

LACTIC ACID BACTERIA CULTURE MEDIUM HAVING SPECIFICITY, CULTURE METHOD THEREFOR AND USE THEREOF

TECHNICAL FIELD

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The present disclosure relates to the field of biotechnologies, and particularly relates to a specific culture medium for lactobacillus, a culture method using the specific culture medium for lactobacillus, and application of the specific culture medium for lactobacillus.

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BACKGROUND

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Researches found that there is no suitable culture methods for 90% or above of microorganisms in nature. Only a small part of them can be cultured through traditional bacterial culture methods. The microorganisms cultured through the traditional methods are known microorganisms. There are few researches and reports on culture medium components, and isolation and culture methods for new microbial species. Microbial culture difficulty is related to special reasons for resisting culture, which include temperature, pondus hydrogenii (pH), oxygen, nutrition and plenty of other factors. Culture of new microbial species is confronted with numerous technical challenges.

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Lactobacillus refers to a type of bacteria that can ferment carbohydrates to produce large amounts of lactic acid. About 200 species of lactobacillus are extensively distributed in nature, so species diversity is rich. The lactobacillus has multiple physiological functions of regulating bacterial colonies of gastrointestinal tracts, improving gastrointestinal functions, improving food digestibility and bioavailability, reducing serum cholesterol, controlling endotoxin, inhibiting growth of putrefying bacteria in intestinal tracts, improving body immunity, and so on.

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Traditionally, vinegar is brewed through open fermentation without sterilization of raw materials and auxiliary materials. There are extremely rich microbial species in a brewing process. Xu Zhenghong and other scholars analyzed microbial community structures in a solid-state vinegar culture of Zhenjiang aromatic vinegar and found that hundreds of microorganisms are involved in fermentation in this process. The lactobacillus is commonly

found in a vinegar fermentation process. Especially in a traditional solid-state vinegar brewing process, a proportion of the lactobacillus can reach 60% or above, which has a vital impact on product quality. It is significant for the vinegar brewing industry to research culture media, and isolation and culture methods for different types of lactobacillus and their application. Thus, it is of huge significance to innovate culture medium formulas and culture methods to culture the lactobacillus better.

At present, plenty of different species of lactobacillus have unique requirements for culture media and culture methods. Even different bacterial strains of the same species of lactobacillus have large different requirements for culture media and culture methods. For example, patent CN 202010877453.8 discloses an isolation medium formula for lactobacillus and a culture method. Although the cultured target lactobacillus has 100% consistency with a 16S ribosomal ribonucleic acid (rRNA) sequence of new lactobacillus HSLZ-75^T, the target lactobacillus cannot grow on a culture medium formula for lactobacillus HSLZ-75^T disclosed in paper (*Lactobacillus jinshani* sp. nov., isolated from solid-state vinegar culture of Zhenjiang aromatic vinegar. Antonie van Leeuwenhoek). Moreover, the target lactobacillus in patent CN 202010877453.8 is required to be cultured on a solid plate for 7 days-14 days, such that culture time is long. The culture medium formula is extremely complex, such that an operation is inconvenient and cost is high. More than 30 types of substances are required to be added in the liquid culture medium, and there are 9 types of vitamins. A large amount of solid-state vinegar culture leaching solution is contained, which is likely to lead to poor stability of the culture medium. Moreover, the solid-state vinegar culture leaching solution is difficult to obtain by ordinary personnel, such that overall applicability is not strong. Patent CN 202011227512.3 discloses a culture medium formula for lactobacillus Z-1 and an isolation method. Culture time is long, and it takes 7 days to form visible bacterial colonies on a solid plate. Culture medium components are complex, and nearly 20 types of substances are required to be added in the liquid culture medium. An operation is complex, and a solution containing a large amount of compound vitamin B is required to be prepared separately. Membrane filtration for sterilization is required before use. A pH value is required to be adjusted by lactic acid and acetic acid. A bran leaching solution is required to be self-prepared, such that stability of the culture medium is poor.

SUMMARY

An objective of the present disclosure is to provide a new specific culture medium for lactobacillus, so as to solve the problems in the prior art. The culture medium in the present disclosure is mainly aimed at new lactobacillus, is a specific, efficient, stable and convenient new culture medium formula for lactobacillus, and can rapidly and efficiently culture the lactobacillus.

The present disclosure further provides a culture method using the specific culture medium for lactobacillus and application of the specific culture medium for lactobacillus.

A technical solution is as follows: in order to realize the above objective, the specific liquid culture medium for lactobacillus in the present disclosure includes raw materials as follows: 8 g -12 g of tryptone, 10 g-15 g of a beef extract, 8 g-12 g of a yeast extract, 30 g-60 g of a malt extract, 10 g-15 g of glucose, 1.5 g-2.5 g of diammonium hydrogen citrate, 1.5 g-2.5 g of potassium phosphate dibasic, 0.1 g-0.2 g of manganese sulfate monohydrate, 0.5 g-0.7 g of magnesium sulfate heptahydrate, 1 mL-2 mL of tween 80, 1 g-2 g of L-cysteine hydrochloride, and 1 mg-3 mg of vitamin B₂ that are added into each 1 L of tap water, and acetic acid that is finally added for adjustment to achieve 4 g/100 mL-6 g/100 mL.

The lactobacillus is lactobacillus *Acetilactobacillus jinshanensis* HSLZ-75^T and *Lactobacillus sp.* HSCY 2073.

