

Research on Lipid-lowing Capability of Three Edible *Streptococcus* from Yogurt in Vitro

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Abstract. Three common edible *streptococcus thermophilus* (St), *streptococcus lactis* (L1) and *streptococcus cremoris* (L2) from yogurt were used to investigate the activity of reducing lipid in vitro. All of the three *streptococcus* could tolerate adverse effects by low acid and high bile salts to survival and reproduction. St had the strongest tolerance with artificial gastric juice and 0.3% porcine bile salt, and still alive 5.02×10^6 CFU/mL. The rates of degrading triglyceride and triglyceride of St were up to 70% and 50%, which were also the best of the three. Next was L1, it could degrade more than 70% cholesterol and 48% triglyceride. And the rates of degrading triglyceride and triglyceride of L2 were 10-20%.

1. Introduction

Streptococcus thermophilus (St), *streptococcus lactis* (L1) and *streptococcus cremoris* (L2), which are known as *streptococcus acidilactici*, usually are found in common yoghurt products. The three *streptococcus* had functions of fermenting sugar into lactic acid, improving food flavor and nutritional value^[1-3]. As probiotics, they could develop a variety of activities in the human body, such as regulating the body normal flora of the gastrointestinal tract, maintaining the ecological balance of intestinal microorganism, reducing serum cholesterol, inhibiting the growth of intestinal pathogen, control endotoxin and improving intestinal immune^[4-7]. Therefore, these lactic acid bacteria usually were added to dairy products to improve nutritional values and healthy functions. However, the precondition of these active functions were that the probiotics should survive and multiply in the intestine, and the breeding environment was vital to their survival and once a change would cause death^[8,9]. The process of entering the intestines, probiotics should pass through saliva and gastric juice with the low acid environment, intestinal juice with high bile salt, stimulation with physical friction and biochemical reactions^[10]. In this research, through simulating stimulation of digestive tract with low acid and high bile salts in vitro, three common edible *Streptococcus acidilactici* (St, L1 and L2) from yogurt were used to investigate the activity against hyperlipaemia.

2. Materials and methods

2.1. Preparation of medium and artificial gastric juice

Cholesterol medium: 0.1g cholesterol was added 0.1g sucrose ester, 1mL Twain-80 and 5 mL acetic acid, and stir well, then MRS medium was added into the mixed liquid with a volume ratio of 1:15 for



0.1g/L cholesterol. The value of pH was adjusted to 6.3-6.5 by sodium acetate, and the medium was autoclaved at 121°C for 15min.

Triglyceride medium: 0.1g triglyceride was added 1mL Twain-80, and stir well. Then MRS medium was added into the mixed liquid, adjusting pH to 6.3-6.5 by sodium acetate and autoclaved at 121°C for 15min.

Artificial gastric juice: 23.1mL hydrochloric acid was diluted with 100mL distilled water, and taking 16.4mL from it to add 800mL water. Then 10g pepsin was added and diluted to 1000mL with distilled water, stored at 4°C.

2.2. The activation of *Streptococcus* and the preparation of seed culture fluid

Streptococcus thermophilus (St), *Streptococcus lactis* (L1) and *Streptococcus cremoris* (L2), which were purchased from Shanghai North Nuo Biotech Corp, were respectively inoculated in MRS medium, and cultured for 48h at 37°C. Then the colony of bacteria with good growth was added in 120mL MRS medium, cultured for 24h at 37°C and stored at 4°C as the seed medium.

2.3. Determination of the optimum temperature and pH for *Streptococcus acidilactici*

The seed medium was added in culture medium for 3 per cent of total volume, and the mixed medium was divided into 5 parts and respectively cultured at 30, 35, 37, 40, 45°C for 48h. OD values of *Streptococcus* culture medium were detected at 600nm, and aseptic medium was used as blank control. The seed medium was divided into 5 parts and respectively added in culture medium with pH 5.5, 6, 6.5, 7, 7.5 for 3 per cent of total volume, and cultured for 24h at 37°C. Then OD values of culture medium were detected at 600nm, and aseptic medium with pH 5.5, 6, 6.5, 7, 7.5 were used as corresponding blank control.

2.4. Determination of tolerance of *Streptococcus acidilactici* to artificial gastric juice

5mL seed medium was centrifuged 10000r/min for 10min, bacteria precipitates were added 5mL normal saline and 5mL artificial gastric juice, stir well and cultured for 3h at 37°C. The culture medium was diluted with normal saline to $10^3 \sim 10^4$ CFU/mL by bacteria count technique.

2.5. Determination of tolerance of *Streptococcus acidilactici* to bile salt

The seed medium was divided into 6 parts and respectively added in MRS medium with 0.1%, 0.2%, 0.3%, 0.4%, 0.5% and 0.6% pig bile salt for 3 per cent of total volume, and the mixed mediums were cultured for 3h at 37°C and measured bacterial concentration with bacteria count technique.

2.6. Determination of the rate of degrading cholesterol of *Streptococcus acidilactici*

The seed medium was added in cholesterol medium for 3 per cent of total volume, cultured at 37°C with 140r/min for 3h, and centrifuged 5000r/min for 10min. The supernatant was used to analyze the contents of cholesterol with cholesterol determination kit.

