Establishment of Isolation Method by Co-culturing of *L.paracasei* HD1.7 and *B.subtilis* in Two Media

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***Abstract*—The co-culture of microorganisms involves at least two microorganisms or more in an environment of confined conditions, which is induced by microbe communities that are ubiquitous in nature. Therefore the attentions of people are increasingly attracted to utilize the co-culture approach in many fields. The growth of microorganisms should be detected starting from the isolation medium and each of them should be recorded respectively which are of significant importance in the process of co-culture. In this paper, L.paracasei HD1.7 and B.subtilis were applied to establish the isolation method. The growth of each of them was determined in MRS and BP medium. The results demonstrated that L.paracasei HD1.7 was not able to grow on BP medium cultivated at 37 °C for 36h, while B.subtilis could not grow on MRS medium incubated at 37 °C for 30h.**

Keywords-co-culture, L.paracasei HD1.7and B.subtilis, isolation method

I. INTRODUCTION

Biological information and methods are being updated rapidly day by day with the coming of biological age. Thus numerous of researchers contribute to biological information and methods not only for interest but also for the global problem needed to be resolved. Co-culture of microorganisms is one of the methods which are helpful to deal with some problems, which has extremely interested the scientific community [1]. Thus many applications of this strategy have been greatly performed to research the interactions among microorganisms by microbiologists for years. Furthermore, analysis of such a complex biological systems has related to the technological advance from physiological and biochemical aspects to study the influence among metabolisms over the last decade.

Actually, the co-culture method can be applied to study a range of fields. Such as the fundamental investigation of the rhizosphere microorganisms in agriculture[2]; to elucidate interaction mechanisms [3]; to investigate gastrointestinal flora interaction [4]; for the induction of interesting secondary metabolites, such as glionitrin A [5]; and to improve the production yields of specific fermentation products, such as bacteriocin synthesis [6]; to limit the growth of spoilage and pathogenic bacteria in food [7].

In this study, the purpose was to establish the isolation method by detecting the growth of each bacterium in a co-culture system. *L.paracasei* HD1.7 was co-cultured with *B.subtilis* in medium which was mixed with modified MRS medium and BHI medium selected from our preciously experiments, where both of *L.paracasei* HD1.7 and *B.subtilis* could grow well in the mixture medium. MRS and BP medium were selected to isolate the two bacteria because of the limitation of MRS and BP medium to either *L.paracasei* HD1.7 or *B.subtilis*. Finally, the isolation method was established by determining the growth of them in MRS or BP medium which could provide reference for further experiments and relative research.

II. MATERIALS AND METHODS

A. Microorganisms

Lactobacillus paracasei HD1.7 was isolated from Chinese sauerkraut juice in 2003, which can produce a non-acid antimicrobial peptide. The strain was maintained at -80℃ in 40% (v/v) glycerol, and grown in De Man-Rogosa-Sharp (MRS) medium at 37℃. *Bacillus subtilis* was stored at -80 ℃ in 40% (v/v) glycerol, and grown in Beef extract –peptone (BP) medium at 37 ℃. They were sub-cultured three times at 37℃ for 18-20h before the experimental into MRS and BP medium, respectively.

B. Medium and cultivation conditions

Fresh MRS and BP medium were inoculated with 1% of an overnight culture of *L.paracasei* HD1.7 (with cell density of 10^8/ml) and the incubation was at 37 ℃,for 60h at a shaking speed of 180rpm. Similarly, fresh MRS and BP medium were inoculated with 1% of an overnight culture of *B.subtilis* (with cell density of 10^8/ml) and the incubation condition was identical.

C. Cell growth in co-culture system

L.paracasei HD1.7 and *B.subtilis* were propagated in modified MRS and BHI medium at 37℃ for 18h before inoculated into 250 ml flasks containing 100 ml fermentation medium which was prepared by mixing equal volumes of modified MRS and BHI medium with initial pH 6.5. And the inoculation volume was in the proportion of 1%, 1% and 2%, 2% and 1% of *L.paracasei* HD1.7 and *B.subtilis* respectively which were incubated at 37℃ for 60h at a shaking speed of 180rpm.

D. Detection methods

Samples of *L.paracasei* HD1.7 and *B.subtilis* from the fermentation broth were taken at 0, 4, 8, 12, 24, 30, 36, 48 and 60h of the fermentation to determine its optical density (OD) at 600nm.

In co-culture system, samples were also harvested for monitoring growth of the cells at 0, 4, 8, 12, 24, 30, 36, 48 and 60h of the fermentation process. The growth was determined by plate counts on MRS and BP agar plates for *L.paracasei* HD1.7 and *B.subtilis*, respectively.

III. RESULTS AND DISCUSSION

A. The growth of L.paracasei HD1.7 in MRS and BP medium

As shown in Fig.1, *L.paracasei* HD1.7 grew well in MRS medium while it grew really poor in BP medium. The maximal biomass of *L.paracasei* HD1.7 was achieved in the end of exponential phase after cultivated for 36 hours in MRS broth. However, the growth of *L.paracasei* HD1.7 began at 36 hours in BP medium which may be explained that the BP broth was shortage of some components which are necessary for *L.paracasei* HD1.7.

