Identification of Microorganisms of Bean Curd Sauce and Its Physiological Characteristics

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Abstract. Morphological identification of molds is usually to observe the colony morphology of molds on the medium, such as the diameter, texture, color and presence of soluble pigments and exudates: as well as their individual morphological characteristics, including the segmentation, the head of the congregation, the branch of the stalk, the top capsule, the hyphae, etc., consult the fungal identification manual, and obtain the identification results. This method is simple in operation, intuitive in results, and has high accuracy and stability. In this paper, the morphological identification of the selected strains was carried out, and after the identification was completed, the growth physiological characteristics of the strains under different temperatures and pH conditions were further studied, which was optimized for the process of making and fermentation of bean paste. Provide a theoretical basis.

1. Introduction
At present, the production mode of traditional Chinese bean paste is mainly based on workshop style. In the open environment, natural inoculation is used to make music, and various strains are mixed and fermented. There are many kinds of microorganisms involved in the process of koji and fermentation. Some of these microorganisms are Fermentation is beneficial, while others may cause certain harm to the body of the consumer, and the natural fermentation process has certain requirements for temperature. The fermentation is mainly concentrated in spring, summer and autumn, the production cycle is long, and the quality of the product is difficult to control. Therefore, the composition of microorganisms in the broad-curved broad bean koji was studied, and the strains suitable for the fermentation of the bean paste were prepared and studied, and the production process was studied to produce a safe bean paste with the unique flavor of traditional natural fermentation. Necessary.

2. Morphological characteristics
The isolated strains were inoculated in a single colony form on PDA medium, Chadian medium, C. cerevisiae medium and Bengal red medium, and each sample was made in three parallels, and cultured at 28 °C ± 1 °C for 3 Observations were made 5 days later, and colony characteristics were recorded. Pick up the hyphae cultured on the medium, make a temporary loading, and observe the division of the recorded bacteria under a microscope.

Microscopic structural features such as stalks, split arms, conids, sacs, and hyphae.

(a). Direct observation
Use a vaccination needle to pick a small amount of hyphae with a stalk, place it in a lactic acid-carbolic acid solution on a clean glass slide, carefully spread the hyphae, and gently cover the
coverslip from one side, taking care not to create bubbles. Made into a temporary loading, low on the microscope.

Microscopic examination under the microscope, if necessary, change the high magnification mirror.

(b). Slide culture

According to the slide culture method, a filter paper having a slightly smaller diameter is placed in the culture vessel, a U-shaped glass rod is placed on the filter paper, and a clean glass slide is placed on the upper surface, and the culture dish is covered and sterilized. 10mL of solid PDA medium was injected into the sterile culture vessel. After solidification, the thin layer of the medium was cut into small pieces with a scalpel and transferred to the center of the above clean glass slide. Then inoculate the stalks to the four sides of the agar medium with the inoculation needle, and cover the coverslips. To avoid drying the agar during the culture, add 2-3 mL of sterile 20% glycerol solution to the filter paper, and place the culture vessels at 28 °C. The culture was carried out in a constant temperature incubator at ±1 °C. After 2 days, a glass slide was taken every 12 h or 24 h to observe the individual morphological characteristics under a microscope.

(c). Bract method

Under sterile conditions, use a knife to open 2 small grooves of about 3 mm wide on the C. sinensis plate, inoculate a low concentration of mold suspension on one side of the trough, and incline the sterile coverslip at 30-45° angle. Insert the medium, place 4 coverslips on each plate, place them in a constant temperature incubator at 28 °C ± 1 °C, and observe the hyphae on the coverslip under the microscope under sterile conditions every 2 hours. growing situation.

3. Identification of Aspergillus spp.

Use a vaccination needle to pick a small amount of No. 1 and No. 9 fungus suspension, inoculate them on PDA, CA, CYA and Bengal red medium in the form of single colonies, and incubate them in a constant temperature incubator at 28 °C ± 1 °C. Their colony morphology on different media is shown in Figure 1.

