



Patent Translate

Powered by EPO and Google

Notice

This translation is machine-generated. It cannot be guaranteed that it is intelligible, accurate, complete, reliable or fit for specific purposes. Critical decisions, such as commercially relevant or financial decisions, should not be based on machine-translation output.

DESCRIPTION CN109810931A

A culture medium for culturing Akkermansia myxophilus and its application method

[0001]

Technical Field

[0002]

This invention belongs to the field of microbial culture medium technology, and particularly relates to a culture medium for culturing *Akkermansia myxophilus* and its method of use.

[0003]

Background Technology

[0004]

Obesity and its associated metabolic diseases, such as diabetes and cardiovascular disease, have posed a significant threat to public health.

The situation in my country is particularly severe, with more than 100 million people currently suffering from diabetes and nearly 500 million people in the prediabetes stage.

Therefore, it is particularly important to study measures for the prevention and treatment of these diseases.

[0005]

The gut microbiota is closely related to human health. Obesity and insulin resistance are two

important early characteristics of metabolic diseases, and the gut microbiota plays an important role in the occurrence and progression of metabolic diseases.

Alterations in the gut microbiota, inflammation, and disruption of the gut barrier function are key characteristics of obesity and type 2 diabetes.

Existing research mainly focuses on the taxonomic level of genus, family, or even phylum, with relatively few studies at the species and strain levels. Studying the relationship between various bacterial strains in the human gut and metabolic diseases can help identify biomarkers with predictive and diagnostic value and provide new targets for effective intervention. Existing research suggests that the gut symbiotic bacterium *Akkermansia muciniphila* may be one such functional bacterium.

[0006]

Akkermansia muciniphila is the first intestinal anaerobic bacterium in the Verrucous phylum to be isolated, and it was successfully isolated in 2004.

Recent research has found that *Akkermansiamuciniphila* (hereinafter referred to as Akk), a species of *Akkermansia*, may be one of the important marker microorganisms for measuring the balance of the gut microbiota. Akk is a functional bacterium with properties that regulate blood glucose homeostasis and is widely present in the gut of healthy individuals. It has the property of degrading mucin and is an important microorganism living on the surface of the host's intestinal mucosa mucus.

[0007]

Because of Akk's metabolic-improving effects, many scholars call it the next generation of probiotics.

Given that Akk is a novel candidate probiotic, microbial preparations with it as the main ingredient are bound to have broad development prospects and a wide market. In fact, there are already experimental studies abroad on human trials of Akk. However, many issues need to be resolved before Akk can be commercially applied.

[0008]

Optimization of Akk synthetic medium is such a necessary and crucial aspect.

Currently, the cultivation of Akk both domestically and internationally largely relies on a culture medium prepared from bovine brain heart infusion agar powder and porcine gastric mucin. Although the culture results are acceptable, the cost is high, and the brain heart infusion agar powder and mucin are derived from animals, which may carry unknown viruses and other dangerous components. The safety of culture medium components from this source cannot be fully guaranteed.

[0009]

There are existing literature reports (Plovier H, Everard A, Druart C, et al. A purified membrane

protein from *Akkermansia muciniphila* or the pasteurized bacterium improves metabolism in obese and diabetic mice).

[J]. *Nature Medicine*, 2016, 23(1):107. The Akk synthesis medium consists of soybean peptone, threonine, glucose, and N-acetylglucosamine. However, the amplification and culture of Akk using the culture medium reported in the literature were unsuccessful, and Akk bacteria could not be cultured.

[0010]

Summary of the Invention

[0011]

In view of this, the purpose of the present invention is to provide a culture medium for

culturing *Akkermansia myxophilus* and a method for using the same. The culture medium provided by the present invention can be used to culture *Akkermansia myxophilus*, and the amount of bacteria cultured can meet the requirements of functional studies.

[0012]

To achieve the above-mentioned objectives, the present invention provides the following technical solution:

[0013]

The present invention provides a culture medium for culturing *Akkermansia myxophilus*, wherein the components of the culture medium include soybean peptone, threonine, glucose, N-acetylglucosamine, sodium chloride and disodium hydrogen phosphate.

[0014]

Preferably, the culture medium uses water as a solvent and comprises per liter: 20-45 mmol glucose, 12-32 g soybean peptone, 3-8 g threonine, 20-35 mmol N-acetylglucosamine, 4-6 g sodium chloride and 2-3.5 g disodium hydrogen phosphate.

[0015]

Preferably, the culture medium uses water as a solvent and comprises per liter: 25-40 mmol glucose, 16-28 g soybean peptone, 4-7 g threonine, 25-30 mmol N-acetylglucosamine, 4.5-5.5 g sodium chloride and 2.5-3 g disodium hydrogen phosphate.

[0016]

Preferably, the culture medium uses water as a solvent and comprises per liter: 30-35 mmol glucose, 20-24 g soybean peptone, 5-6 g threonine, 35 mmol N-acetylglucosamine, 5 g sodium chloride and 2.6-2.8 g disodium hydrogen phosphate.

[0017]

The present invention also provides a method for using the culture medium described in the above technical solution, comprising: inoculating the bacterial suspension of *Akkermansia myxophilus* into the culture medium described in the above technical solution for anaerobic culture.

[0018]

Preferably, the instrument used for the anaerobic culture includes an anaerobic chamber containing 85% CO₂, 10% N₂, and 5% H₂.

[0019]

Preferably, the content of *Akkermansia myxophilus* in the bacterial solution is 2.6×10^4 CFU/mL.

