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DESCRIPTION CN110551658A

A hypoxic culture method for *Akkermansia myxophilus*

[0001]

Technical Field

[0002]

This invention belongs to the field of culture technology of *Akkermansia myxophilus*, and in particular, a hypoxic culture method for *Akkermansia myxophilus*.

[0003]

Background Technology

[0004]

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 bacteria), a species of Akkermansia, may be one of the important marker microorganisms for measuring the balance of the gut microbiota.

Akk bacteria are functional bacteria with properties that regulate blood glucose homeostasis and are 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. Recent studies have shown that Akk bacteria are associated with many metabolic diseases in humans, and their abundance is one of the important markers of gut microbiota balance. Akk bacteria abundance is negatively correlated with human weight, type 1 diabetes, and type 2 diabetes, and is also negatively

correlated with waist circumference, weight, and body mass index. Many scholars refer to it as the next generation of probiotics. Given that Akk bacteria is a novel candidate probiotic, its cultivation is of particular importance.

[0005]

Based on current research findings, Akk bacteria are strict anaerobic bacteria and require special anaerobic culture media for cultivation.

For example, patent 201910265701.0 provides a culture medium for culturing Akkermansia myxophilus and a method for using it. The anaerobic culture environment selected in this method is 85% CO₂, 10% N₂ and 5% H₂, and the instrument used is an anaerobic chamber. Another example is Derrien M et al. (Derrien M. Akkermansia muciniphila gen.nov.sp.nov.a humanintestinal mucin-degrading bacterium[J]. Int. J.Syst. Evol. Microbiol. 2004, 54. The invention discloses that Akkermansia myxophilus can grow on a basic anaerobic medium containing porcine gastric mucin as the sole carbon and nitrogen source, and the culture atmosphere is N₂/CO₂ (80:20, v/v); the inventors conducted shake flask culture using the above culture conditions and

conventional anaerobic chamber culture conditions (95% N₂, 5% H₂).

[0006]

However, Janneke P.Ouwerkerk et al. (Adaptation of *Akkermansia muciniphila* to the Oxic-Anoxic Interface of the Mucus Layer[J].

The study of Akk bacteria's oxygen tolerance in Applied and Environmental Microbiology (2016, 82) mentioned that Akk bacteria can not only tolerate but even benefit from low levels of oxygen. However, this article only studied the oxygen concentration (0.1%) during the stationary phase of Akk bacteria, without investigating the hypoxic culture atmosphere during the logarithmic phase when Akk bacteria grow most vigorously. Using the oxygen level described in this paper, the culture time required for Akk bacteria to enter the stabilizer is relatively long. For Akk bacteria research, it is necessary to promote rapid growth and quickly reach the stationary phase to obtain a larger quantity of Akk bacteria. Obviously, the technique in this paper cannot achieve the above-mentioned effect and cannot improve the culture efficiency of Akk bacteria. In addition, the 0.1% oxygen level requirement is still

relatively strict for Akk bacteria culture, and the gas atmosphere is prone to fluctuations during culture, affecting Akk bacteria growth.

[0007]

Summary of the Invention

[0008]

To address the shortcomings of the existing technology, this invention provides a low-oxygen culture method for Akkella myxophilus. Through further research on the production environment of Akkella, this method found that within the range of 1-5% O₂ volume, the production and reproduction of Akkella were not only not inhibited by O₂, but were significantly accelerated. That is, within this oxygen concentration range, Akkella not only showed good tolerance, but also its growth was stimulated.

By measuring OD₆₀₀ at fixed time intervals, it can be seen that the culture generally enters the stationary phase after 24 to 36 hours.

Specifically, this is achieved through the following technologies.

[0009]

A method for low-oxygen culture of *Akkermansia myxophilus*, wherein the volume ratio of the mixed gas for low-oxygen culture is: 70-94% N₂, 1-5% O₂, and 5-25% CO₂.

[0010]

The above-mentioned culture method breaks with the conventional thinking of researchers

in this field. It is different from the traditional anaerobic culture conditions of Akk bacteria and the microaerobic conditions recorded in existing literature. It was found that Akk bacteria can also grow rapidly under the above-mentioned low oxygen concentration conditions.

Further in-depth research on the hypoxic environment revealed that, under the same conditions such as the culture medium used, Akk bacteria grew more vigorously in the above-mentioned mixed gas atmosphere compared to the existing absolute anaerobic environment, and the culture time required from inoculation to entering the stationary phase was significantly shortened to 24-36 hours.

This achievement provides industry professionals with a novel method for culturing Akk bacteria, with more relaxed culture conditions. It also avoids the impact of accidental oxygen introduction on bacterial growth, thus shortening the time required for Akk bacteria-based scientific research and improving research efficiency.

[0011]

Preferably, the volume ratio of the mixed gas is: 84-90% N₂, 2% O₂, and 7-14% CO₂.

[0012]

Preferably, the volume ratio of the mixed gas is: 88% N₂, 2% O₂, and 10% CO₂.

[0013]

Preferably, the incubation time in the mixed gas is 24 to 36 hours.

[0014]

More preferably, the incubation time in the mixed gas is 14 hours.

[0015]

More preferably, the temperature for cultivation in the mixed gas is 35–40°C.

