mainly focus on antioxidants and antibacterial. The application value of this research results still needs to be further developed (35, 36).

S. aureus is an important pathogen, which belongs to *S.* and is representative of Gram-positive bacteria. Under the microscope, the grapes are usually arranged in clusters, without spores and flagella, and generally do not form a capsule. They do not have high requirements for nutrition. They are aerobic and facultatively anaerobic. The optimum pH for growth is 7.4, and the optimum temperature for growth is 37 ℃. *S. aureus* can endure low water index, survive for several months in a dry environment, and is highly salt resistant. At present, the research on bacterial biofilm at home and abroad is basically concentrated in the fields of medicine, sewage treatment, bioengineering materials and other disciplines, but the research on bacterial biofilm is less in the food industry. In fact, the phenomenon of bacterial biofilm has more important significance than other industries in food processing, food enterprise health management, food safety, food preservation and so on.

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