

Screening and Identification of Staphylococcus Aureus Endophytic Strains with Antibacterial Activity

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Abstract: Objective: The screening and identification methods of endophytic strains of Staphylococcus aureus with antimicrobial activity were expounded, and the effect of identification was observed. Method: Staphylococcus aureus and other strains were prepared, isolated and purified, and endophytic strains with antimicrobial activity were screened and identified by dot grafting. Result: Through the observation of the preliminary screening results, the subject found that Staphylococcus aureus has antibacterial activity endogenous strain A-2, which is positive for contact enzyme, yellow pigment, acetate, acetamide hydrolysis, V-P and hydrolyzed starch. The antibacterial titer of A-2 strain against Staphylococcus aureus was 60 mm/ml. When the concentration of the diluent was 10⁻¹, 10⁻⁴, A-2 had antibacterial activity against Staphylococcus aureus. Conclusion: Screening and identification of endophytic strains of Staphylococcus aureus bacteriostatic activity by point-and-click method can effectively identify the type of corresponding strain and provide reference for the treatment of Staphylococcus aureus infection.

Keywords: Staphylococcus aureus; antibacterial activity; endophytic strain.

1. INTRODUCTION

Staphylococcus aureus is one of the most important pathogens in the human body. It is widely involved in the occurrence and progress of diseases of the digestive system and respiratory infections, and has a negative impact on human health. Screening and identifying the endophytic strains of Staphylococcus aureus with antimicrobial activity is the main way to improve the treatment efficiency of various infectious diseases. Experiments were carried out from January 2017 to January 2018 to observe the screening and identification of endophytic strains of Staphylococcus aureus with antimicrobial activity. The report is as follows:

2. MATERIALS AND INSTRUMENTS

2.1 Material science

Staphylococcus aureus, Bacillus subtilis, Lactobacillus plantarum and Staphylococcus epidermidis should be prepared as the main experimental materials to screen and identify the endophytic strains with antibacterial activity of Staphylococcus aureus.

2.2 Instrument

The instruments needed for the experiment include autoclave, constant temperature incubator, electronic balance, pipette, 3730 sequencing analyzer, etc.

2.3 Method

2.3.1 Separation and purification methods

All the strains were isolated and purified by the following methods: (1) Staphylococcus aureus dilution was obtained and inoculated uniformly on the medium by coating plate method. (2) Incubate for 4 to 7 days at 28 °C. (3) Picking up a single colony for separation and purification. (4) Preservation, to be used in the experiment.

2.3.2 Screening and identification method

The screening and identification process of endophytic strains with antibacterial activity of Staphylococcus aureus includes activation of indicator bacteria, primary screening of strains, and rescreening. The details are as follows: (1) Activation of indicator bacteria: The relevant personnel should first inoculate the indicator bacteria on the medium and shake for 36 hours at 37 °C. Diluted with sterile water, when the concentration of suspended bacteria reaches 107CFU/ml, a nutritious solid plate is made. (2) Screening: first, the strains were screened by point joining method and 72h was observed after screening. 72 hours later, the strain was inoculated into the medium, shaken at 37 C for 5 days, centrifuged at 4000 R / min for 15 minutes, and supernatant was taken for reserve. (3) Determination of inhibitory concentration: take fermentation broth 9 ml diluted to 10 times the liquid, continue to dilute until the liquid concentration of 1-7.

2.4 Observation index

(1) Observe Staphylococcus aureus with antibacterial activity of endophytic strains. (2) Observe the bacteriostatic titer of the strain. (3) Observe the bacteriostatic effect of the strain under different dilution. (4) Observe the characteristics of the strain.

3. RESULT

3.1 Staphylococcus aureus has antibacterial activity and endophytic strains

Through the observation of the preliminary screening results, we found that Staphylococcus aureus has antibacterial activity of endophytic strain A-2.