A specific solid culture medium for lactobacillus described in the present disclosure includes raw materials as follows: 8 g -12 g of tryptone, 10 g-15 g of a beef extract, 8 g-12 g of a yeast extract, 30 g-60 g of a malt extract, 10 g-15 g of glucose, 1.5 g-2.5 g of diammonium hydrogen citrate, 1.5 g-2.5 g of potassium phosphate dibasic, 0.1 g-0.2 g of manganese sulfate monohydrate, 0.5 g-0.7 g of magnesium sulfate heptahydrate, 1 mL-2 mL of tween 80, 1 g-2 g of L-cysteine hydrochloride, and 1 mg-3 mg of vitamin B₂ that are added into each 1 L of tap water, 20 g-25 g of agar powder that is added, and acetic acid that is finally added for adjustment to achieve 4 g/100 mL-6 g/100 mL.

The lactobacillus is lactobacillus *Acetilactobacillus jinshanensis* HSLZ-75^T and *Lactobacillus sp.* HSCY 2073.

A culture method using the specific liquid culture medium for lactobacillus in the present disclosure includes steps as follows:

inoculating a bacterial strain in the culture medium, where a height of residual air above a liquid level of the culture medium is less than 0.5 cm, a liquid filling amount of the culture medium is greater than 90%, an airtight effect is achieved, and a culture temperature is 30°C-35°C.

A culture method using the specific solid culture medium for lactobacillus in the present disclosure includes steps as follows:

inoculating a bacterial strain in the culture medium, and carrying out culture in an anaerobic culture box, where an oxygen content in the anaerobic culture box is less than 10 ppm, culture humidity is 75%-85% and a culture temperature is 30°C-35°C.

Application of the specific liquid culture medium for lactobacillus or the solid culture medium for lactobacillus described in the present disclosure in rapid mass culture of lactobacillus is as follows:

Lactobacillus Acetilactobacillus jinshanensis HSLZ-75^T in the present disclosure is a commercial bacterial strain (CICC 6269), and is a new species (*Lactobacillus jinshani* sp. nov., isolated from solid-state vinegar culture of Zhenjiang aromatic vinegar. Antonie van Leeuwenhoek) of lactobacillus.

Lactobacillus Lactobacillus sp. HSCY 2073 is deposited in China General Microbiological Culture Collection Center. deposit place is Institute of Microbiology, Chinese Academy of Sciences, No.3, Yard 1, Beichen West Road, Chaoyang District, Beijing, China. Accession number of deposit is CGMCC No. 18982. Deposit date is 21 November 2019. The above lactobacillus is lactobacillus that has strong acetic acid and ethanol tolerance and excellent effect of producing non-volatile acid, and can produce high contents of 5 aroma substances including acetaldehyde, ethyl acetate, isobutyl acetate, isoamyl acetate and ethyl 3-methylvalerate that have fruity aromas. The lactobacillus can adapt to high-acid, high-alcohol and oligotrophic harsh environments, etc. of vinegar brewing, and has strong ability of high acid tolerance. That is, the lactobacillus can produce non-volatile acid to improve softness of taste of vinegar, can increase aroma substances such as aldehydes and esters that have excellent aromas, and finally can greatly improve overall flavor quality of vinegar

products. The lactobacillus can tolerate total acid of 8.0 g/100 mL to a maximum extent, and can tolerate ethanol of 13%. The lactobacillus can be metabolized at 15°C, and an optimum growth temperature is 30°C. Bacterial strain HSCY 2073 is obvious in industrial application effect in vinegar brewing, convenient in operation and low in cost, and can obviously increase contents of non-volatile acid and aroma substances in vinegar. By using the bacterial strain in Zhenjiang aromatic vinegar brewing, in a solid-state vinegar culture, a content of total acid can be increased by 4.71%, a content of non-volatile acid can be increased by 24.86%, and a product yield can be increased by 5.14%. In cases of the 5 flavor substances having fruity aromas, a content of acetaldehyde can be increased by 9.05%, a content of ethyl acetate can be increased by 25.83%, a content of isobutyl acetate can be increased by 1.51%, a content of isoamyl acetate can be increased by 7.39%, and a content of ethyl 3-methylvalerate can be increased by 34.27%. Taste and aromas of products are significantly improved, and overall flavor quality is high. Moreover, flavor quality of Shanxi aged vinegar can also be effectively enhanced by using the bacterial strain in Shanxi aged vinegar brewing. A 16S rRNA gene sequence is shown as SEQ ID NO.1.

At present, there is no culture medium formula and culture method that are strong in specificity, high in culture efficiency, stable in culture effect, simple in component, convenient in use and low in cost for *Acetilactobacillus jinshanensis* HSLZ-75^T and *Lactobacillus* sp. HSCY 2073. *Acetilactobacillus jinshanensis* HSLZ-75^T is a special and newly discovered new lactobacillus species. It is an urgent technical problem to be solved in the field to develop a specific, efficient, stable and convenient culture medium formula and culture method that can be commercially applied. At present, a de man, rogosa and sharpe (MRS) culture medium or conventionally improved MRS culture medium is a main culture medium used to isolate and culture lactobacillus, but generally cannot be used to isolate and culture *Acetilactobacillus jinshanensis* HSLZ-75^T and *Lactobacillus* sp. HSCY 2073, which is also a reason why the lactobacillus was not discovered as a new species. Moreover, a current MRS or improved MRS culture medium can be used to isolate conventional lactobacillus, but a culture effect and selectivity are poor. An isolation effect is likely to be affected by other lactobacillus, bacillus, saccharomycetes and other microorganisms.

The specific culture medium in the present disclosure can be used to rapidly and efficiently culture specific bacterial strains *Acetilactobacillus jinshanensis* HSLZ-75^T and *Lactobacillus* sp. HSCY 2073, and has beneficial effects of specificity, high efficiency,

stability and convenience.