[0020]

Preferably, the inoculation volume of the bacterial solution is 100 µL/200 µL.

[0021]

Preferably, the anaerobic culture temperature is 35–40°C.

[0022]

Preferably, the anaerobic culture time is 3 to 4 days.

[0023]

The present invention provides a culture medium for culturing *Akkermansia myxophilus*, wherein the components of the culture medium include soybean peptone, threonine, glucose, N-acetylglucosamine, sodium chloride and disodium hydrogen phosphate.

Existing culture media contain soybean peptone, threonine, glucose, and N-acetylglucosamine, which cannot cultivate *Akkermansia myxophilus*. This invention adds sodium chloride and disodium hydrogen phosphate to the existing culture media, which solves the problem that the lack of buffering substances in the existing culture media prevents the growth of *Akkermansia myxophilus*. After adding sodium chloride and disodium hydrogen phosphate, *Akkermansia myxophilus* can grow normally, and the amount of bacteria required for functional studies of this bacterium can be obtained.

[0024]

Attached Figure Description

[0025]

Figure 1 shows the microscopic morphology of *Akkermansia myxophilus*;

[0026]

Figure 2 shows the colony morphology and purity results of *Akkermansia myxophilus*;

[0027]

Figure 3 shows the imaging results of the amplification products of *Akkermansia myxophilus* after agarose gel electrophoresis;

[0028]

Figure 4 shows the bacterial precipitates obtained by centrifugation after inoculating equal volumes of mucin medium and the medium provided by the present invention with equal amounts of Akk in the same environment for the same time. The centrifuge tube on the left is the result obtained by culturing in the mucin medium, and the centrifuge tube on the right is the result obtained by culturing in the medium provided by the present invention.

[0029]

Figure 5 shows the results of inoculating equal volumes of mucin medium and the medium provided by the present invention with equal amounts of Akk, culturing them in the same environment for the same time, centrifuging them, resuspending them with equal volumes of sterile PBS, staining and counting the viable bacteria in the bacterial solutions. In the figure, the upper left is the uninoculated medium of the present invention as a negative control, the upper right is the viable bacteria staining of Akk after culturing in the medium of the present invention, the lower left is the uninoculated mucin medium as a negative control, and the lower right is the viable bacteria staining of Akk after culturing in the mucin medium.

[0030]

Detailed Implementation

[0031]

The present invention provides a culture medium for culturing *Akkermansia myxophilus*, wherein the components of the culture medium include soybean peptone, threonine, glucose, N-acetylglucosamine, sodium chloride and disodium hydrogen phosphate.

In this invention, the culture medium uses water as a solvent, and preferably comprises per liter: 20-45 mmol glucose, 12-32 g soybean peptone, 3-8 g threonine, 20-35 mmol N-acetylglucosamine, 4-6 g sodium chloride, and 2-3.5 g disodium hydrogen phosphate; optionally, it comprises: 25-40 mmol glucose, 16-28 g soybean peptone, 4-7 g threonine, 25-30

mmol N-acetylglucosamine, 4.5-5.5 g sodium chloride, and 2.5-3 g disodium hydrogen phosphate; or optionally, it comprises: 30-35 mmol glucose, 20-24 g soybean peptone, 5-6 g threonine, 35 mmol N-acetylglucosamine, 5 g sodium chloride, and 2.6-2.8 g disodium hydrogen phosphate.

In this invention, sodium chloride and disodium hydrogen phosphate are used as buffer substances in the culture medium, which solves the problem that *Akkermansia myxophilus* cannot be obtained in a culture medium containing soybean peptone, threonine, glucose and N-acetylglucosamine, and enables the bacteria to be obtained.

In this invention, when the concentration of sodium chloride in each liter of culture medium is higher than 6g, it is not conducive to the growth of *Akkermansia myxophilus*.

[0032]

The present invention also provides a method for using the culture medium described in the above technical solution, comprising: inoculating the bacterial suspension of *Akkermansia*

myxophilus into the culture medium described in the above technical solution for anaerobic culture.

[0033]

In this invention, the anaerobic culture is preferably carried out at 85% CO₂, 10% N₂ and 5% H₂, and the instrument used for the anaerobic culture is preferably an anaerobic chamber.

In this invention, the anaerobic culture temperature is preferably 35-40°C, and the anaerobic culture time is preferably 3-4 days.

[0034]

In this invention, the inoculation amount of the Akkermansia myxophilus bacterial solution is

preferably 100 μ L/200 μ L, and the Akkermansia myxophilus content in the bacterial solution is 2.6×10 CFU/mL.

The present invention does not have any particular limitation on the source of the bacterial culture of Akkermansia myxophilus, which can be prepared by conventional preparation methods.

The present invention does not impose any special limitations on the inoculation method and tools used; conventional methods are acceptable.

[0035]

The technical solutions provided by the present invention will be described in detail below with reference to the embodiments, but they should not be construed as limiting the scope of protection of the present invention.

[0036]

Example 1

[0037]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 0.72g glucose, 2.4g soybean peptone, 0.6g threonine, 0.88g N-acetylglucosamine, 0.9g sodium chloride, and 0.4g disodium hydrogen phosphate. Mix thoroughly and autoclave the culture medium. After cooling, inoculate the medium with 100μL of *Akkermansia myxophilus* bacterial suspension (the bacterial count of *Akkermansia myxophilus* in the suspension is 2.6×10^8 CFU). The inoculum was inoculated with 200 μL of cysteine and 85% CO₂ (85% CO₂, 10% N₂, and 5% H₂) and cultured in an anaerobic incubator (37°C) for 3 days. After incubation, the inoculum was centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD₆₀₀ value of the bacterial culture was measured using NanoDrop. The OD₆₀₀ value was 0.0183.