![Figure 1. Colony morphology of strains on different media](image_url)

On the PDA medium, the colonies grew faster. After 24 hours, white fungus with a diameter of 0.2 cm to 0.3 cm was formed on the PDA. After 5 days, the colony diameter was 5.0 cm to 5.5 cm. The texture was loose. In the form of flocculent, the color of the colony changes from the initial white to yellowish green, eventually becoming greenish brown, and the colony is colorless on the back. Obvious lawns were observed after 24 hours on the Bengal red medium. The composition of the Bengal red medium had a certain inhibitory effect on the spread of the fungus. After 5 days, the colony diameter was 4.2 cm to 4.7 cm. The texture was loose and the middle protrusion The color of the colony gradually changed from the initial white to yellow, and the colony was colorless on the back. It
grew relatively slowly on CA medium. After 48 hours, obvious lawn was observed on the plate. After 5 days, the colony diameter was 2.8 cm to 3.6 cm. The texture was loose and the colony color gradually changed from the initial white to yellow. The back is colorless. On CYA medium, white fungus was observed after 24h. After 5 days, the diameter of the colony was 5.6 cm to 6.0 cm. The texture was loose and velvet. The color of the colony gradually changed from the initial white to yellowish green, and finally turned brown. The back of the colony was light yellowish brown with no exudate.

On the PDA medium, the colonies grew faster. After 5 days of culture, the colonies were 4.0 cm to 4.7 cm in diameter. The texture was loose and the central protrusions were velvety. The color was initially white or yellow, and turned yellowish brown after maturity. There is a circle of white hyphae and the back is colorless. On the Bengal red medium, the colony diameter was 3.5 cm to 4.0 cm after 5 days of growth, the colony texture was velvety, the middle part was slightly white flocculent, the stalked area was yellowish brown, surrounded by a circle of white hyphae, and there was no colony on the back. color. On the CA medium, the growth was relatively slow. After 48 hours, the obvious lawn was observed. After 5 days, the colony diameter was 2.7 cm to 3.0 cm. The texture was loose, the hyphae were yellow, and the colony was colorless. On the CYA medium, after 5 days, the colony diameter was 4.5 cm to 5.0 cm, which was velvety and centrally protruding. The color of the colony gradually changed from the initial white to the greenish brown, and the back was pale yellow.

4. Physiological study
In a 250 mL flask containing 50 mL of natural pH liquid PDA medium, 0.5 mL of the same concentration of Aspergillus sp. suspension was placed in a constant temperature water bath shaker at a temperature of 28 °C and a rotation speed of 120 rpm/min. Shake culture, every 2 hours from the 4th hour, every 4 hours after 24 hours, 2 hours after 48 hours, filter out the filter paper or centrifuge to collect the mycelium every 8 hours, and dry at 105 °C to constant weight. Calculate the dry weight and find the average. The measured dry weight of the hyphae was plotted on the ordinate and the culture time was plotted on the abscissa.

According to the growth curve, in the liquid PDA medium at 28 °C natural pH, the dry weight of mycelium in the first 16 hours of No.1 and No.9 strains remained basically unchanged. At this time, the cells just entered the medium and were in the adaptation stage. With the increase of time, the dry weight of mycelium increased rapidly from 16 hours to 40 hours, and it was in the logarithmic growth phase. After 40 hours of culture, the dry weight of mycelium tended to reach a stable period. At 48 hours, the bacteria decreased. The dry weight of silk reached a maximum value of 0.488g, and then showed a slow downward trend. When the No. 9 strain was cultured until the 44th hour, the dry weight of the hyphae reached a maximum of 0.385g, and then the same strain as the No. 1 strain. The dry weight of the silk began to decrease slowly, which was caused by the depletion of the nutrient matrix in the medium, which caused the mycelial growth rate of the strain to be lower than the autolysis rate and entered the autolysis waning period.

In a 250 mL flask containing 50 mL of natural pH liquid PDA medium, 0.5 mL of the same concentration of Aspergillus sp. suspension was placed, and the temperature was set to 25 °C according to the optimum growth temperature of Aspergillus oryzae studied by Wang Tao. The mixture was shaken and cultured in a constant temperature water bath shaker at a rotation speed of 120 rpm/min. The temperature was made two parallels. After 24 hours, the mycelium was collected by filtration or centrifugation, dried at 105 °C to constant weight, and the dry weight was calculated. To obtain the average value: prepare a PDA plate, inoculate a single colony at the center of the plate, incubate at the above temperature, and make two parallels for each temperature. After 48 hours, measure the diameter of the colony and average it to measure the dried hyphae. The weight and colony diameter are plotted on the ordinate, and the culture temperature is plotted on the abscissa.

According to the results of the study, the dry weight of mycelium of No.1 and No.9 strains increased with the increase of culture temperature before 30 °C, reached the maximum at 30 °C, and then increased with the temperature. The growth of the strain was inhibited to some extent, and the dry
weight of the mycelium began to decrease. From the experimental results, the optimum growth temperature of the strains No. 1 and No. 9 was 30 °C.

