

# Isolation and Identification of Streptococcus Suis

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**Keywords:** Streptococcus suis serotype 2; Identification; Virulence; Drug sensitivity test.

**Abstract.** This study mainly isolated and identified a Streptococcus suis strains by the methods of disease material separation, microscopic examination and PCR identification, drug sensitivity test, biochemical test, animal attack to toxicity test and so on. Get A preliminary assessment of its virulence and drug resistance to provide a reference basis for further study of Streptococcus suis.

## Introduction

Streptococcus suis is a major disease causing harm to the pig industry. The pathogen is detected by the nose fluid, urine, blood, muscle, visceral and joint fluid of the pig. The death of pork, viscera and waste without the harmless treatment are the important source of the disease. Respiratory tract is the major route of transmission. The disease can occur in the four seasons, but the incidence of 4-10 months, often endemic, the short term can spread to the entire group, the incidence, mortality is high. This experiment is mainly from pig disease viscera disease data collection of strains, selection and validation of Streptococcus suis, test the resistance and pathogenic of further prevention and treatment of swine diseases and deep research provide basic information and materials.

## Materials and methods

**Isolation and microscopic examination of pathogen.** The brain, lung and liver disease collect dead pigs inoculated in the normal tissues of sheep blood agar plate. After bacterial staining, microscopic examination, purification, 1 strains of suspected strains, named DYL-22. And hemolysis test is conducted. Among them, there are gram stain, staining, microscopic examination, purification steps are: smear, drying, fixation, staining, microscopic examination, purification.

**Sugar fermentation experiment.** The experimental strains access glucose, maltose, fructose, lactose, beta galactosidase (ONPC), inulin, arabinose, rhamnose, inositol, Dulcitol, mannitol, sorbitol, xylose biochemical reagent tube sealing 37°C 24h and observe the result of the experiment.

**Drug sensitivity test.** According to standard paper agar diffusion (K-B) operation, the drug sensitive paper is attached to the blood of the pathogenic bacteria on the blood plate, keep the appropriate distance between the paper, 37 temperature boxes 24 hours. The experiment shared 9 drugs: AN, GM, CRO, AMS, N, LVF, SMX, FOS, F.

**Identification of pathogenic bacteria PCR.** The content of PCR identification in this experiment primarily aims at the test whether the strain DYL-22 contained GDH gene. Streptococcus suis glutamate dehydrogenase belongs to GDH protein family, which raises on the cell surface. Nucleic acid sequence analysis shows that GDH genes includes an open reading frame, encoding 448 amino acid residues and expressed on the cell surface. The enzyme activity is dependent NAD (P) h, substrate for L-glutamic acid, its coding gene is SS2 (type 2 Streptococcus suis) conserved. SS2 GDH has conserved antigenic and infection of virulent Streptococcus suis in pigs serum reaction, detection of Streptococcus suis as an important indicator of the antigen. If the GDH gene is achieved, then the strain of Streptococcus suis is possible for Streptococcus suis type 2. Synthesis of Streptococcus suis type 2 specific primers, respectively gdh688a: CCATGGACAGATAAAGATGG, gdh689b: GCAGCGTATTCTGTCAAACG.

PCR system, condition and detection

PCR reaction system (20ul system) is: Taq 10ul Premix, template 1ul, upstream and downstream primer 1ul, Ultra pure water 7ul, a total of 20ul.

The reaction conditions of PCR were: at 94°C, the denaturation of 5 min, then entered into 35 cycles: 94°C 1 min, 51°C 1 min, 72°C 1 min, at last 7 min extends at 72°C, and the product of PCR is kept at 4°C for a brief. Agarose gel electrophores is detection.