In the present disclosure, due to specific bacterial strains *Acetilactobacillus jinshanensis* HSLZ-75^T and *Lactobacillus sp.* HSCY 2073 require specific culture media, it is found that conventional lactobacillus do not grow on the specific culture medium set in the present disclosure. Addition of vitamin B₂ and an addition amount thereof are extremely important. In addition, compared with the improved MRS culture medium, trace vitamins are specifically added in the present disclosure, such that a usage amount of a malt extract is effectively reduced. Moreover, acidity is adjusted, such that a culture effect is obviously better. The culture medium in the present disclosure is mainly aimed at new lactobacillus, is a specific, efficient, stable and convenient new culture medium formula for lactobacillus, and can rapidly and efficiently culture the lactobacillus.

Beneficial effects are as follows: compared with the prior art, the present disclosure has advantages as follows:

(1) The culture medium formula and culture method in the present disclosure can better satisfy isolation and culture requirements of *Acetilactobacillus jinshanensis* HSLZ-75^T and *Lactobacillus sp.* HSCY 2073.

(2) The culture medium formula and culture method in the present disclosure are strong in specificity, high in culture efficiency, stable in culture effect, simple in component, convenient in use, low in cost and easy in commercialization for culture of *Acetilactobacillus jinshanensis* HSLZ-75^T and *Lactobacillus sp.* HSCY 2073.

(3) According to the present disclosure, it is proved through comprehensive comparison experiments of multiple dimensions that the culture medium formula and culture method in the present disclosure have excellent specificity, high efficiency and stability.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a photograph of a shape of bacterial strain HSCY 2073 according to the present disclosure.

FIG. 2 shows a bacterial colony shape of HSLZ-75^T on a solid plate of a culture medium in the present disclosure.

DETAILED DESCRIPTION OF THE EMBODIMENTS

5 The present disclosure will be further described below in combination with accompanying drawings and examples.

Raw materials and reagents in the present disclosure are commercially available unless otherwise specified.

10 Model bacteria and conventional lactobacillus in the present disclosure are purchased from corresponding bacterial strain libraries or commercially available.

Improved de man, rogosa and sharpe (MRS) culture medium: 10 g of tryptone, 10 g of a beef extract, 5 g of a yeast extract, 150 g of a malt extract, 20 g of glucose, 2 g of diammonium
15 hydrogen citrate, 5 g of sodium acetate, 2 g of potassium phosphate dibasic, 0.19 g of manganese sulfate monohydrate, 0.58 g of magnesium sulfate heptahydrate, 1.0 mL of tween 80, 1 g of L-cysteine, 20 g of agar and 1 L of distilled water are provided, a temperature is 115°C, sterilization is carried out for 25 min, and acetic acid having a final concentration of 2%(v/v) is added.

20 MRS culture medium formula 1: 10 g of casein peptone, 10 g of a beef extract, 5 g of a yeast extract, 5 g of glucose, 5 g of sodium acetate, 2 g of diammonium citrate, 2 g of potassium phosphate dibasic, 0.2 g of magnesium sulfate heptahydrate, 0.05 g of manganese sulfate monohydrate, 1 g of tween 80, 15 g of agar and 1 L of distilled water are provided, a
25 pondus hydrogenii (pH) value is 6.8, a temperature is 115°C, and sterilization is carried out for 25 min.

MRS culture medium formula 2: 10 g of casein peptone, 8 g of beef powder, 4 g of a yeast extract, 20 g of glucose, 0.2 g of magnesium sulfate, 5 g of sodium acetate, 2 g of
30 triammonium citrate, 2 g of potassium phosphate dibasic, 0.05 g of manganese sulfate, 1 g of tween 80, 20 g of agar and 1000 mL of distilled water are provided, a pH value is 5.0, a temperature is 115°C, sterilization is carried out for 25 min.

MRS culture medium formula 3: 400 mL of tomato juice, 10 g of glucose, 5 ml of salt

solution A, 5 ml of salt solution B, 10 g of a yeast extract, 20 g of agar and 600 mL of distilled water are provided, a pH value is 6.9, a temperature is 115°C, and sterilization is carried out for 25 min. Salt solution A formula: 2.5 g of potassium phosphate dibasic, 2.5 g of potassium dihydrogen phosphate and 250 mL of distilled water are provided. Salt solution B formula: 10 g of magnesium sulfate heptahydrate, 5 g of ferric sulfate, 5 g of sodium chloride, 5 g of manganese sulfate tetrahydrate and 250 mL of distilled water are provided.

Luria-bertani (LB) culture medium formula: 10 g of tryptone, 5 g of a yeast extract, 10 g of sodium chloride, 15 g of agar and 1 L of water are provided, a temperature is 115°C, and sterilization is carried out for 25 min.

Beef extract-agar culture medium formula: 3 g of a beef extract, 10 g of peptone, 5 g of sodium chloride, 15 g of agar and 1 L of water are provided, a temperature is 115°C, and sterilization is carried out for 25 min.

Culture medium formula 100239: 10 g of tryptone, 10 g of a beef extract, 5 g of a yeast extract, 20 g of glucose, 1 g of tween 80, 2 g of potassium phosphate dibasic, 5 g of sodium acetate, 2 g of triammonium citrate, 0.2 g of magnesium sulfate heptahydrate, 0.05 g of manganese sulfate monohydrate, 15 g of agar and 1 L of distilled water are provided, a pH value is 6.2-6.5, a temperature is 115°C, and sterilization is carried out for 20 min.