Based on 50mL liquid PDA medium, adjust the medium to a certain pH value with NaOH and HCL solution, sterilize at 121 °C for 15min, and then add 0.5mL of the same concentration of the sclerotium suspension. Parallel, placed in a constant temperature water bath shaker at a temperature of 30 ° C and a rotational speed of 120 rpm / min. After 24 hours, remove the filter paper or centrifuge to collect the mycelium, and dry at 105 ° C to constant weight to calculate the dry weight. , to find the average. Taking the measured dry weight of the hyphae as the ordinate, the initial pH is plotted on the abscissa.

It can be seen from the research results that the No.1 strain can grow well under acidic environment. With the increase of pH value, the dry weight of mycelium reaches the maximum value at pH 6.0, and then the growth amount decreases with the increase of pH. Trend, when the pH is higher than 9.0, the growth is obviously inhibited. The optimum pH range of No.1 strain is 6.0-7.0. The growth of No.9 strain in the acidic environment is not good, and it has some fluctuation with the change of pH. Sexuality, with the increase of pH value, the dry weight of mycelium reached the maximum at pH 7.0, and the growth was inhibited when the pH was higher than 9.0, but the growth condition was slightly better than that in the acidic environment. The pH range is 6.0-8.0.

The pH of the broad bean and the seed koji after cooking and sterilizing in the process of making the bean paste is about 6.5 to 7.1, which is suitable for the growth of the No. 1 and No. 9 strains in a weak acid environment of pH 6.0-7.0. In agreement, the pH environment of the broad bean paste is conducive to the growth of the koji strain, making the broad bean koji. The pH of the finished bean paste after the mixed fermentation of broad bean curd and brine

Between 5.0 and 5.7, the No. 1 strain can maintain good growth conditions in this pH range, and the growth of No. 9 strain is obviously inhibited. It is speculated that the role of No. 9 strain is mainly reflected in the system. In the koji stage, the No. 1 strain plays an important role in the process of koji and Shijin fermentation.

5. Study on Protease Characteristics of Aspergillus

In the fermentation process of bean paste, the microorganisms produce abundant enzymes acting on the raw materials, decomposing the components in the raw materials, and producing various flavor substances. Protease is a kind of enzyme with a key function, and the protease can catalyze the multi-peptide in the raw materials. Or protein hydrolysis, the production of various amino acids, improve the amino acid content. Therefore, it is necessary to study the protease-producing properties of the strains. 50 mL liquid PDA medium was placed in a 250 mL flask, 0.5 mL of the sterile inoculum suspension was placed, and cultured in a constant temperature water bath shaker at a temperature of 30 ° C and a rotation speed of 120 rpm/min, and sampled every 8 hours. After sufficient shaking, the crude enzyme solution was filtered, and the protease activity was measured for 96 hours, and each sample was made in parallel.

According to the results of the study, with the growth of strains No. 1 and No. 9, the proteases produced by them also increased rapidly, which showed a rapid increase in protease activity.

No.1 strain had no obvious protease activity when cultured for 12 hours to 36 hours: after 36h, the enzyme activity increased sharply and reached a maximum at 72 hours. At this time, the growth of No. 1 strain had entered a stable period: 72 after a few hours, the protease activity was maintained at a relatively high level. Compared with the previous growth curve, it was found that the production of protease had a certain retardation with respect to growth.

No. 9 strain had no obvious protease activity when cultured for 12-24h: after 24 hours, the enzyme activity increased sharply and reached the maximum at 48 hours. At this time, the growth of the strain had entered the stable phase: 48 to 60 hours protease the viability remained at a relatively high level, and the protease activity began to decline after 60 hours, so the growth trend of the strains was consistent.
6. Conclusion
In this paper, by observing the morphological and microscopic characteristics of colonies, the traditional strains were used to identify the two strains of the strains. The results showed that the strain No. 1 was Aspergillus oryzae and the No. 9 strain was soy sauce. Aspergillus. The growth characteristics of two strains of Aspergillus strains were studied, and the optimal growth temperature of the two strains was determined to be 30 °C. The optimum pH range of strain No. 1 was 6.0-7.0. The pH range is 6.0-8.0; the optimum carbon source for the No. 1 strain is sucrose, and the No. 9 strain is not sensitive to the carbon source, and the growth of the different carbon sources is basically the same: the No. 1 and No. 9 strains are extracted with yeast. The growth of the medium in which the powder and protein porpoise are nitrogen sources is much better than other nitrogen sources. The protease-producing properties of two strains of koji strains were studied. Under the conditions of shaking culture, the protease production time of strain No. 1 was concentrated at 48 to 72 hours, and the optimum enzyme temperature was 30 °C. The time is concentrated between 24 and 48 hours, and the optimum enzyme temperature is 27 °C.

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