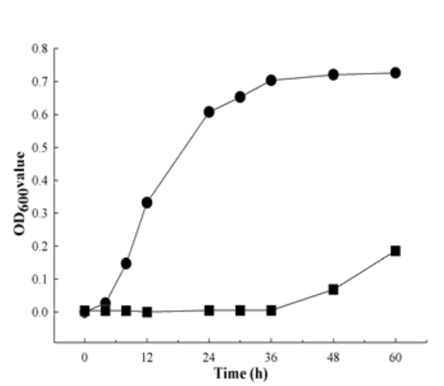

Figure 1. The growth of *L.paracasei* HD1.7 in MRS (●) and BP (■) broth. The ***values were the mean of three independent samples.***

B. The growth of B.subtilis in BP and MRS broth

As shown in Fig.2, *B.subtilis* grew well in BP medium, while it grew really poor in MRS medium. The maximal biomass of *B.subtilis* was obtained in the end of exponential phase after cultivated for 30 hours in BP medium. However, the growth of *B.subtilis* began at 30 hours in MRS medium which may be explained that the components in MRS medium could retard the growth of *B.subtilis.*

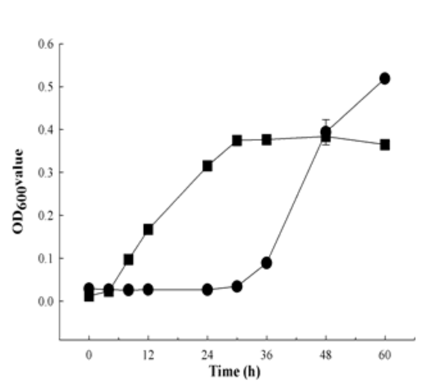

Figure 2. The growth of *B.subtilis* in BP (■) and MRS (●) broth. The values were the mean of three independent samples.

The pH and compositions of culture media are crucial for bacteria growth. Compared with the MRS media, the BP media lacks a large amount of carbon sources such as glucose and inorganic salts which are necessary for the growth of *L.paracasei* HD1.7. Simultaneously, the pH of BP media (7.0) is not suitable for *L.paracasei* HD1.7. Thus, *L.paracasei* HD1.7 needs to take dozens of hours to adapt itself to BP media. While the MRS media contains some compositions such as Diammonium citrate, $MgSO_4$, $MnSO_4$, Tween-80 and $CH_3COONa·3H_2O$, they are the inhibitory factors for the growth for *B.subtilis* and many other bacteria. Furthermore, these factors are necessary for the growth of *L.paracasei* HD1.7, thus, the MRS medium is a medium only suitable for *L.paracasei* HD1.7.

C. Cell growth of L.paracasei HD1.7 and B.subtilis in co-culture system

As shown in Fig.3A, *L.paracasei* HD1.7 was able to grow well and the maximum biomass was observed at 36h (about 8.5log CFU/ml) while the growth of *B.subtilis* was inhibited after 30h in co-culture system. Compared with the result in Fig.3A, *B.subtilis* went to stationary phase rapidly at 24h and the maximum cell density was about 8.5log CFU/ml because of the twice more of inoculum volume (Fig.3B). Furthermore, *L.paracasei* HD1.7 grew well and went to stationary phase at 30h, however, the growth of *B.subtilis* was seriously inhibited after 24h (Fig.3C).

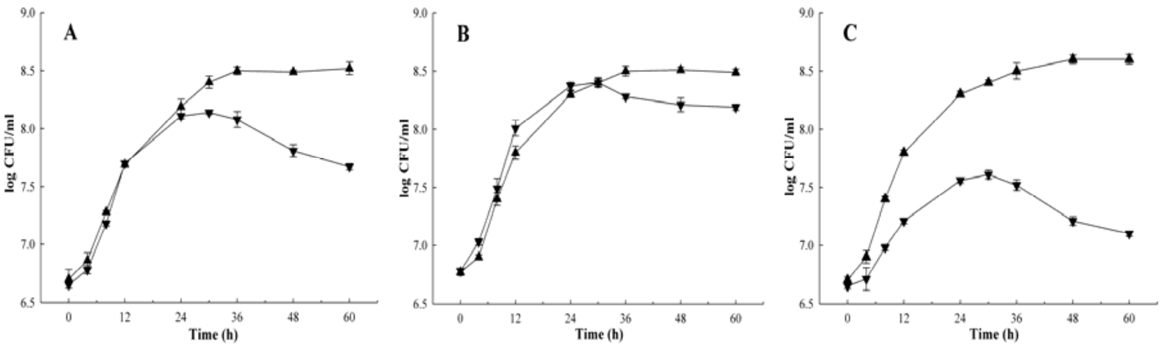

Figure 3: Cell growth of *L.paracasei* HD1.7 (▲) and *B.subtilis* (▼) with the different inoculation volumes incubated at 37℃ in co-culture system. A: the inoculation volume was in the proportion of 1% of *L.paracasei* HD1.7 and *B.subtilis*. B: the inoculation volume was in the proportion of 1% and 2% of *L.paracasei* HD1.7 and *B.subtilis*. C: the inoculation volume was in the proportion of 2% and 1% of *L.paracasei* HD1.7 and *B.subtilis*. The values were the mean of three independent samples.

Actually, *B.subtilis* existed as spores at later stages of stationary phase which could resist the inhibition from *L.paracasei* HD1.7 in formation of spores. The inhibition to growth of *B.subtilis* was caused by bacteriocin that was produced by *L.paracasei* HD1.7 in stationary phase. The bacteriocin was named paracin 1.7 which could be applied to food industry as biopreservatives due to its broad inhibition spectrum with wider tolerance to pH and better heat stability [8-9]. The inhibition could also be resulted from many other factors such as competition for nutrients and existing space, production of organic acids in the fermentation process [10]. The organic acids such as lactic acid, acetic acid and citric acid produced by *L.paracasei* HD1.7 could lead to decrease of pH value and thus the growth of *B.subtilis* was inhibited [11].

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