2.7. Determination of the rate of degrading triglyceride of *Streptococcus acidilactici*

The seed medium was added in triglyceride medium for 3 per cent of total volume, cultured at 37°C with 140r/min for 3h, and centrifuged 5000r/min for 10min. The supernatant was used to analyze the contents of triglyceride with cholesterol determination kit.

3. Results

3.1. Growth of the optimum of temperature and pH of *Streptococcus acidilactici*

As shown in Figures 1 and 2, the optimum temperature of three *Streptococcus acidilactici* was 37°C, and the growth of L1 and L2 were similar at different temperatures. However, growth of three *Streptococcus* were different with different pH, and the optimum pH of L1 and St were 6.5, and L2 was 6.

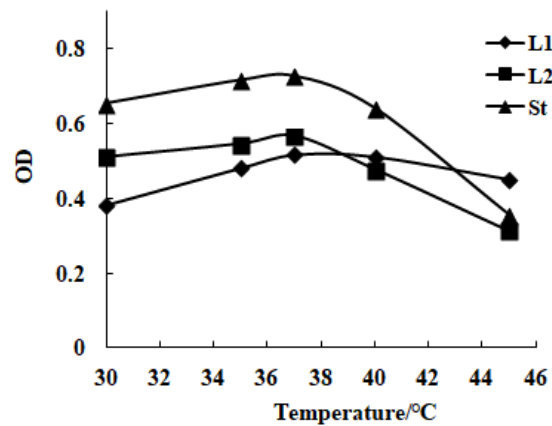


Figure 1. Effects of temperature on the activity of three *Streptococcus*.

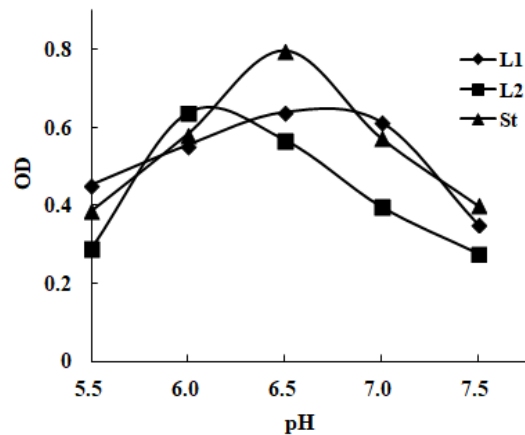


Figure 2. Effects of pH on the activity of three *Streptococcus*.

3.2. Tolerance of *Streptococcus acidilactici* to bile salt and artificial gastric juice

The levels of bile salt usually were 0.03~0.3%, and probiotics should survive, grow and reproduce with 10^6 CFU/mL for appearance functions in this condition. As shown in Table 1, three *Streptococcus acidilactici* were still alive at 0.3% bile salt, L2 and St of survival were more than 10^6 CFU/mL and both were relatively well tolerated. The strongest tolerance of three was St, which was only one survived and alive with 2.39×10^5 CFU/mL at 0.6% bile salt. Three *Streptococcus acidilactici* had tolerance of artificial gastric juice, both L1 and St were more than 10^6 CFU/mL and well-tolerated. Moreover, under the condition of artificial gastric juice and 0.3% porcine bile salt, St still alive with 5.02×10^6 CFU/mL and had the best tolerance for bile salt and artificial gastric juice.

Table 1. Tolerance of three *Streptococcus acidilactici* to bile salt.

Source	<i>Streptococcus</i>		
	L1 (CFU/mL)	L2 (CFU/mL)	St (CFU/mL)
0.0% bile salt	5.76×10^7	5.24×10^7	5.34×10^7
0.1% bile salt	3.71×10^6	8.56×10^6	5.01×10^7
0.2% bile salt	2.85×10^6	3.49×10^6	9.87×10^6
0.3% bile salt	7.74×10^5	1.52×10^6	5.27×10^6
0.4% bile salt	3.52×10^4	8.6×10^4	4.87×10^6
0.5% bile salt	—	4.43×10^4	5.72×10^5
0.6% bile salt	—	—	2.39×10^5
Artificial gastric juice	5.72×10^6	7.65×10^5	7.86×10^6
Artificial gastric juice + 0.3% Bile salt	3.32×10^4	4.13×10^5	5.02×10^6

3.3. Cholesterol degradation rate of three *Streptococcus acidilactici*

Figure 3 shows the cholesterol degradation rate of three *Streptococcus acidilactici*. There were activities of degrading cholesterol in all of the three. After 24 hours of the culture, L1 and St had higher rate of cholesterol degradation than L2. Both L1 and St could degrade 60% cholesterol at 32h, and after 48h the rates of cholesterol degradation were more than 70% in L1 and St.