[0038]

After inoculating the bacterial culture into mucin liquid medium, culture for 2-3 days, centrifuge, and Gram stain the precipitate for microscopic examination to observe the microscopic morphology of the preserved bacteria (the results are shown in Figure 1).

Meanwhile, the live bacteria precipitate was streaked onto mucin plates and cultured for 2 days. The colony morphology and purity of the preserved bacteria were then observed (the results are shown in Figure 2).

Finally, using the bacterial culture as a template, PCR amplification was performed using *Akkermansia myxophilus* primers. The amplification products were subjected to agarose gel electrophoresis, and the molecular weight of the original template DNA was determined using a fully automated gel imaging system (the results are shown in Figure 3).

[0039]

As shown in Figure 1, the bacteria in the bacterial solution are Gram-negative short rods, which is consistent with the microscopic morphological characteristics of *Akkermansia myxophilus*. Moreover, no other obvious contaminating bacteria were observed under the microscope, proving that the bacteria in the bacterial solution are pure bacteria.

As can be seen from Figure 2, the colonies in the bacterial solution are small, white and transparent, which is consistent with the colony morphology of *Akkermansia myxophilus*. Moreover, there are no other bacteria growing on the plate, which proves that the bacteria in the bacterial solution are pure bacteria.

As shown in Figure 3, after PCR amplification using bacterial culture as a template, the molecular weight of the DNA is between 250 and 500 bp, located in the lower middle part, slightly close to the 250 bp line. This is consistent with previous reports that the molecular weight of *Akkermansia myxophilus* DNA after amplification with the same primers is 327 bp.

In conclusion, the bacteria in the bacterial solution are pure *Akkermansia myxophila*.

[0040]

Cysteine is a reducing agent that can consume oxygen in the culture medium, which is beneficial to the anaerobic growth of *Akkermansia myxophilus*.

In the following examples and comparative examples, the role of cysteine is the same as in Example 1.

[0041]

Example 2

[0042]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 0.72g glucose, 3.2g soybean peptone, 0.8g threonine, 1.056g N-acetylglucosamine, 1g sodium chloride, and 0.5g disodium hydrogen phosphate. Mix thoroughly and autoclave the culture medium. After cooling, inoculate the medium with 100μL of *Akkermansia myxophilus* bacterial suspension (the bacterial count of *Akkermansia myxophilus* in the suspension is 2.6×10^1 NER15). The inoculum was inoculated with 200 μL of cysteine and placed in an anaerobic incubator (85% CO₂, 10% N₂, and 5% H₂) at 37°C for 4 days. After inoculation, the inoculum was centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD₂₀ value of the bacterial culture was measured using NanoDrop. The OD₂₀ value was 0.0343.

[0043]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0044]

Example 3

[0045]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 0.72g glucose, 4g soybean peptone, 1g threonine, 1.328g N-acetylglucosamine, 1.1g sodium chloride, and 0.6g disodium hydrogen phosphate. Mix thoroughly and autoclave the culture medium. After cooling, inoculate the medium with 100μL of *Akkermansia myxophilus* bacterial suspension (the bacterial count of *Akkermansia myxophilus* in the suspension is 2.6×10^8). The inoculum was

inoculated with 200 μ L of cysteine and placed in an anaerobic incubator (85% CO₂, 10% N₂, and 5% H₂) at 37°C for 4 days. After inoculation, the inoculum was centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD₂₅ value of the bacterial culture was measured using NanoDrop. The OD₂₆ value was 0.0423.

[0046]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0047]

Example 4

[0048]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 0.72g glucose, 4.8g soy peptone, 1.2g threonine, and 1.548g... N-acetylglucosamine, 1.2 g sodium chloride, and 0.7 g disodium hydrogen phosphate were thoroughly mixed and then autoclaved. After the culture medium cooled, 100 μ L of *Akkermansia myxophilus* bacterial suspension (containing 2.6×10 cfu/mL of *Akkermansia myxophilus*) and 200 μ L of cysteine were inoculated into the culture medium. After inoculation, the culture was placed in an anaerobic chamber (85% CO, 10% N, and 5% H) and cultured at 37°C for 4 days. After incubation, the culture was removed, centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD value of the bacterial suspension was determined using NanoDrop. The OD value was 0.063.

[0049]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0050]

Example 5

[0051]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 0.9g glucose, 2.4g soy peptone, 0.6g threonine, and 1.056g... N-acetylglucosamine, 1.1 g sodium chloride, and 0.7 g disodium hydrogen phosphate were thoroughly mixed and then autoclaved. After the culture medium cooled, 100 μ L of *Akkermansia myxophilus* bacterial suspension (containing 2.6×10^{33} cfu/mL) and 200 μ L of cysteine were inoculated into the culture medium. After

inoculation, the culture medium was placed in an anaerobic chamber (85% CO₂, 10% N₂, and 5% H₂) and cultured at 37°C for 4 days. After inoculation, the culture medium was removed, centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD value of the bacterial suspension was measured using NanoDrop. The OD value was 0.0143.

[0052]

The determination of whether the bacteria in the bacterial solution are *Akkermansia* *myxophilus* and the purity of the bacteria are the same as in Example 1.