Culture medium formula 20203: 10 g of tryptone, 10 g of a beef extract, 5 g of a yeast extract, 20 g of glucose, 1 g of tween 80, 2 g of potassium phosphate dibasic, 5 g of sodium acetate, 2 g of triammonium citrate, 0.2 g of magnesium sulfate heptahydrate, 0.05 g of manganese sulfate monohydrate, 15 g of agar and 1 L of distilled water are provided, a pH value is 6.2-6.5, a temperature is 115°C, and sterilization is carried out for 20 min.

Culture medium formula 15814: 10 g of tryptone, 10 g of a beef extract, 15 g of a yeast extract, 20 g of glucose, 10 g of maltose, 1 g of tween 80, 2 g of potassium phosphate dibasic, 5 g of sodium acetate, 2 g of triammonium citrate, 0.2 g of magnesium sulfate heptahydrate, 0.05 g of manganese sulfate monohydrate, 15 g of agar and 1 L of distilled water are provided, a pH value is 5.6, a temperature is 115°C, and sterilization is carried out for 20 min.

Culture medium formula 20331: 10 g of tryptone, 10 g of a beef extract, 5 g of a yeast

extract, 20 g of glucose, 1 g of tween 80, 2 g of potassium phosphate dibasic, 5 g of sodium acetate, 2 g of triammonium citrate, 0.2 g of magnesium sulfate heptahydrate, 0.05 g of manganese sulfate monohydrate, 0.5 g of cysteine hydrochloride, 15 g of agar and 1 L of distilled water are provided, a pH value is 5.2, a temperature is 115°C, and sterilization is carried out for 20 min.

All the above culture media without agar are corresponding liquid culture media.

1. Activated culture of bacterial strains

(1) Activated culture of bacterial strains *Acetilactobacillus jinshanensis* HSLZ-75^T and *Lactobacillus* sp. HSCY 2073.

HSLZ-75^T and HSCY 2073 that grew to logarithmic phases were inoculated into a sealable container containing the liquid culture medium in the present disclosure according to an inoculation amount of 10%(v/v). The liquid culture medium formula was as follows: 10 g of tryptone, 12 g of a beef extract, 10 g of a yeast extract, 50 g of a malt extract, 13 g of glucose, 2 g of diammonium hydrogen citrate, 2 g of potassium phosphate dibasic, 0.15 g of manganese sulfate monohydrate, 0.5 g of magnesium sulfate heptahydrate, 1.5 mL of tween 80, 1.5 g of L-cysteine hydrochloride, 2 mg of vitamin B₂ and 1 L of tap water were provided, a temperature was 115°C, sterilization was carried out for 25 min, and acetic acid was added for adjustment to achieve total acid of 5 g/100 mL.

A height of residual air above a liquid level of the culture medium in the sealable container was about 0.3 cm, a liquid filling amount of the culture medium was about 95%, an airtight effect was achieved, and culture was carried out at 30°C for 5 days.

The above methods were repeated. The bacterial strains were activated for 3 times. Last culture time was 5 days for later use. Bacterial strain shape of HSCY 2073 was as shown in FIG. 1.

(2) Activated culture of 4 model strains of which 16S rRNA is similar to that of HSLZ-75^T

Corresponding liquid culture media without agar were selected to carry out activation 3 times for later use.

Lactobacillus caviae DSM 100239^T was cultured by using a culture medium formula of culture medium 100239 at 37°C in an ordinary culture box for 2 days.

Lactobacillus fructivorans DSM 20203^T was cultured by using a culture medium formula of culture medium 20203 at 30°C in an ordinary culture box for 2 days.

Lactobacillus rossiae DSM 15814^T was cultured by using a culture medium formula of culture medium 15814 at 30°C in an ordinary culture box for 2 days.

Pediococcus damnosus DSM 20331^T was cultured by using a culture medium formula of culture medium 20331 at 26°C in an ordinary culture box for 2 days.

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(3) Activated culture of 4 strains of conventional lactobacillus

Corresponding liquid culture media without agar were selected to carry out activation 3 times for later use.

10 *Lactobacillus helveticus* CICC 20243 and *Lactobacillus acidipiscis* CICC 10822 were cultured by using MRS culture medium formula 1 at 37°C in an ordinary culture box for 1 day.

Lactobacillus acetotolerans CICC10774 was cultured by using MRS culture medium formula 2 at 37°C in an ordinary culture box for 1 day.

15 *Lactobacillus fermentum* CICC 20176 was cultured by using MRS culture medium formula 3 at 37°C in an ordinary culture box for 1 day.

2. Coating of bacterial strains

20 A culture solution of each bacterial strain activated 3 times in the above steps was diluted in a gradient manner with a sterile physiological saline solution. 0.1 mL of diluted solution having a viable bacteria count of 1000 CFU/mL-2000 CFU/mL was selected to coat a corresponding solid plate in Example 1 below.

Example 1

25 Solid culture medium formula: 10 g of tryptone, 12 g of a beef extract, 10 g of a yeast extract, 50 g of a malt extract, 13 g of glucose, 2 g of diammonium hydrogen citrate, 2 g of potassium phosphate dibasic, 0.15 g of manganese sulfate monohydrate, 0.5 g of magnesium sulfate heptahydrate, 1.5 mL of tween 80, 1.5 g of L-cysteine hydrochloride, 2 mg of vitamin B₂, 22 g of agar powder and 1 L of tap water were provided, a temperature was 115°C, sterilization was carried out for 25 min, cooling was carried out to 50°C-55°C, and acetic acid
30 was added for adjustment to achieve total acid of 5 g/100 mL.

Specifically, 15 mL of the above culture medium was poured into a culture dish having a diameter of 9 cm. Coating was carried out with 0.1 mL of the above corresponding diluted bacterial strain solution (1000 CFU/mL-2000 CFU/mL) after cooling. Culture was carried out

in an anaerobic culture box, where an oxygen content was about 8 ppm, culture humidity was 75%, a culture temperature was 33°C, culture time was 15 days. Growth situations of bacterial colonies on a plate were observed every day.

