乳酸菌の増殖及び乳酸発酵に対するホエーシロップの促進効果

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原　報

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Stimulatory Effect of Whey Syrup on Growth of Lactic Acid Bacteria and Lactic Acid Fermentation

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Abstract

Whey syrup, prepared by immobilized β-galactosidase, and whey were added separately to 10% skim milk culture media, and then incubated with 2% of a fresh culture starter of *Lactobacillus delbrueckii* subsp. *bulgaricus* at 37°C for 24 hr. The growth of *Lb. bulgaricus* and change in acidity were observed during the incubation. The addition of whey syrup and whey both stimulated the growth of *Lb. bulgaricus* in the skim milk culture media. However, the addition of whey syrup was more effective than that of whey. The lag phase of growth of *Lb. bulgaricus* with the addition of whey syrup was 2 hr shorter than that with the addition of whey. When whey syrup and whey were added to skim milk culture media, the acidities were higher than the control sample during incubation, suggesting that both whey syrup and whey had a good stimulatory effect on lactic acid fermentation. Furthermore, the lactic acid production was higher with the addition of whey syrup than with the addition of whey within the logarithmic phase of growth of *Lb. bulgaricus*. The results show that the whey syrup is a better substrate than whey for stimulating lactic acid fermentation.

Key words: Whey syrup, Growth of *Lb. bulgaricus*, Lactic acid fermentation.

The cheese and casein industry produces large amounts of whey, 27 million tonnes/yr in the USA alone[1]. Whey is used in infant formula, dairy products, animal feed and several industrial fermentations[2]. However, despite its many uses, there is a huge surplus of whey because of the continuous increase in cheese production. The dumping of whey with a high BOD into rivers and streams has caused serious environmental pollution[3]. On the other hand, the unused volume of whey, which corresponds to approximately 90% of whole milk, is the reason for high production costs in the cheese industry.

As a way to solve the whey surplus problem, Wierzbicki and Kosikowski (1972) first reported the preparation of whey syrup from acid whey using *A. niger* β-galactosidase[4,5]. Recently, more studies have been carried out on the production of whey syrup from whey using immobilized β-galactosidase. These studies have shown that immobilized β-galactosidase can be used to produce whey syrup from whey in the dairy industry[6~8].

Lactobacillus delbrueckii subsp. *bulgaricus* is a thermophilic lactic acid bacterium, which is mainly used in the preparation of yogurt (in association with *Streptococcus thermophilus*) and various fermented milks, and for the manufacture of various types of cheese[9]. The main compounds responsible for stimulating growth of *Lb. bulgaricus* have been identified as amino acid, peptides and various purines[10]. Champagne et al. have produced starter cultures on whey concentrates[11]. Baig and Prasad reported that incorporation of whey solids stimulated the growth of *Lb. bulgaricus* in yogurt[12]. However, there have been no reports on the stimulatory effects of whey syrup on the growth of lactic acid bacteria and lactic acid fermentation.

The aim of the present experiment was to investigate the stimulatory effects of whey syrup on the growth of *Lb. bulgaricus* and lactic acid fermentation in skim milk. A comparison of the stimulatory effects of whey syrup and whey was also made.

Materials and Methods

Bacterial strain

The lactic acid bacterium used in this study was *Lactobacillus delbrueckii* subsp. *bulgaricus* B–5b, which was obtained from the Institute of Physical and Chemical Research (RIKEN), Japan. It was subcultured three times in reconstituted sterilized 10% (w/v) skim milk containing 0.3% yeast extract for 20 h at 37℃.

Whey and whey syrup

In the present experiment, all the whey and whey syrup were prepared from a demineralized whey powder, which was obtained from Domo Food Ingredients Ltd., the Netherlands. The whey powder was dissolved in deionized water, and whey proteins were removed by acidifying with 0.5 M HCl until pH reached 4.2, followed by heating (55℃, 5 min) and centrifugation (7000 × G, 5 min). The composition of the resultant whey was 13.50% lactose, 0.47% protein, 0.96% minerals and 84.48% water. As a substrate, whey was diluted to a lactose concentration of 13.3%, and pH was adjusted back to 4.5 prior to use.