[0053]

Example 6

[0054]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 0.9g glucose, 3.2g soy peptone, 0.8g threonine, and 0.884g... N-acetylglucosamine, 1.2g sodium chloride, and 0.6g disodium hydrogen phosphate were thoroughly mixed and then autoclaved. After the culture medium cooled, 100μL of *Akkermansia myxophilus* bacterial suspension (containing 2.6×10^3 cfu/mL of *Akkermansia myxophilus*) and 200μL of cysteine were inoculated into the culture medium. After inoculation, the culture was placed in an anaerobic chamber (85% CO₂, 10% N₂, and 5% H₂) and cultured at 37°C for 3 days. After incubation, the culture was removed, centrifuged at 3000rpm for 10min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5mL of sterile PBS, and the OD value of the bacterial suspension was determined using NanoDrop. The OD value was 0.0253.

[0055]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0056]

Example 7

[0057]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 0.9g glucose, 4g soybean peptone, 1g threonine, 1.548g N-acetylglucosamine, 0.9g sodium chloride, and 0.5g disodium hydrogen phosphate. Mix thoroughly, then autoclave the culture medium. After cooling, inoculate the medium with 100μL of *Akkermansia myxophilus* bacterial suspension (the bacterial count of *Akkermansia myxophilus* in the suspension is 2.6×10^8 CFU/mL). The inoculum

was inoculated with 200 μ L of cysteine and placed in an anaerobic incubator (85% CONER46, 10% NNER47 and 5% HNER48) at 37°C for 3 days. After inoculation, the inoculum was centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD49 value of the bacterial culture was measured using NanoDrop. The OD50 value was 0.027.

[0058]

The determination of whether the bacteria in the bacterial solution are Akkermansia myxophilus and the purity of the bacteria are the same as in Example 1.

[0059]

Example 8

[0060]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 0.9g glucose, 4.8g soybean peptone, 1.2g threonine, 1.328g N-acetylglucosamine, 1g sodium chloride, and 0.4g disodium hydrogen phosphate. Mix thoroughly and autoclave the culture medium. After cooling, inoculate the medium with 100μL of *Akkermansia myxophilus* bacterial suspension (the bacterial count of *Akkermansia myxophilus* in the suspension is 2.6×10^8 CFU). The inoculum was inoculated with 200 μL of cysteine and placed in an anaerobic incubator (85% CONER52, 10% NNER53 and 5% HNER54) at 37°C for 3 days. After inoculation, the inoculum was centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD55 value of the bacterial culture was determined using NanoDrop. The OD56 value was 0.0513.

[0061]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0062]

Example 9

[0063]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.08g glucose, 2.4g soy peptone, 0.6g threonine, and 1.328g... N-acetylglucosamine, 1.2g sodium chloride, and 0.5g disodium hydrogen phosphate were thoroughly mixed and then autoclaved. After the culture medium cooled, 100μL of *Akkermansia myxophilus* bacterial suspension (containing 2.6×10 cfu/mL of *Akkermansia myxophilus*) and 200μL of cysteine were inoculated into the culture

medium. After inoculation, the culture was placed in an anaerobic chamber (85% CO₂, 10% N₂, and 5% H₂) and cultured at 37°C for 3 days. After inoculation, the culture was removed, centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5mL of sterile PBS, and the OD value of the bacterial suspension was determined using NanoDrop. The OD value was 0.0087.

[0064]

The determination of whether the bacteria in the bacterial solution are *Akkermansia* *myxophilus* and the purity of the bacteria are the same as in Example 1.

[0065]

Example 10

[0066]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.08g glucose, 3.2g soy peptone, 0.8g threonine, and 0.884g... N-acetylglucosamine, 1.1 g sodium chloride, and 0.4 g disodium hydrogen phosphate were thoroughly mixed and then autoclaved. After the culture medium cooled, 100 μ L of *Akkermansia myxophilus* bacterial suspension (containing 2.6×10 CFU/mL) and 200 μ L of cysteine were inoculated into the culture medium. After inoculation, the culture medium was placed in an anaerobic chamber (85% CO, 10% N, and 5% H) and cultured at 37 °C for 3 days. After inoculation, the culture medium was removed, centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD value of the bacterial suspension was determined using NanoDrop. The OD value was 0.035.

[0067]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0068]

Example 11

[0069]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.08g glucose, 4g soy peptone, 1.25g threonine, and 1.548g... N-acetylglucosamine, 1g sodium chloride, and 0.7g disodium hydrogen phosphate were thoroughly mixed and then autoclaved. After the culture medium cooled, 100μL of *Akkermansia myxophilus* bacterial suspension (containing 2.6×10 CFU/mL) and 200μL of cysteine were inoculated into the culture medium. After inoculation,

the culture medium was placed in an anaerobic chamber (85% CO₂, 10% N₂, and 5% H₂) and cultured at 37°C for 3 days. After 3 days, the culture medium was removed, centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5mL of sterile PBS, and the OD value of the bacterial suspension was determined using NanoDrop. The OD value was 0.0687.

[0070]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0071]

Example 12

[0072]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.08g glucose, 4.8g soy peptone, 1.2g threonine, and 1.056g... N-acetylglucosamine, 0.9 g sodium chloride, and 0.6 g disodium hydrogen phosphate were thoroughly mixed and then autoclaved. After the culture medium cooled, 100 μ L of *Akkermansia myxophilus* bacterial suspension (containing 2.6×10 cfu/mL of *Akkermansia myxophilus*) and 200 μ L of cysteine were inoculated into the culture medium. After inoculation, the culture was placed in an anaerobic chamber (85% CO, 10% N, and 5% H) and cultured at 37°C for 3 days. After incubation, the culture was removed, centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD value of the bacterial suspension was determined using NanoDrop. The OD value was 0.087.