A group in which coating was carried out with HSLZ-75^T was Example 1-1, and a bacterial colony shape was as shown in FIG. 2. A group in which coating was carried out with HSCY 2073 was Example 1-2. A group in which coating was carried out with DSM 100239^T was Example 1-3. A group in which coating was carried out with DSM 20203^T was Example 1-4. A group in which coating was carried out with DSM 15814^T was Example 1-5. A group in which coating was carried out with DSM 20331^T was Example 1-6. A group in which coating was carried out with CICC 20243 was Example 1-7. A group in which coating was carried out with CICC 10822 was Example 1-8. A group in which coating was carried out with CICC10774 was Example 1-9. A group in which coating was carried out with CICC 20176 was Example 1-10.

Example 2

Culture medium formula: 8 g of tryptone, 10 g of a beef extract, 8 g of a yeast extract, 60 g of a malt extract, 10 g of glucose, 1.5 g of diammonium hydrogen citrate, 2.5 g of potassium phosphate dibasic, 0.1 g of manganese sulfate monohydrate, 0.5 g of magnesium sulfate heptahydrate, 1 mL of tween 80, 1 g of L-cysteine hydrochloride, 1 mg of vitamin B₂, 20 g of agar powder and 1 L of tap water were provided, a temperature was 115°C, sterilization was carried out for 25 min, cooling was carried out to 50°C-55°C, and acetic acid was added for adjustment to achieve total acid of 4 g/100 mL.

Specifically, 15 mL of the above culture medium was poured into a culture dish having a diameter of 9 cm. Coating was carried out with 0.1 mL of the above corresponding diluted bacterial strain solution after cooling. Culture was carried out in an anaerobic culture box, where an oxygen content was about 8 ppm, culture humidity was 85%, a culture temperature was 30°C, culture time was 15 days. Growth situations of bacterial colonies on a plate were observed every day.

A group in which coating was carried out with HSLZ-75^T was Example 2-1. A group in which coating was carried out with HSCY 2073 was Example 2-2. A group in which coating was carried out with DSM 100239^T was Example 2-3. A group in which coating was carried

out with DSM 20203^T was Example 2-4. A group in which coating was carried out with DSM 15814^T was Example 2-5. A group in which coating was carried out with DSM 20331^T was Example 2-6. A group in which coating was carried out with CICC 20243 was Example 2-7. A group in which coating was carried out with CICC 10822 was Example 2-8. A group in which coating was carried out with CICC10774 was Example 2-9. A group in which coating was carried out with CICC 20176 was Example 2-10.

Example 3

Culture medium formula: 12 g of tryptone, 15 g of a beef extract, 12 g of a yeast extract, 30 g of a malt extract, 15 g of glucose, 2.5 g of diammonium hydrogen citrate, 1.5 g of potassium phosphate dibasic, 0.2 g of manganese sulfate monohydrate, 0.7 g of magnesium sulfate heptahydrate, 2 mL of tween 80, 2 g of L-cysteine hydrochloride, 3 mg of vitamin B₂, 25 g of agar powder and 1 L of tap water were provided, a temperature was 115°C, sterilization was carried out for 25 min, cooling was carried out to 50°C-55°C, and acetic acid was added for adjustment to achieve total acid of 6 g/100 mL.

Specifically, 15 mL of the above culture medium was poured into a culture dish having a diameter of 9 cm. Coating was carried out with 0.1 mL of the above corresponding diluted bacterial strain solution after cooling. Culture was carried out in an anaerobic culture box, where an oxygen content was about 8 ppm, culture humidity was 80%, a culture temperature was 35°C, culture time was 15 days. Growth situations of bacterial colonies on a plate were observed every day.

A group in which coating was carried out with HSLZ-75^T was Example 3-1. A group in which coating was carried out with HSCY 2073 was Example 3-2. A group in which coating was carried out with DSM 100239^T was Example 3-3. A group in which coating was carried out with DSM 20203^T was Example 3-4. A group in which coating was carried out with DSM 15814^T was Example 3-5. A group in which coating was carried out with DSM 20331^T was Example 3-6. A group in which coating was carried out with CICC 20243 was Example 3-7. A group in which coating was carried out with CICC 10822 was Example 3-8. A group in which coating was carried out with CICC10774 was Example 3-9. A group in which coating was carried out with CICC 20176 was Example 3-10.

Comparative Example 1

The following solid plates of culture media were coated with HSLZ-75^T. The improved MRS culture medium was Comparative Example 4-1. MRS culture medium formula 1 was Comparative Example 4-2. MRS culture medium formula 2 was Comparative Example 4-3. MRS culture medium formula 3 was Comparative Example 4-4. The LB culture medium formula was Comparative Example 4-5. The beef extract-agar culture medium formula was Comparative Example 4-6. Culture medium 100239 was Comparative Example 4-7. Culture medium 20203 was Comparative Example 4-8. Culture medium 15814 was Comparative Example 4-9. Culture medium 20331 was Comparative Example 4-10.

The following solid plates of culture media were coated with HSCY 2073. The improved MRS culture medium was Comparative Example 5-1. MRS culture medium formula 1 was Comparative Example 5-2. MRS culture medium formula 2 was Comparative Example 5-3. MRS culture medium formula 3 was Comparative Example 5-4. The LB culture medium formula was Comparative Example 5-5. The beef extract-agar culture medium formula was Comparative Example 5-6. Culture medium 100239 was Comparative Example 5-7. Culture medium 20203 was Comparative Example 5-8. Culture medium 15814 was Comparative Example 5-9. Culture medium 20331 was Comparative Example 5-10.