**Pathogenic bacteria virulence experiment.** Blood agar inoculum activation is picked from a single colony from Streptococcus vaccination in Ma Dingtang (plus 4% heme) and 37°C culture 16-18 h, viable bacterial counts. Take soup Martin medium 0.2ml (including bacteria about 40 million) intravenous injection of 1.5 ~ 2.0kg rabbits, two rabbits are immunized. At the same time, live in bacteria count; blank control group is set to 2 and injected with saline. After the death of the rabbit, rabbit anatomy, and take blood inoculation blood incline 37°C, cultured for 24 h. At the same time, the rabbit blood filling small tube, -20°C preservation.

## Results and analysis

**The results and analysis of the mirror.** The diseased inoculates in the blood culture medium, the bacteria separation and purification, observe suspicious strains grew well, colonies are round, smooth transparent ridges, grey dew like small colonies and produce obvious beta hemolysis (Fig. 1). Suspicious colonies on the blood culture base are inoculated on the agar medium of wheat Kang Kai agar and has no bacterial growth. The growth of colonies on a common medium is poor. Picking blood culture built on the typical colonies, Gram staining, microscopic examination, visible gram positive, short chains or in single coccus cell diameter 0.1-0.2um (Fig. 2).



Fig. 1 hemolysis test

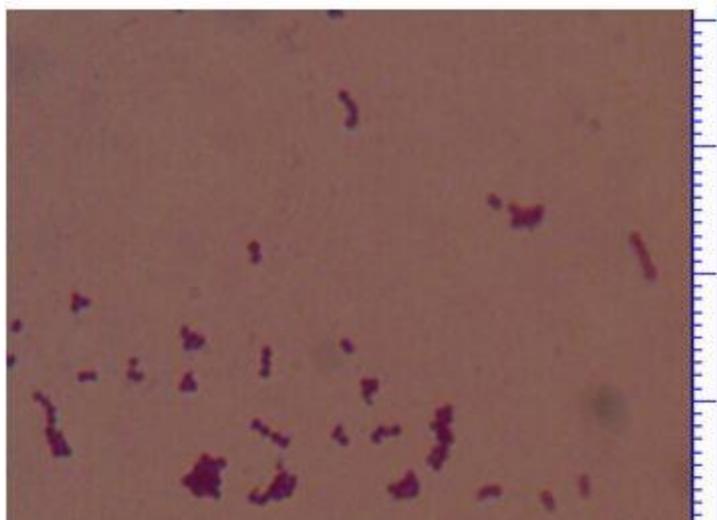


Fig. 2 gram stain amplification factor of 1000

**Biochemical test results and analysis.** Mainly for the sugar fermentation of *Streptococcus suis* biochemical tests, from Fig. 3, Fig. 4 and Fig. 5, it can be seen that biochemical test of glucose, fructose, maltose, lactose pipe by blue and purple turn yellow, inulin from light pink and the color of biochemical test tube are not found in the gas. Thus, the pathogen can be fermented more than five carbohydrates, but acid production but not gas.

The ONPC, arabinose, rhamnose, inositol, Dulcitol, mannitol, sorbitol, xylose biochemical test tube did not change also do not produce gas, indicating that the pathogenic bacteria cannot glycolysis above carbohydrates.

The results of the experiment are basically the same as the expected results of the experiment.



Fig. 3 Pathogen inoculation 0 h



Fig. 4 Pathogen inoculation 24h



Fig. 5 Pathogen inoculation 24h

**Drug sensitivity test results and analysis.** Fig. 6 : drug sensitive paper from the clockwise direction are CRO, AN, N, FOS, AMX, F, LVF, SMX. In the middle is: GM.

The neomycin (N), amikacin (AN), gentamicin (GM), sulfamethoxazole (SMX) of the pathogen without or inhibitory effect is weak and other antibacterial drugs ring diameter respectively is: CRO:3.040cm, F:2.460cm, FOS:2.360cm, AMX:1.610cm, LVF:1.734cm.