According to the optimum reaction conditions (13.3% lactose content, 45℃ and pH4.5) for immobilized β–galactosidase (Sumitomo Chemical Co., Ltd., Japan), 2 g of enzymes per 100 m*l* of whey was added and shaken in an oil bath at 45℃. Whey syrup was obtained 40 min after the start of the reaction and filtered rapidly to remove immobilized enzymes. HPLC analysis of the sugar constituents in the whey syrup showed the sugar composition to be 4.30% lactose, 4.43% glucose, 2.56% galactose and 2.01% oligosaccharide.

Preparation of samples

All experiments were carried out in a 10% reconstituted skim milk medium consisting of 5.24% (w/v) lactose, 3.48% protein, 0.08% fat, 0.80% minerals and 90.40% water. To prepare each sample, 10 m*l* of whey syrup was added to 90 m*l* of the skim milk culture medium and was then pasteurized at 65℃ for 30 min. Two milliliters of active (10^8–10^9 cfu/g) starter cultures of *Lb. delbrueckii* subsp. *bulgaricus* B–5b were inoculated and incubated at 37℃ according to Thornhill and Cogan[13]. The same amount of whey or water was added to the skim milk culture media as controls. All samples were allowed to stand overnight at 4℃ and used for the determination of acidity and the enumeration of bacterial counts.

Enumeration of bacteria and determination of acidity

Bacteria in the samples were counted on Standard Plate Count Agar (Oxoid, Ltd., USA). The plates were incubated at 37°C for 12 hr as described by Marshall[14]. Different colonies were randomly selected from countable plates. The acidities of the samples were determined every 2 hr over a period of 24 hr by the standard method[15].

Results and Discussion

Effect of whey syrup on the growth of *Lb. bulgaricus*

At first, we studied the effects of different amounts of whey syrup on the growth of *Lb. bulgaricus*. The results are shown in Fig. 1. Without the addition of whey syrup, the *Lb. bulgaricus* count was 6.51×10^8 cfu/m*l* in 10% skim milk culture media incubated at 37°C for 6 hr. As the amount of whey syrup added to the culture medium was increased, the *Lb. bulgaricus* counts also increased, suggesting that the addition of whey syrup to a skim milk culture medium stimulates the growth of *Lb. bulgaricus*. With an addition of 1–4% whey syrup, the *Lb. bulgaricus* counts increased slowly; however, when the amount of whey syrup was increased to 5–10%, the *Lb. bulgaricus* count increased remarkably. The rate of increase slowed when the amount of whey syrup was increased to more than 10%. It was therefore concluded that the best amount of added whey syrup for stimulating the growth of *Lb. bulgaricus* in skim milk medium was 10%.

Thus, 10% of whey syrup or whey was added to the skim milk culture media and incubated at 37°C for 24 hr; the growth curves of *Lb. bulgaricus* are shown in Fig. 2. Before incubation, the *Lb. bulgaricus* count in all samples was about 5×10^6 cfu/m*l*. When the whey syrup was added, the first 2 hr of incubation was the lag phase of the growth of *Lb. bulgari-*

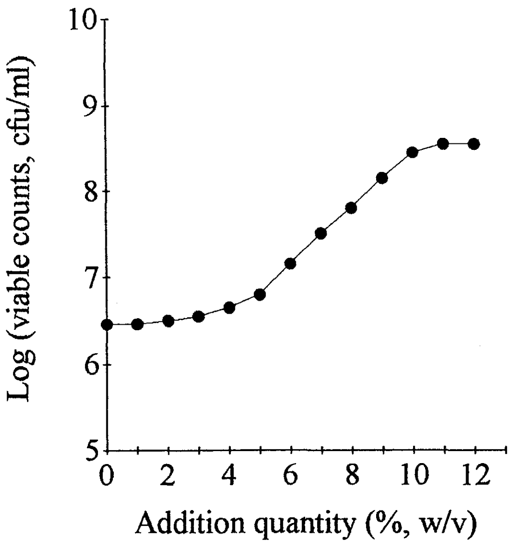

Fig. 1 Effect of the addition of various quantities of whey syrup on the growth of *Lb. bulgaricus* in 10% skim milk culture media incubation for 6 hr at 37°C