[0073]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0074]

Example 13

[0075]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.26g glucose, 2.4g soybean peptone, 0.6g threonine, 1.548g N-acetylglucosamine, 1g sodium chloride, and 0.6g disodium hydrogen phosphate. Mix thoroughly and autoclave the culture medium. After cooling, inoculate the medium with 100μL of *Akkermansia myxophilus* bacterial suspension (the bacterial count of *Akkermansia myxophilus* in the suspension is 2.6×10^8 CFU). The

inoculum was inoculated with 200 μ L of cysteine and 85% CO₂ (85% CO₂, 10% N₂, and 5% H₂) and cultured in an anaerobic incubator (37°C) for 3 days. After incubation, the inoculum was centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD₈₅ value of the bacterial culture was determined using NanoDrop. The OD₈₆ value was 0.0093.

[0076]

The determination of whether the bacteria in the bacterial solution are Akkermansia myxophilus and the purity of the bacteria are the same as in Example 1.

[0077]

Example 14

[0078]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.26g glucose, 3.2g soy peptone, 0.8g threonine, and 1.328g... N-acetylglucosamine, 0.9 g sodium chloride, and 0.7 g disodium hydrogen phosphate were thoroughly mixed and then autoclaved. After the culture medium cooled, 100 μ L of *Akkermansia myxophilus* bacterial suspension (containing 2.6×10 CFU/mL) and 200 μ L of cysteine were inoculated into the culture medium. After inoculation, the culture medium was placed in an anaerobic chamber (85% CO, 10% N, and 5% H) and cultured at 37 °C for 3 days. After 3 days, the culture medium was removed, centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD value of the bacterial suspension was determined using NanoDrop. The OD value was 0.0187.

[0079]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0080]

Example 15

[0081]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.26g glucose, 4g soybean peptone, 1g threonine, 1.056g N-acetylglucosamine, 1.2g sodium chloride, and 0.4g disodium hydrogen phosphate. Mix thoroughly and autoclave the culture medium. After cooling, inoculate the medium with 100μL of *Akkermansia myxophilus* bacterial suspension (the bacterial count of *Akkermansia myxophilus* in the suspension is 2.6×10^9 CFU). The inoculum

was inoculated with 200 μ L of cysteine and placed in an anaerobic incubator (85% CONER94, 10% NNER95 and 5% HNER96) at 37°C for 4 days. After inoculation, the inoculum was centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD97 value of the bacterial culture was measured using NanoDrop. The OD98 value was 0.0043.

[0082]

The determination of whether the bacteria in the bacterial solution are Akkermansia myxophilus and the purity of the bacteria are the same as in Example 1.

[0083]

Example 16

[0084]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.26g glucose, 4.8g soy peptone, 1.2g threonine, and 0.884g... N-acetylglucosamine, 1.1 g sodium chloride, and 0.5 g disodium hydrogen phosphate were thoroughly mixed and then autoclaved. After the culture medium cooled, 100 μ L of *Akkermansia myxophilus* bacterial suspension (containing 2.6×10 CFU/mL) and 200 μ L of cysteine were inoculated into the culture medium. After inoculation, the culture medium was placed in an anaerobic chamber (85% CO, 10% N, and 5% H) and cultured at 37 °C for 4 days. After incubation, the culture medium was removed, centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD value of the bacterial suspension was determined using NanoDrop. The OD value was 0.097.

[0085]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0086]

Example 17

[0087]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.08g glucose, 4g soybean peptone, 1g threonine, 1.056g N-acetylglucosamine, 0.8g sodium chloride, and 0.5g disodium hydrogen phosphate. Mix thoroughly and autoclave the culture medium. After cooling, inoculate the medium with 100μL of *Akkermansia myxophilus* bacterial suspension (the bacterial count of *Akkermansia myxophilus* in the suspension is 2.6×10^8 cfu). The inoculum was

inoculated with 200 μ L of cysteine and placed in an anaerobic incubator (85% CO₂ 106, 10% N₂ 107 and 5% H₂ 108) at 37°C for 4 days. After inoculation, the inoculum was centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD₂₀₉ value of the bacterial culture was measured using NanoDrop. The OD₂₀₁₁₀ value was 0.1083.

[0088]

The determination of whether the bacteria in the bacterial solution are Akkermansia myxophilus and the purity of the bacteria are the same as in Example 1.

[0089]

Example 18

[0090]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.08g glucose, 4.8g soy peptone, 1.2g threonine, and 1.056g... N-acetylglucosamine, 0.9 g sodium chloride, and 0.5 g disodium hydrogen phosphate were thoroughly mixed and then autoclaved. After the culture medium cooled, 100 μ L of *Akkermansia myxophilus* bacterial suspension (containing 2.6×10^{11} cfu/mL of *Akkermansia myxophilus*) and 200 μ L of cysteine were inoculated into the culture medium. After inoculation, the culture was placed in an anaerobic chamber (85% CO₂, 10% N₂, and 5% H₂) and cultured at 37°C for 4 days. After incubation, the culture was removed, centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD value of the bacterial suspension was determined using NanoDrop. The OD value was 0.1193.