The pathogen of neomycin, amikacin, gentamicin, sulfamethoxazole sensitive degree is low, highly resistant, high sensitivity to ceftriaxone, fluorescein test, tobramycin, amoxicillin and levitation, resistance is weak, the clinical medication can be for reference.

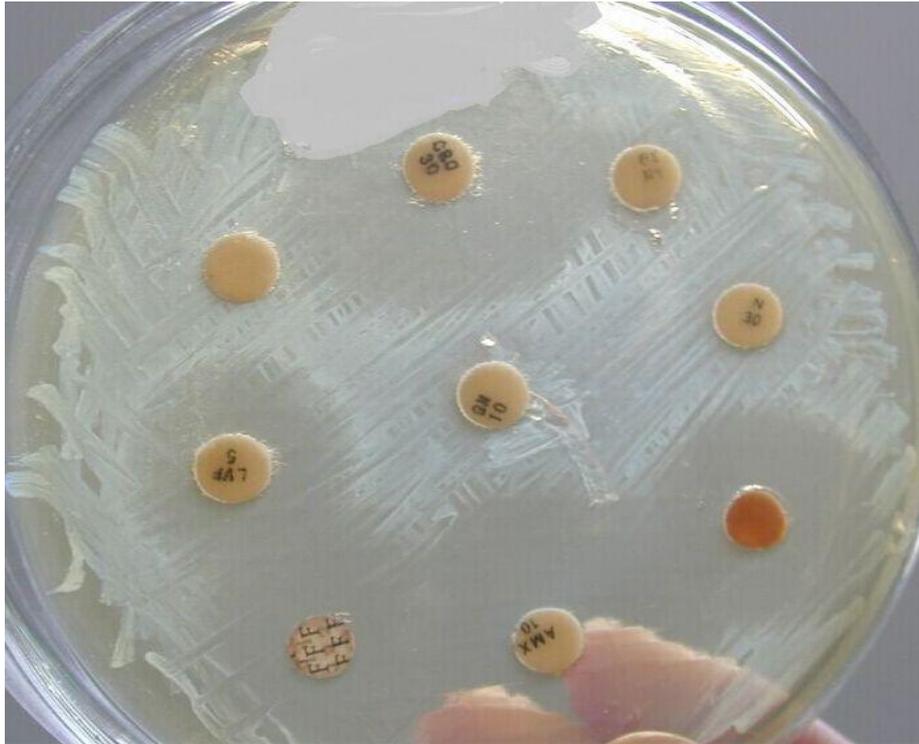


Fig. 6 Pathogenic bacteria drug sensitivity test

**PCR electrophoresis results and analysis.** PCR products are segregated on agarose gel electrophoresis. The results show that the standard of *Streptococcus suis* and isolates DYL-22 are about 700bp appear to strip, consistent with the expected fragment.

**The results and analysis of virulence experiments.** The symptoms of respiratory distress were around 36h, and the death of 72h was around. Necropsy: typical pneumonia, meningitis, myocardial hemorrhage, mesenteric hemorrhage, necrosis of spleen enlargement, renal hemorrhage and necrosis; blank control, observe two weeks Houjian live.

## Conclusions

*Streptococcus suis* usually in pig tonsils settlement can be divided into 35 serotypes. In pathogenic serovars, type 2 is the most virulent, incidence of a disease distinguished, wide range of popular, followed by type 1 and type 9.

Microscopic examination of colony morphology and hemolysis and *Streptococcus* compliance; biochemical test results, in addition to the ONPC and inulin and other reagent tube in line with expectations; by PCR detection of the pathogen indeed with *gdh* gene, *gdh* gene characteristic of *Streptococcus suis* genes. Therefore, pathogenic bacteria for *Streptococcus suis*.

The animal virus attacking test and rabbit organs show obviously *Streptococcus suis* type 2 infection symptoms, lung hemorrhage, mesenteric lymph node hemorrhage, enlargement of the spleen, brain hemorrhage and edema, cardiac and renal lesions.

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