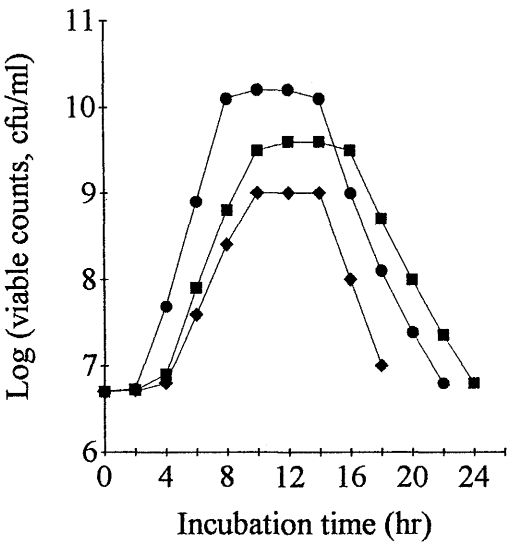

Fig. 2 Growth curves of *Lb. bulgaricus* in 10% skim milk culture media incubation for 24 hr at 37°C.
●: 10% whey syrup-added. ■: 10% whey-added.
◆: control.

cus, and the increase in *Lb. bulgaricus* counts was low. The logarithmic growth phase, characterized by a constant specific growth rate, started after 2 hr of incubation. After 8 hr of incubation, the *Lb. bulgaricus* count reached 1.52×10^{10} cfu/m*l* and maintained this level with little variation until 14 hr. The final growth phase of *Lb. bulgaricus* was the decline phase, which started after 14 hr of incubation. The *Lb. bulgaricus* count decreased rapidly in this phase.

The growth curves of *Lb. bulgaricus* in the control samples also showed the same four phases as those observed with the addition of whey syrup. When 10% whey was added and incubated at 37°C, the length of the lag phase was 4 hr. After 4 hr of incubation, the growth of *Lb. bulgaricus* entered the logarithmic phase. The stationary phase was from 10 to 16 hr, with a maximum *Lb. bulgaricus* count of 4.85×10^{9} cfu/m*l*. In the control samples, the maximum *Lb. bulgaricus* counts did not exceed 8.91×10^{8} cfu/m*l* during the incubation period. Thus, these results suggest that whey syrup and whey in the skim milk culture media have stimulatory effects on the growth of *Lb. bulgaricus* and that the addition of whey syrup is more effective than the addition of whey.

As shown above, the addition of whey syrup shortened the lag phase by 2 hr compared to the controls, indicating that whey syrup could have a significant role in fermented milk production. According to a report by Hayes[16], bacteria multiply during the lag phase due to division of cells. The rate of division depends upon a number of factors, which will be considered later, but under favorable conditions, divisions could occur approximately every 20 min.

Effect of whey syrup on lactic acid fermentation

The increases in acidity during the growth of *Lb. bulgaricus* in skim milk culture media with the addition of whey syrup or whey are shown in Fig. 3. Before incubation, the acidity of 10% skim milk culture media was low, i. e., about 0.15% (g/100 m*l*). With the addition of whey syrup, the acidity increased slowly during the first 2 hr of incubation. After 2 hr of incubation, the acidity increased rapidly at a constant rate. The final acidity with the addition of whey syrup reached 1.25% after 20 hr of incubation.

With the addition of whey, the acidity increased rapidly after 4 hr of incubation, and the final acidity was almost the same as that with the addition of whey syrup at the end of incubation. In the control sample, the final acidity after 24 hr of incubation was only 0.89%. Because *Lb. bulgaricus* is a homofermentative lactic acid bacterium, an increase in acidity implies production of lactic acid and the occurrence of lactic acid fermentation in