[0091]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0092]

Example 19

[0093]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.08g glucose, 5.6g soybean peptone, 1.4g threonine, 1.056g N-acetylglucosamine, 1g sodium chloride, and 0.5g disodium hydrogen phosphate. Mix thoroughly and autoclave the culture medium. After cooling, inoculate the medium with 100μL of *Akkermansia myxophilus* bacterial suspension (the bacterial count of *Akkermansia myxophilus* in the suspension is 2.6×10^{11} c). The

inoculum was inoculated with 200 μ L of cysteine and placed in an anaerobic incubator (85% CO₂, 10% N₂, and 5% H₂) at 37°C for 4 days. After inoculation, the inoculum was centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD₁₂₁ value of the bacterial culture was measured using NanoDrop. The OD₁₂₂ value was 0.137.

[0094]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0095]

Example 20

[0096]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.08g glucose, 6.4g soy peptone, 1.6g threonine, and 1.056g... N-acetylglucosamine, 1.1 g sodium chloride, and 0.5 g disodium hydrogen phosphate were thoroughly mixed and then autoclaved. After the culture medium cooled, 100 μ L of *Akkermansia myxophilus* bacterial suspension (containing 2.6×10^{12} cfu/mL of *Akkermansia myxophilus*) and 200 μ L of cysteine were inoculated into the culture medium. After inoculation, the culture was placed in an anaerobic chamber (85% CO₂, 10% N₂, and 5% H₂) and cultured at 37 °C for 4 days. After incubation, the culture was removed, centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD value of the bacterial suspension was determined using NanoDrop. The OD value was 0.1427.

[0097]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0098]

Example 21

[0099]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.26g glucose, 4g soybean peptone, 1g threonine, 1.056g N-acetylglucosamine, 0.9g sodium chloride, and 0.5g disodium hydrogen phosphate. Mix thoroughly and autoclave the culture medium. After cooling, inoculate the medium with 100μL of *Akkermansia myxophilus* bacterial suspension (the bacterial count of *Akkermansia myxophilus* in the suspension is 2.6×10^{12} cf). The inoculum

was inoculated with 200 μ L of cysteine and placed in an anaerobic incubator (85% CO₂, 10% N₂, and 5% H₂) at 37°C for 4 days. After inoculation, the inoculum was centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD₃₃ value of the bacterial culture was determined using NanoDrop. The OD₄₄ value was 0.1050.

[0100]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0101]

Example 22

[0102]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.26g glucose, 4.8g soybean peptone, 1.2g threonine, 1.056g N-acetylglucosamine, 1g sodium chloride, and 0.5g disodium hydrogen phosphate. Mix thoroughly and autoclave the culture medium. After cooling, inoculate the medium with 100μL of *Akkermansia myxophilus* bacterial suspension (the bacterial count of *Akkermansia myxophilus* in the suspension is 2.6×10^{13} c). The inoculum was inoculated with 200 μL of cysteine and placed in an anaerobic incubator (85% CO₂, 10% N₂, and 5% H₂) at 37°C for 3 days. After inoculation, the inoculum was centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD139 value of the bacterial culture was measured using NanoDrop. The OD140 value was 0.1120.

[0103]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0104]

Example 23

[0105]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.26g glucose, 5.6g soy peptone, 1.4g threonine, and 1.056g... N-acetylglucosamine, 1.1 g sodium chloride, and 0.5 g disodium hydrogen phosphate were thoroughly mixed and then autoclaved. After the culture medium cooled, 100 μ L of *Akkermansia myxophilus* bacterial suspension (containing 2.6×10^4 CFU/mL) and 200 μ L of cysteine were inoculated into the culture medium. After

inoculation, the culture medium was placed in an anaerobic chamber (85% CO₂, 10% N₂, and 5% H₂) and cultured at 37 °C for 3 days. After 3 days, the culture medium was removed, centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD value of the bacterial suspension was determined using NanoDrop. The OD value was 0.1423.

[0106]

The determination of whether the bacteria in the bacterial solution are *Akkermansia* *myxophilus* and the purity of the bacteria are the same as in Example 1.

[0107]

Example 24

[0108]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.26g glucose, 6.4g soy peptone, 1.6g threonine, and 1.056g... N-acetylglucosamine, 0.8 g sodium chloride, and 0.5 g disodium hydrogen phosphate were thoroughly mixed and then autoclaved. After the culture medium cooled, 100 μ L of *Akkermansia myxophilus* bacterial suspension (containing 2.6×10 CFU/mL) and 200 μ L of cysteine were inoculated into the culture medium. After inoculation, the culture medium was placed in an anaerobic chamber (85% CO, 10% N, 10% H, 150) and cultured at 37 °C for 3 days. After 3 days, the culture medium was removed, centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD value of the bacterial suspension was determined using NanoDrop. The OD value was 0.1367.

[0109]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0110]

Example 25

[0111]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.44g glucose, 4g soybean peptone, 1g threonine, 1.056g N-acetylglucosamine, 1g sodium chloride, and 0.5g disodium hydrogen phosphate. Mix thoroughly and autoclave the culture medium. After cooling, inoculate the medium with 100μL of *Akkermansia myxophilus* bacterial suspension (the bacterial count of *Akkermansia myxophilus* in the suspension is 2.6×10^{13} cfu). The inoculum

was inoculated with 200 µL of cysteine and placed in an anaerobic incubator (85% CO₂, 10% N₂ and 5% H₂) and cultured at 37°C for 3 days. After 3 days, the inoculum was removed, centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD₆₀₀ value of the bacterial culture was measured using NanoDrop. The OD₆₀₀ value was 0.1063.