Specifically, 15 mL of the above culture medium was poured into a culture dish having a diameter of 9 cm. Coating was carried out with 0.1 mL of the above corresponding diluted bacterial strain solution after cooling. Culture was carried out in an anaerobic culture box, where an oxygen content was about 8 ppm, culture humidity was 75%, a culture temperature was 33°C, culture time was 15 days. Growth situations of bacterial colonies on a plate were observed every day.

Comparative Example 2

The culture medium formula in the present disclosure in Example 1 was selected and placed in an ordinary (non-anaerobic) culture box in culture, culture was carried out at 33°C, culture time was 15 days, and growth situations of bacterial colonies on a plate were observed every day. A group in which coating was carried out with HSLZ-75^T was Example 6-1. A group in which coating was carried out with HSCY 2073 was Example 6-2.

The culture medium formula in the present disclosure in Example 2 was selected and

placed in an ordinary culture box in culture, culture was carried out at 33°C, culture time was 15 days, and growth situations of bacterial colonies on a plate were observed every day. A group in which coating was carried out with HSLZ-75^T was Example 6-3. A group in which coating was carried out with HSCY 2073 was Example 6-4.

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The culture medium formula in the present disclosure in Example 3 was selected and placed in an ordinary culture box in culture, culture was carried out at 33°C, culture time was 15 days, and growth situations of bacterial colonies on a plate were observed every day. A group in which coating was carried out with HSLZ-75^T was Example 6-5. A group in which coating was carried out with HSCY 2073 was Example 6-6.

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A coating method was the same as that in Comparative Example 1.

Comparative Example 3

The culture medium formula in the present disclosure in Example 1 was selected, an oxygen content in a culture box in culture was about 20 ppm, culture was carried out at 33°C, culture time was 15 days, and growth situations of bacterial colonies on a plate were observed every day. A group in which coating was carried out with HSLZ-75^T was Example 7-1. A group in which coating was carried out with HSCY 2073 was Example 7-2.

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The culture medium formula in the present disclosure in Example 2 was selected, an oxygen content in a culture box in culture was about 20 ppm, culture was carried out at 30°C, culture time was 15 days, and growth situations of bacterial colonies on a plate were observed every day. A group in which coating was carried out with HSLZ-75^T was Example 7-3. A group in which coating was carried out with HSCY 2073 was Example 7-4.

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The culture medium formula in the present disclosure in Example 3 was selected, an oxygen content in a culture box in culture was about 20 ppm, culture was carried out at 35°C, culture time was 15 days, and growth situations of bacterial colonies on a plate were observed every day. A group in which coating was carried out with HSLZ-75^T was Example 7-5. A group in which coating was carried out with HSCY 2073 was Example 7-6.

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A coating method was the same as that in Comparative Example 1.

Comparative Example 4

The above improved MRS culture medium, which is derived from a culture medium and culture method for a lactobacillus bacterial strain in reference document, was selected. The reference document is as follows: YuYJ ,LiX ,Zhang JH ,Chai LJ ,Lu ZM ,Xu ZH(2019)*Lactobacillus jinshani* sp. nov.,isolated from solid-state vinegar culture of Zhenjiang aromatic vinegar .113(1):43-54. According to conditions in the above document, culture time was 15 days, and growth situations of bacterial colonies on a plate was observed every day. A group in which coating was carried out with HSLZ-75^T was Example 8-1. A group in which coating was carried out with HSCY 2073 was Example 8-2.

A coating method was the same as that in Comparative Example 1.

Comparative Example 5

Solid culture medium formula: 10 g of tryptone, 12 g of a beef extract, 10 g of a yeast extract, 50 g of a malt extract, 13 g of glucose, 2 g of diammonium hydrogen citrate, 2 g of potassium phosphate dibasic, 0.15 g of manganese sulfate monohydrate, 0.5 g of magnesium sulfate heptahydrate, 1.5 mL of tween 80, 1.5 g of L-cysteine hydrochloride, 22 g of agar powder and 1 L of tap water were provided, a temperature was 115°C, sterilization was carried out for 25 min, cooling was carried out to 50°C-55°C, and acetic acid was added for adjustment to achieve total acid of 5 g/100 mL.

A group in which coating was carried out with HSLZ-75^T was Example 9-1. A group in which coating was carried out with HSCY 2073 was Example 9-2.

A coating method was the same as that in Comparative Example 1.

Example 4

The culture medium formula in Example 1 was selected to be prepared in to a liquid culture medium without agar. Bacterial strain HSLZ-75^T cultured to a logarithmic phase was inoculated into a sealable container containing the liquid culture medium according to an inoculation amount of 10%(v/v). A height of residual air above a liquid level of a culture medium in the sealable container was 0.3 cm, a liquid filling amount of the culture medium was 95%, an airtight effect was achieved, and culture was carried out at 33°C for 3 days, which was Example 10-1. A height of residual air above a liquid level of a culture medium

was 1.0 cm, a liquid filling amount of the culture medium was 80%, an airtight effect was achieved, and a culture temperature was 33°C for 3 days, which was Example 10-2.

The culture medium formula in Example 1 was selected to be prepared into a liquid culture medium without agar. Bacterial strain HSCY 2073 cultured to a logarithmic phase was inoculated into a sealable container containing the liquid culture medium according to an inoculation amount of 10%(v/v). A height of residual air above a liquid level of a culture medium in the sealable container was 0.3 cm, a liquid filling amount of the culture medium was 95%, an airtight effect was achieved, and culture was carried out at 33°C for 3 days, which was Example 10-3. A height of residual air above a liquid level of a culture medium was 1.0 cm, a liquid filling amount of the culture medium was 80%, an airtight effect was achieved, and a culture temperature was 33°C for 3 days, which was Example 10-4.