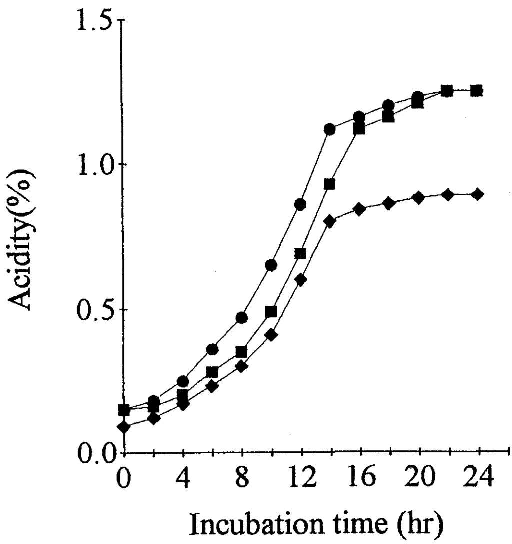

Fig. 3 Changes in acidity in 10% skim milk culture media with *Lb. bulgaricus* during 24 hr incubation at 37°C.
●: 10% whey syrup-added. ■: 10% whey-added.
◆: control.

milk. The above results show that the whey syrup and the whey have a better stimulatory effect on the lactic acid fermentation. Furthermore, when whey syrup was added, lactic acid production was higher than that of whey within the first 12 hr of incubation (Fig. 3). This indicates that the whey syrup is a better substrate than whey for stimulating lactic acid production and lactic acid fermentation.

Thus, whey syrup has a greater stimulatory effect than dose whey on the growth of *Lb. bulgaricus* and on lactic acid fermentation. This can be explained by the nutritional requirements of lactic acid bacteria and the mechanism of lactic acid fermentation. Lactic acid bacteria differ greatly in their nutritional requirements, but certain materials in milk are essential and growth is impossible in their absence. The most important materials for the growth of lactic acid bacteria are carbohydrates, amino acid and small quantities of iron. Carbohydrates are required by lactic acid bacteria not only as a source of energy but also for manufacturing protoplasm and structural materials. When whey syrup or the whey was added, the concentrations of nutritional materials in the skim milk culture medium increased. As a result, the *Lb. bulgaricus* grew better and acidity was higher than without addition.

The most prominent carbohydrate in milk is lactose, which has a constant concentration of between 4.5 and 5%. However, according to the mechanism of lactic acid fermentation, lactic acid bacteria can not utilize lactose directly to produce lactic acid. The first step in lactic acid fermentation is hydrolysis of lactose to glucose and galactose. Most homofermentative lactic acid bacteria have a phosphotransferase system in the cell membrane that phosphorylates lactose at C–6 of the galactose moiety as it enters the cell. A β–D–phosphogalactosidase then hydrolyzes the lactose–P to glucose and galactose–6–P. The glucose is phosphorylated at C–6 and metabolized to lactic acid via the Embden-Meyerhof pathway of glycolysis[17]. When whey syrup was added to the skim milk culture media, glucose produced by the hydrolysis of lactose could be directly utilized and entered the Embden-Meyerhof pathway to produce lactic acid; therefore, the lag phase of growth of *Lb. bulgaricus* was shorter and the acidity was higher than with the addition of whey.

The results of this study showed that whey syrup can stimulate the growth of lactic acid bacteria and lactic acid fermentation. Based on the mechanism of lactic acid fermentation, we assume that this stimulatory effect is caused mainly by glucose in the whey syrup. On the other hand, some researchers have suggested that oligosaccharide can stimulate the growth of bacteria[18,19], such as *Bifidobacterium*. Therefore, further study is needed to elucidate the effects of glucose and oligosaccharide.

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乳酸菌の増殖及び乳酸発酵に対するホエーシロップの促進効果

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チーズホエーを固定化β-ガラクトシダーゼで処理して調製したホエーシロップ及び未処理ホエーを還元脱脂乳に添加し，乳酸菌スターター（*Lactobacillus delbrueckii* sp. *bulgaricus* B-5b）2%を加えて37℃, 24時間培養を行い，乳酸菌増殖及び酸度の変化について検討した。その結果，ホエーシロップ及び未処理ホエーの添加によって乳酸菌増殖の促進効果を認めた。また，ホエーシロップの添加は未処理ホエーよりも乳酸菌増殖の促進効果が著しく，乳酸菌増殖の誘導期が約2時間短縮されることを明らかにした。ホエーシロップ及び未処理ホエーの添加によって乳酸発酵が促進されて酸度が上昇するが，乳酸の生成量は前者が後者よりも多く，そのため，固定化β-ガラクトシダーゼでチーズホエーを処理して調製したホエーシロップは乳酸菌の増殖及び乳酸発酵の促進効果が顕著であることを認めた。