[0112]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0113]

Example 26

[0114]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.44g glucose, 4.8g soy peptone, 1.2g threonine, and 1.056g... N-acetylglucosamine, 1.1 g sodium chloride, and 0.5 g disodium hydrogen phosphate were thoroughly mixed and then autoclaved. After the culture medium cooled, 100 μ L of *Akkermansia myxophilus* bacterial suspension (containing 2.6×10^1 cfu/mL) and 200 μ L of cysteine were inoculated into the culture medium. After inoculation, the culture was placed in an anaerobic chamber (85% CO, 10% N, and 5% H) and cultured at 37°C for 3 days. After incubation, the culture was removed, centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD value of the bacterial suspension was determined using NanoDrop. The OD value was 0.1337.

[0115]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0116]

Example 27

[0117]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.44g glucose, 5.6g soy peptone, 1.4g threonine, and 1.056g... N-acetylglucosamine, 0.8 g sodium chloride, and 0.5 g disodium hydrogen phosphate were thoroughly mixed and then autoclaved. After the culture medium cooled, 100 μ L of *Akkermansia myxophilus* bacterial suspension (containing 2.6×10 CFU/mL) and 200 μ L of cysteine were inoculated into the culture medium. After inoculation,

the culture medium was placed in an anaerobic chamber (85% CO₂, 10% N₂, and 5% H₂) and cultured at 37°C for 3 days. After incubation, the culture medium was removed, centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD value of the bacterial suspension was determined using NanoDrop. The OD value was 0.1407.

[0118]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0119]

Example 28

[0120]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.44g glucose, 6.4g soy peptone, 1.6g threonine, and 1.056g... N-acetylglucosamine, 0.9 g sodium chloride, and 0.5 g disodium hydrogen phosphate were thoroughly mixed and then autoclaved. After the culture medium cooled, 100 μ L of *Akkermansia myxophilus* bacterial suspension (containing 2.6×10 CFU/mL) and 200 μ L of cysteine were inoculated into the culture medium. After inoculation, the culture medium was placed in an anaerobic chamber (85% CO, 10% N, and 5% H) and cultured at 37°C for 3 days. After 3 days, the culture medium was removed, centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD value of the bacterial suspension was determined using NanoDrop. The OD value was 0.1400.

[0121]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0122]

Example 29

[0123]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.44g glucose, 4g soybean peptone, 1g threonine, 1.056g N-acetylglucosamine, 1.1g sodium chloride, and 0.5g disodium hydrogen phosphate. Mix thoroughly and autoclave the culture medium. After cooling, inoculate the medium with 100μL of *Akkermansia myxophilus* bacterial suspension (the bacterial count of *Akkermansia myxophilus* in the suspension is 2.6×10^1 cfu). The inoculum

was inoculated with 200 µL of cysteine and placed in an anaerobic incubator (85% CO₂, 10% N₂ and 5% H₂) and cultured at 37°C for 3 days. After 3 days, the inoculum was removed, centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD₆₀₀ value of the bacterial culture was determined using NanoDrop. The OD₆₀₀ value was 0.1143.

[0124]

The determination of whether the bacteria in the bacterial solution are Akkermansia myxophilus and the purity of the bacteria are the same as in Example 1.

[0125]

Example 30

[0126]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.44g glucose, 4.8g soybean peptone, 1.2g threonine, 1.056g N-acetylglucosamine, 1g sodium chloride, and 0.5g disodium hydrogen phosphate. Mix thoroughly and autoclave the culture medium. After cooling, inoculate the medium with 100μL of *Akkermansia myxophilus* bacterial suspension (the bacterial count of *Akkermansia myxophilus* in the suspension is 2.6×10^8 cfu). The inoculum was inoculated with 200 μL of cysteine and placed in an anaerobic incubator (85% CO₂, 10% N₂, and 5% H₂) at 37°C for 3 days. After inoculation, the inoculum was centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD₇ value of the bacterial culture was measured using NanoDrop. The OD₈₈ value was 0.1043.

[0127]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0128]

Example 31

[0129]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.44g glucose, 5.6g soy peptone, 1.4g threonine, and 1.056g... N-acetylglucosamine, 0.9 g sodium chloride, and 0.5 g disodium hydrogen phosphate were thoroughly mixed and then autoclaved. After the culture medium cooled, 100 μ L of *Akkermansia myxophilus* bacterial suspension (containing 2.6×10^1 CFU/mL) and 200 μ L of cysteine were inoculated into the culture medium. After

inoculation, the culture medium was placed in an anaerobic chamber (85% CO, 10% N, and 5% H) and cultured at 37 °C for 3 days. After 3 days, the culture medium was removed, centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD value of the bacterial suspension was determined using NanoDrop. The OD value was 0.1470.

[0130]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0131]

Example 32

[0132]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 1.44g glucose, 6.4g soybean peptone, 1.6g threonine, 1.056g N-acetylglucosamine, 1g sodium chloride, and 0.5g disodium hydrogen phosphate. Mix thoroughly and autoclave the culture medium. After cooling, inoculate the medium with 100μL of *Akkermansia myxophilus* bacterial suspension (the bacterial count of *Akkermansia myxophilus* in the suspension is 2.6×10^1 c). The inoculum was inoculated with 200 μL of cysteine and placed in an anaerobic incubator (85% CO₂, 10% N₂, and 5% H₂) at 37°C for 3 days. After inoculation, the inoculum was centrifuged at 3000 rpm for 10 min, and the amount of precipitated bacteria was observed. The precipitate was then resuspended in 5 mL of sterile PBS, and the OD₂₀₀ value of the bacterial culture was measured using NanoDrop. The OD₂₀₀ value was 0.1470.