[0016]

Compared with the prior art, the advantages of the present invention are:

[0017]

1. This patent differs from existing patents and literature in that it discovers a novel low-oxygen mixed gas atmosphere with a higher oxygen concentration, suitable for the growth of Akk bacteria. Compared to using an absolutely anaerobic gas environment, Akk bacteria can grow more rapidly under this specific oxygen concentration, and the culture time for Akk bacteria to enter the stationary phase is significantly shortened. This breaks the conventional and fixed thinking of those skilled in the art that Akk bacteria are strict anaerobic bacteria. It not only greatly reduces the difficulty of Akk bacteria cultivation, but also provides another experimental design idea and theoretically feasible technical solution for the cultivation of Akk bacteria in a low-oxygen environment, achieving unexpected technical results.

[0018]

2. When culturing Akk bacteria using existing culture media, the low-oxygen environment

provided by this patented technology has a more significant promoting effect than using an absolutely anaerobic environment.

[0019]

Attached Figure Description

[0020]

Figure 1 shows the colony morphology of Akk bacteria cultured in Example 1 during the stationary phase;

[0021]

Figure 2 shows the colony morphology of Akk bacteria cultured in Example 1 during the stationary phase;

[0022]

Figure 3 shows the colony morphology of Akk bacteria cultured in Example 1 during the logarithmic phase;

[0023]

Figure 4 shows the OD_{NER20} values of Akk bacteria cultured in Examples 1-4 and Comparative Examples 1-3 at different time periods.

[0024]

Detailed Implementation

[0025]

The technical solution of the present invention will be clearly and completely described below with reference to the accompanying drawings. Obviously, the described embodiments are only some embodiments of the present invention, and not all embodiments.

Based on the embodiments of the present invention, all other embodiments obtained by those skilled in the art without inventive effort are within the scope of protection of the present invention.

[0026]

The Akk bacteria used in all the following examples and comparative examples were purchased from the China Center for Type Culture Collection.

[0027]

Examples 1-3 and Comparative Examples 1-3 used BHI (brain heart infusion) medium, which was purchased from OXOID, UK, lot number CM1135.

[0028]

Example 4 uses culture medium I, selected from the reference (Derrien M. Akkermansia muciniphilagen.nov.sp.nov.a human intestinal mucin-degrading bacterium[J].

Int.

J.Syst.

Evol.

Microbiol. 2004, 54.

The same mucin medium.

[0029]

Examples 1-3 and Comparative Examples 1-3

[0030]

The Akk bacteria culture method used in this embodiment is as follows: Akk bacteria are activated for 48 hours, inoculated into BHI medium at an inoculum of 1%, and cultured at a constant temperature of 37°C; the volume ratio of the mixed gas is shown in Table 1 below.

[0031]

Table 1. Composition and volume ratio of mixed gas in Examples 1-3 and Comparative Examples 1-3 using Culture Medium A

[0032]

N₂O₂CO₂Example 1 88% 2% 10% Example 2 70% 5%
25% Example 3 94% 1% 5% Comparative Example 1 90% 0 10% Comparative Example 2 90%
0.1% 9.9% Comparative Example 3 68% 7% 25%

[0033]

Example 4

[0034]

The Akk bacteria culture method used in this embodiment is as follows: Akk bacteria are activated for 48 hours, inoculated into culture medium I at an inoculation rate of 1%, and

cultured at a constant temperature of 37°C; the volume ratio of the mixed gas is the same as in Example 1.

[0035]

Application example: The effect of gas mixture composition and volume ratio on the growth of Akk bacteria.

[0036]

The colony composition of Akk bacteria cultured in the hypoxic mixed gas environment of Example 1 is shown in Figures 1, 2, and 3. The Akk bacteria cultured in the mixed gas environments of Examples 1-4 and Comparative Examples 1-3 were measured at 6h, 8h, 10h, 12h, 14h, 16h, 24h, 30h, and 36h after culture using a spectrophotometer at a wavelength of 600nm.

The results are shown in Table 1 below.

[0037]

Table 1. Effects of gas mixture composition and volume ratio on Akk bacteria growth
(OD_{600nm})

[0039]

The above Table 1 is plotted as a curve graph, as shown in Figure 4.

In Table 1, when OD₆₀₀ is between 0.2 and 0.3, Akk bacteria have just entered the logarithmic phase; when it is between 0.35 and 0.4, Akk bacteria are in the logarithmic phase; and when it is between 0.5 and 0.6, Akk bacteria are in the stationary phase.

As shown in Table 1 and Figure 1, using the mixed gas environment of Examples 1-5 and Comparative Examples 1-3, and using different culture media in Examples 1-4, all of them were able to enter the stable period after 24 hours and reach their maximum value after 32 or 36 hours.

[0040]

The bacteria cultured in Comparative Examples 1–3 were relatively slower; when in an absolutely anaerobic environment (i.e., Comparative Example 1), they entered the stationary phase after more than 32 hours of culture; when in a microaerobic environment (i.e., Comparative Example 2), they just entered the stationary phase after about 32 hours of culture, and reached their maximum value after about 36 hours.

[0041]

The above data shows that, under the same low-oxygen gas atmosphere, different culture media all significantly promote the growth of Akk bacteria.

However, using existing micro-oxygen or absolute anaerobic concentrations, Akk bacteria require a relatively long time to enter the stationary phase, and the time it takes for OD₆₀₀ to reach its maximum value after entering the stationary phase is also correspondingly delayed. This further illustrates that the low-oxygen environment of the mixed gas in this patent has a better promoting effect on the growth of Akk bacteria than the traditional anaerobic environment and the existing micro-oxygen environment, and can enter the stationary phase more quickly.