Shaking up was carried out after culture, a gradient dilution method was used, culture was carried out by using a culture medium and culture method in Example 1-1 after gradient dilution, and counting was carried out after gradient dilution.

Example 5

In Examples 5, the culture medium formula in Example 2 was selected to be prepared into a liquid culture medium without agar. Bacterial strain HSLZ-75^T or HSCY 2073 cultured to a logarithmic phase was inoculated into a sealable container containing the liquid culture medium according to an inoculation amount of 10%(v/v). A height of residual air above a liquid level of the culture medium in the sealable container was 0.3 cm, a liquid filling amount of the culture medium was 95%, an airtight effect was achieved, and culture was carried out at 30°C for 3 days.

Example 6

In Examples 6, the culture medium formula in Example 3 was selected to be prepared into a liquid culture medium without agar. Bacterial strain HSLZ-75^T or HSCY 2073 cultured to a logarithmic phase was inoculated into a sealable container containing the liquid culture medium according to an inoculation amount of 10%(v/v). A height of residual air above a liquid level of the culture medium in the sealable container was 0.3cm, a liquid filling amount of the culture medium was 95%, an airtight effect was achieved, and culture was carried out at 35°C for 3 days.

Effects of the above culture medium formulas and culture methods in the present disclosure were compared. Results were as shown in Tables 1 and 2.

5 Table 1 Comparison table of culture effects of solid plates

Serial number	Time for bacterial colonies to grow to be visible	Is there any bacterial colony grows at a 15th day	A total number of bacterial colonies on a plate
Example 1-1	3rd day	Yes	138
Example 1-2	3rd day	Yes	107
Example 1-3 to Example 1-10	/	No	0
Example 2-1	4th day	Yes	134
Example 2-2	4th day	Yes	104
Example 2-3 to Example 2-10	/	No	0
Example 3-1	4th day	Yes	129
Example 3-2	4th day	Yes	104
Example 3-3 to Example 3-10	/	No	0
Comparative Example 4-1	7th day	Yes	93
Comparative Example 4-2- Comparative Example 4-10	/	No	0
Comparative Example 5-1	7th day	Yes	79
Comparative Example 5-2- Comparative Example 5-10	/	No	0
Comparative Example 6-1- Comparative Example 6-6	/	No	0
Comparative Example 7-1	7th day	Yes	121
Comparative Example 7-2	7th day	Yes	91
Comparative Example 7-3	8th day	Yes	120
Comparative Example 7-4	8th day	Yes	92
Comparative Example 7-5	8th day	Yes	119
Comparative Example 7-6	8th day	Yes	87
Comparative Example 8-1	7th day	Yes	75

Comparative Example 8-2	7th day	Yes	56
Comparative Example 9-1	9th day	Yes	73
Comparative Example 9-2	9th day	Yes	55

It could be seen from Examples 1-1 to 1-10, 2-1 to 2-10 and 3-1 to 3-10 that other conventional lactobacillus could not grow on the culture medium in the present disclosure. It was indicated that the culture medium formula in the present disclosure had excellent specificity and stability.

It could be seen from Comparative Examples 4-1 to 4-10 and Comparative Examples 5-1 to 5-10 that target lactobacillus *Acetilactobacillus jinshanensis* HSLZ-75^T and *Lactobacillus* sp. HSCY 2073 in the present disclosure could not grow on the above conventional culture media. It was indicated that the target lactobacillus had strong particularity. Although the target lactobacillus could grow on the improved MRS culture medium, a growth speed was slow, the lactobacillus could be visible to naked eyes on a 7th day, and few bacterial colonies grow. It was indicated that the culture medium formula in the present disclosure had excellent high efficiency.

It could be seen from Comparative Examples 6-1 to 6-6 that the target lactobacillus in the present disclosure could not grow through the above conventional culture method. It could be seen from Comparative Examples 7-1 to 7-6 that although the target lactobacillus in the present disclosure could grow through the above culture method in which an oxygen content was 20 ppm, a growth speed was slow, and the lactobacillus was visible to naked eyes on 7th and 8th days, and few bacterial colonies grow. It could be seen in combination with Comparative Examples 8-1 and 8-2 and Comparative Examples 9-1 and 9-2 that the culture medium and culture method in the present disclosure had excellent high efficiency. In addition, it could be seen from Comparative Examples 9-1 and 9-2 that vitamin B₂ had a great influence on a growth rate of a bacterial strain and a number of bacterial colonies in the culture medium in the present disclosure. Vitamin B₂ was not additionally added in a common culture medium for lactobacillus. Since a content of other substances was low, growth of conventional strains was not influenced whether the vitamin B₂ was additionally added or not. Moreover, by adding trace vitamins in the present disclosure, a consumption usage of a malt extract could be greatly reduced, and a culture effect was obviously better.

Therefore, it can be seen through comprehensive comparison of multiple dimensions in Table 1 that the specific culture medium formula for lactobacillus and culture method in the present disclosure have excellent specificity, high efficiency and stability.

5 Table 2 Comparison table of liquid culture effects

Serial number	Number of viable bacteria CFU/mL
Example 10-1	6.3×10^8
Comparative Example 10-2	1.5×10^7
Example 10-3	7.8×10^8
Comparative Example 10-4	2.4×10^7

It can be seen from Table 2 mentioned above that the culture method using the specific culture medium for lactobacillus in the present disclosure has better growth suitability.

10 **Example 7**

The culture medium in the present disclosure is stable in component. Substances in the culture medium can be mixed, and when in use, water is added, such that a liquid or solid culture medium is prepared. Thus, application is more convenient.