[0133]

The determination of whether the bacteria in the bacterial solution are *Akkermansia myxophilus* and the purity of the bacteria are the same as in Example 1.

[0134]

Example 33

[0135]

Take a 250ml Erlenmeyer flask, add 200ml of pure water, then add 1.44g glucose, 6.4g soybean peptone, 1.6g threonine, 1.056g N-acetylglucosamine, 1g sodium chloride, and 0.5g disodium hydrogen phosphate. Mix thoroughly, then autoclave the culture medium. After cooling, inoculate the medium with 100μL of *Akkermansia myxophilus* bacterial suspension (the bacterial count of *Akkermansia myxophilus* in the suspension should be 2.6×10^8 NE¹).

Inoculate with R201 (cfu/mL) and 200 μ L of cysteine, and then incubate in an anaerobic chamber (85% CO₂, 10% N₂O₃ and 5% H₂O₄) at 37°C for 3 days. After incubation, remove the culture, centrifuge at 3000 rpm for 10 min, and observe the amount of precipitated bacteria (results are shown in Figure 4). Resuspend the precipitate in 5 mL of sterile PBS and then use NanoDrop to determine the OD₂₀₅ value of the bacterial culture.

The OD_NER206 values in the three parallel experiments were 1.494, 1.395, and 1.412, respectively.

[0136]

The determination of whether the bacteria in the bacterial solution are Akkermansia myxophilus and the purity of the bacteria are the same as in Example 1.

[0137]

The bacterial count in the bacterial solution was determined using a urine analyzer in the laboratory. The bacterial count of *Akkermansia myxophilus* in 200 μL of the bacterial solution was 8.8×10^7 cfu.

[0138]

Fluorescent staining of *Akkermansia myxophilus* was performed using the following procedure: Take 5 μL each of dyes A and B from the kit (NER208 BacLight NER209 Bacterial Viability Kits), mix well, and store in the dark. Dilute the Akk culture broth 500-fold with sterile PBS. Add 3 μL of the mixed dye to 1 mL of the diluted Akk culture broth, shake thoroughly to mix, and incubate at room temperature in the dark for 15 min. Then, take 5 μL of the stained live bacterial suspension onto a glass slide, cover with a coverslip, and observe under a fluorescence microscope.

The result is shown in Figure 5.

As can be seen from Figure 5, when equal amounts of Akk are cultured in the culture medium

provided by this invention, a greater number of viable bacteria can be obtained than when cultured in mucin culture medium. This indicates that the culture medium provided by this invention can fully realize the in vitro amplification culture of Akk and can realize subsequent functional research and industrialization development.

[0139]

Comparative Example 1

[0140]

Take a 250ml Erlenmeyer flask, add 200mL of pure water, then add 0.4g of mucin and 7.4g of brain heart extract powder, stir thoroughly, and autoclave the culture medium. After the culture medium cools, inoculate it with 100 μ L of *Akkermansia myxophilus* bacterial suspension (the content of *Akkermansia myxophilus* in the bacterial suspension is 2.6

$\times 10^8$ cfu/mL) and 200 μ L of cysteine. After inoculation, place it in an anaerobic chamber (85% CO₂, 10% N₂ and 5% H₂) and incubate at 37°C for 3 days. After that, take it out, centrifuge at 3000rpm for 10min, and observe the amount of precipitated bacteria (the result is shown in Figure 4). Then resuspend the precipitate with 5mL of sterile PBS and use NanoDrop to determine the OD₆₀₀ value of the bacterial suspension.

The OD_{NER215} values for the three parallel experiments were 0.915, 0.945, and 0.976, respectively.

[0141]

The determination of whether the bacteria in the bacterial solution are Akkermansia myxophilus and the purity of the bacteria are the same as in Example 1.

[0142]

The bacterial count in the bacterial solution was determined using a urine analyzer in the laboratory. The bacterial count of *Akkermansia myxophilus* in 200 μL of the bacterial solution was 4.2×10^1 CFU.

[0143]

Fluorescent staining of *Akkermansia myxophilus* with fluorescent dyes was performed as follows: 5 μL each of dyes A and B from the kit (NER217 BacLight NER218 Bacterial Viability Kits) were mixed thoroughly and kept in the dark. The Akk culture was diluted 500-fold with sterile PBS. 3 μL of the mixed dye was added to 1 mL of the diluted Akk culture, and the mixture was thoroughly shaken and incubated at room temperature in the dark for 15 min. Then, 5 μL of the stained live bacterial suspension was placed on a glass slide, covered with a coverslip, and observed under a fluorescence microscope.

The result is shown in Figure 5.

[0144]

As can be seen from Figure 5, when equal amounts of Akk are cultured in the culture medium provided by this invention, a greater number of viable bacteria can be obtained than when cultured in mucin culture medium. This indicates that the culture medium provided by this invention can fully realize the in vitro amplification culture of Akk and can realize subsequent functional research and industrialization development.

[0145]

As can be seen from the above examples and comparative examples, the culture medium of the present invention can be used to culture Akkermansia myxophilus, the components in the culture medium are safe and there are no potential risks, and it can enable subsequent functional research and industrial development.

[0146]

The above description is only a preferred embodiment of the present invention. It should be noted that for those skilled in the art, several improvements and modifications can be made without departing from the principle of the present invention, and these improvements and modifications should also be considered within the scope of protection of the present invention.