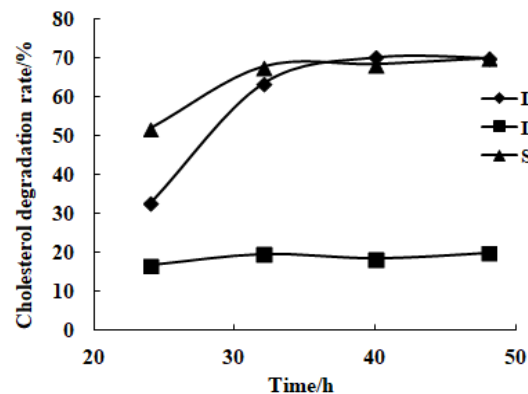


Figure 3. Cholesterol degradation rate of three *Streptococcus*.

3.4. Triglyceride degradation rate of three *Streptococcus acidilactici*

As shown in Figure 4, L2 had a lower level of triglyceride degradation than L1 and St, and the rate of triglyceride degradation was only 0-10%. L2 could degrade more than 45% triglyceride after 40h, and St had the strongest activities of degrading triglyceride at 24-36h or 48h.

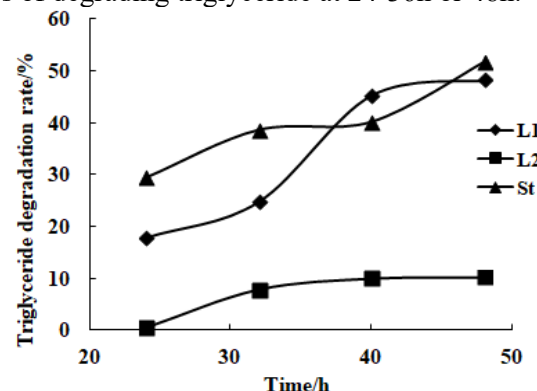


Figure 4. Triglyceride degradation rate of three *Streptococcus*.

4. Conclusion and analysis

Streptococcus lactis, as probiotics, should pass the digestive with low pH value, high bile salt and other effect of adverse stimulative to entry into the intestines smoothly, and reproduced in a particular area and multiplied until sufficient numbers to exert bioactivity^[11]. There was a research shown that *Streptococcus thermophilus* could tolerate adverse effects by low acid and high bile salts, and successful survival and reproduction^[12,13]. At present, there were two views on lipid-lowering function of probiotics: first, the bacteria could absorb and degrade cholesterol and free triglyceride in the environment; second, bile salt hydrolase of bacteria could decompose cholic acid and combine with cholesterol to be caused precipitation^[14-16].

In this study, *Streptococcus thermophilus* (St), *Streptococcus lactis* (L1) and *Streptococcus cremoris* (L2), which were common *streptococci* in fermented milk, had the tolerance for bile salt and artificial gastric juice, and St had the best tolerance under the condition of artificial gastric juice and 0.3% porcine bile salt and still alive with 5.02×10^6 CFU/mL. Moreover, there were activities of degrading cholesterol and triglyceride in all of the three. St had the best lipid-lowering function, the

rate of cholesterol degradation was up to 70% and the triglyceride degradation rate was 50%. Followed by L1, it could degrade more than 70% cholesterol and 48% triglyceride. And the rates of degrading triglyceride and triglyceride still were 10-20% in L2. The results showed that St, L1 and L2 could tolerate the low acid and high bile salt, degrade cholesterol and triglyceride, and show effective activity of reducing lipid.

Acknowledgments

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References

- [1] Waters D M, Mauch A, Coffey A, Arendt E K and Zannini E 2015 *Crit. Rev. Food. Sci* **55** 503-20
- [2] Garrigues C, Loubiere P, Lindley N D and Coccagnibousquet M 1997 *J. Bacteriol* **179** 5282
- [3] Bonestroo M H, Kusters B J and Wit J C 1992 *Int. J. Food Microbio* **15** 365
- [4] Chang G S, LI G P and Liu Z 2011 *J. Dairy Sci. Tech* **34** 288-92
- [5] Yang F Y, Peng L U, Lan X H and Wu F U 2014 *Chinese. J. Microecolo* **26** 735-9
- [6] Hassan M R, Choe H S and Ryu K S 2012 *Korean. J. Poultryence* **39** 253-60
- [7] Mei L, Tang Y, Li M, Yang P and Liu Z 2015 *Plos One* **10** 1-16
- [8] Vries M C D, Vaughan E E, Kleerebezem M and Vos W M D 2006 *Int. Dairy. J* **16** 1018-28
- [9] Tachon S, Lee B and Marco M L 2015 *Environ. Microbiol* **16** 2915-26
- [10] Ashraf R and Smith S C 2016 *International Food Res. J* **23** 777-89
- [11] Ridlon J M, Kang D J and Hylemon P B 2006 *J. Lipid. Res* **47** 241-59
- [12] Hatice B, Belma A and Gulcin A 2010 *Arch. Biol. Sci* **62** 323-8
- [13] Khalil R 2009 *Pol. J. Microbiol* **58** 49-55
- [14] Marhamatizade M H 2015 *Original Article* **14** 10-5
- [15] Liao X, Guo L, Qiu L, Gu F, Lin J, etc 2016 *J. Chin. Inst. Food Sci* **16** 11-16
- [16] Wang S C, Chang C K, Chan S C, Shieh J S, Chiu C K, etc 2014 *Asian Pac. J. Tropical Bio* **4** 523-8