3.2 Bacteriostatic titer of strain

The antibacterial potency of A-2 strain against Staphylococcus aureus is 60mm/ml, as shown in Table 1:

Table 1. Bacteriostatic titer of strain

Indicative bacteria	Staphylococcus aureus (mm/ml)	Bacillus subtilis (mm/ml)	Lactobacillus plantarum (mm/ml)	Staphylococcus epidermidis (mm/ml)
Bacteriostasis titer	60.00	0	44.01	55.01

3.3 Bacteriostatic effect of strains under different dilution

3.3.1 Bacteriostatic effect under 10⁻¹ concentration

At 10⁻¹ concentration, A-2 has antibacterial activity against Staphylococcus aureus, as shown in Table 2:

Table 2. Antibacterial effect at 10⁻¹ concentration

Indicator bacteria	Staphylococcus aureus	Bacillus subtilis	Lactobacillus plant	Staphylococcus epidermidis
Antibacterial activity	+	-	+	+

(+ means the bacteriostatic effect is positive, - means the bacteriostatic effect is negative)

3.3.2 Antibacterial effect at 10⁻⁴ concentration

At a concentration of 10⁻⁴, A-2 has antibacterial activity against Staphylococcus aureus, as shown in Table 3:

Table 3. Antibacterial effect at 10⁻⁴ concentration

Indicator bacteria	Staphylococcus aureus	Bacillus subtilis	Lactobacillus plant	Staphylococcus epidermidis
Antibacterial activity	+	-	+	+

(+ means the bacteriostatic effect is positive, - means the bacteriostatic effect is negative)

3.3.3 Antibacterial effect at 10⁻⁷ concentration

At 10⁻⁷ concentration, A-2 has no antibacterial activity against Staphylococcus aureus, as shown in Table 4:

Table 4. Antibacterial effect at 10⁻⁷ concentration

Indicator bacteria	Staphylococcus aureus	Bacillus subtilis	Lactobacillus plant	Staphylococcus epidermidis
Antibacterial activity	-	-	-	-

(+ means the bacteriostatic effect is positive, - means the bacteriostatic effect is negative)

3.4 Characteristics of strains

A-2 contact enzyme, yellow pigment, acetate, acetamide hydrolysis, V-P, hydrolyzed starch were positive, as shown in Table 5:

Table 5. Characteristics of strains

Feature	Contact enzyme	Yellow pigment	Acetate	Hydrolyzed gelatin
Nature	+	+	+	-
Feature	Acetamide hydrolysis	V-P	Hydrolyzed starch	NO ₃ -NO ₂
Nature	+	+	+	-

(+ stands for positive, - stands for negative)

4. DISCUSS

Staphylococcus aureus, also known as *Staphylococcus aureus*, is common in all kinds of bacteria, belonging to the main pathogens of human infectious diseases, also belongs to the main cause of food pollution [1]. Clinical studies have found that penicillin can achieve good results in inhibiting *Staphylococcus aureus* [2]. In order to further improve the efficiency of treatment of infectious diseases, this topic again with antibacterial activity strains were screened and identification of *Staphylococcus aureus*. As a result, the A-2 strain was found, and it was further observed that the strain was composed of a contact enzyme, yellow pigment, acetate, acetamide hydrolysis, V-P, hydrolyzed starch and the like. The antibacterial potency against *Staphylococcus aureus* is 60 mm/ml. It has higher antibacterial potency than strains such as *Lactobacillus plantarum*. By observing the concentration of A-2 bacteriostatic activity, it was found that A-2 had antibacterial activity against *Staphylococcus aureus* when the concentration of the diluent was 10⁻¹, 10⁻⁴. The above research results show that A-2 is a kind of endophytic strain with antibacterial activity of *Staphylococcus aureus*.

In summary, the selection and identification of endophytic strains of *Staphylococcus aureus* bacteriostatic activity by point-and-click method can effectively identify the type of corresponding strains and provide reference for the treatment of *Staphylococcus aureus* infections.

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