15 Specifically, 100 g of tryptone, 120 g of a beef extract, 100 g of a yeast extract, 500 g of a malt extract, 130 g of glucose, 20 g of diammonium hydrogen citrate, 20 g of potassium phosphate dibasic, 1.5 g of manganese sulfate monohydrate, 5 g of magnesium sulfate heptahydrate, 15 mL of tween 80, 15 g of L-cysteine hydrochloride and 20 mg of vitamin B₂ were evenly mixed and placed in a refrigerator at -20°C. When in use, 1 L of tap water was
20 added into 125 g of the above mixture for dissolution, a temperature was 115°C, sterilization was carried out for 25 min, cooling was carried out to 50°C-55°C, and acetic acid was added for adjustment to achieve total acid of 5 g/100 mL. That is, a liquid culture medium was quickly obtained.

25 Specifically, 100 g of tryptone, 120 g of a beef extract, 100 g of a yeast extract, 500 g of a malt extract, 130 g of glucose, 20 g of diammonium hydrogen citrate, 20 g of potassium phosphate dibasic, 1.5 g of manganese sulfate monohydrate, 5 g of magnesium sulfate heptahydrate, 15 mL of tween 80, 15 g of L-cysteine hydrochloride, 20 mg of vitamin B₂ and 220 g of agar powder were evenly mixed and placed in a refrigerator at -20°C. When in use,

1 L of tap water was added into 125 g of the above mixture for dissolution, a temperature was 115°C, sterilization was carried out for 25 min, cooling was carried out to 50°C-55°C, and acetic acid was added for adjustment to achieve total acid of 5 g/100 mL. That is, a solid plate culture medium was quickly obtained.

5

Bran vinegar samples were collected. With reference to a bran vinegar sample, culture medium and method in Example 1 in patent CN 202011227512.3, time for visible bacterial colonies to grow was 7 days. By using the culture medium and culture method in the present disclosure (a solid culture medium and a liquid culture medium in Example 1 were used as culture media, and a solid culture method and a liquid culture method in Example 4 were used as culture methods), time for visible bacterial colonies to grow was 3 days in a case that similar bran vinegar samples were used. Moreover, a total number of viable bacteria detected was increased by 8.3%. It is indicated that a growth speed in cases of the culture medium and method in the present disclosure was faster.

15

Therefore, the culture medium and the culture method in the present disclosure have excellent commercial application feasibility.

The above examples are merely illustrative of embodiments of the present disclosure, and do not limit the scope of the present disclosure. Those skilled in the art can make modifications to the above description, but all such modifications should fall within the scope of protection defined in the claims of the present disclosure.

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CLAIMS

1. A specific liquid culture medium for lactobacillus, comprising raw materials as follows: 8 g -12 g of tryptone, 10 g-15 g of a beef extract, 8 g-12 g of a yeast extract, 30 g-60 g of a malt extract, 10 g-15 g of glucose, 1.5 g-2.5 g of diammonium hydrogen citrate, 1.5 g-2.5 g of potassium phosphate dibasic, 0.1 g-0.2 g of manganese sulfate monohydrate, 0.5 g-0.7 g of magnesium sulfate heptahydrate, 1 mL-2 mL of tween 80, 1 g-2 g of L-cysteine hydrochloride, and 1 mg-3 mg of vitamin B₂ that are added into each 1 L of tap water, and acetic acid that is finally added for adjustment to achieve 4 g/100 mL-6 g/100 mL.

2. The specific liquid culture medium for lactobacillus according to claim 1, wherein the lactobacillus is lactobacillus *Acetilactobacillus jinshanensis* HSLZ-75^T and *Lactobacillus sp.* HSCY 2073.

3. A specific solid culture medium for lactobacillus, comprising raw materials as follows: 8 g -12 g of tryptone, 10 g-15 g of a beef extract, 8 g-12 g of a yeast extract, 30 g-60 g of a malt extract, 10 g-15 g of glucose, 1.5 g-2.5 g of diammonium hydrogen citrate, 1.5 g-2.5 g of potassium phosphate dibasic, 0.1 g-0.2 g of manganese sulfate monohydrate, 0.5 g-0.7 g of magnesium sulfate heptahydrate, 1 mL-2 mL of tween 80, 1 g-2 g of L-cysteine hydrochloride, and 1 mg-3 mg of vitamin B₂ that are added into each 1 L of tap water, 20 g-25 g of agar powder that is added, and acetic acid that is finally added for adjustment to achieve 4 g/100 mL-6 g/100 mL.

4. The specific solid culture medium for lactobacillus according to claim 3, wherein the lactobacillus is lactobacillus *Acetilactobacillus jinshanensis* HSLZ-75^T and *Lactobacillus sp.* HSCY 2073.

5. A culture method using the specific liquid culture medium for lactobacillus according to claim 1, comprising steps as follows:

inoculating a bacterial strain in the culture medium, wherein a height of residual air above a liquid level of the culture medium is less than 0.5 cm, a liquid filling amount of the culture medium is greater than 90%, an airtight effect is achieved, and a culture temperature is 30°C-35°C.

6. A culture method using the specific solid culture medium for lactobacillus according to claim 3, comprising steps as follows:

inoculating a bacterial strain in the culture medium, and carrying out culture in an anaerobic culture box, wherein an oxygen content in the anaerobic culture box is less than 10 ppm, culture humidity is 75%-85% and a culture temperature is 30°C-35°C.

7. Application of the specific liquid culture medium for lactobacillus according to claim 1 or the solid culture medium for lactobacillus according to claim 3 in rapid mass culture of lactobacillus.

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