Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(4):617-622

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Effect of carbon sources including lactose, glucose and maltose on growth of *B. bifidum* in the extract of *Fructus tribuli*

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ABSTRACT

In this study, the effect of three kinds of carbon sources including lactose, glucose and maltose on growth of Bifidobacteria bifidum BB01 and BB28 in the extract of Fructus tribuli was studied by measuring optical density at 600nm (OD_{600}) and pH. The concentration of three carbon sources was 0.00%, 1.60%, 1.80%, 2.00%, 2.20% and 2.40%. Results were as follows: addition of glucose, maltose and lactose in the extract of Fructus tribuli could promote the growth of Bifidobacteria bifidum BB01, maltose and lactose added to the extract of Fructus tribuli could significantly promote the growth of Bifidobacterium bifidum BB01 but inhibit the growth of Bifidobacterium bifidum BB28. The optimum carbon source on growth of two Bifidobacteria in the extract of Fructus tribuli was glucose. The optimum concentration of Glucose in the extract of Fructus tribuli was 2.40% for Bifidobacterium bifidum BB01and 2.20% for Bifidobacterium bifidum BB28, respectively.

Keywords: lactose; glucose; maltose; *Bifidobacteria bifidum*; *Fructus tribuli*

INTRODUCTION

Bifidobacterium is among the friendly microorganisms that have been shown to alleviate symptoms of inflammatory bowel disease. Bifidobacterium is a Gram-positive, anaerobic, branched rod-shaped bacterium [1]. They are ubiquitous, endosymbiotic inhabitants of the gastrointestinal tract, vagina and mouth of mammals and other animals [2]. Bifidobacteria is one of the major genera of bacteria that make up the colon flora in mammals. Bifidobacteria is used in treatment as so-called "probiotics," the opposite of antibiotics [3]. Antibiotics disrupt the balance of natural intestinal flora. Bifidobacterium promotes good digestion, boosts the immune system. In the intestines, they ferment sugars to produce lactic and acetic acid that controls intestinal pH [4]. These bacteria also inhibit the growth of pathogenic and putrefactive bacteria such as *Candida albicans*, *Escherichia coli*, *Helicobacter pylori*, *Clostridium difficile*. Bifidobacteria is used for many conditions affecting the intestines, including prevention of infectious diarrhea (traveler's diarrhea, children's acute viral diarrhea), antibiotic- associated diarrhea, and irradiation-associated diarrhea [5]. Some people take bifidobacteria to restore "good bacteria" in the gut that have been killed or removed by diarrhea, radiation, chemotherapy, antibiotics, or other causes [6]. Some Bifidobacterium strains are also used in the food industry. Different species or strains of Bifidobacteria may exert a range of beneficial health effects, including the regulation of intestinal microbial homeostasis, the inhibition of pathogens and harmful bacteria that colonize or infect the gut mucosa, the modulation of local and systemic immune responses [7]. The most recognized species of bifidobacteria used as probiotics are *Bifidobacterium animalis*, *Bifidobacterium adolescentis*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium longum and Bifidobacterium lactis.*

Tribulus terrestris has long been a constituent in tonics in Indian ayurveda practice, where it is known by its Sanskrit name, "gokshura ". In China, it is offen called "Jili". It is an annual herb that grows all over the world,

especially in the Africa, Europe, America and Australia [8], subtropical area of Asia. It has been reported that saponins, polysaccharide, alkaloids, amides, flavonoids and cinammic acid occurred at fruits of *T. terrestris* [9-12].

In our previous research, we found the hot water extracts from fruits of *T. terrestris* added to MRS broth can promote the growth *Bifidobacterium bifidum, L.acidophilus, L.bulgaricus, L. casei, L.reuteri, L.rhamnosus* [13-16], so we thought that the extracts from fruits of *T. terrestris* can be used to cultured probiotics and maybe a useful alternative to MRS broth through adding some nutrients such as carbon sources, nitrogen source, salts and growth factor. The purpose of the present work was to study the effect of three carbon sources including glucose, maltose and lactose on growth of Bifidobacteria bifidum BB01 and BB28 in the extract of *Fructus tribuli*.

EXPERIMENTAL SECTION

Microorganism

Bifidobacterium bifidum BB01 and *BB28* used in the study were obtained from College of Life Science & Engineering, Shaanxi University of Science & Technology.

Preparation of the extract from *Fructus tribuli*

Dried fruits of *Fructus tribuli* were bought from a local herb market (Xi'an, China) and was ground in a high speed disintegrator (Model SF-2000, Shanghai, China) to obtain a fine powder, then was extracted distilled water (liquid/solid ratio (ml/g)14/1, While the temperature (95℃) of the water bath was kept steady, The extraction in a 1.0 l stainless steel boiler in the water bath was stirred with an electric mixing paddle for 1.5h, then was centrifuged (3000g, 15 min), the supernatant was separated and obtained the extract of *Fructus tribuli*.

Culture conditions

The extract of *Fructus tribuli* act as natural medium, the addition of various carbon sources were all 1.60%, 1.80%, 2.00%, 2.20% and 2.40%, respectively. The media without adding carbon sources was included in this experiment as the control.

Biomass and pH evaluation

The biomass concentration was determined by optical density at 600nm (OD_{600}) through a spectrophotometer (SP-756PC, Shanghai Spectrum Co., Ltd., Shanghai, China). The pH of culture media was measured through a pH meter (pHS-3C Shanghai Precision Scientific Instrument Co., Ltd, Shanghai, China).

RESULTS AND DISCUSSION

Effect of glucose on growth of *B. bifidum BB01* and *BB28*

The effect of glucose on growth of B. *bifidum BB01* showed in Figure1.With the increasing concentration of glucose, OD value increased gradually from 0.480 at glucose 0% to 0.612 at glucose 2.40%, the pH decreased gradually from 4.3 at glucose 0% to 4.12 at glucose 2.40%, which indicated that glucose could improve the growth of *Bifidobacterium bifidum BB01* in the extract of *Fructus tribuli.*

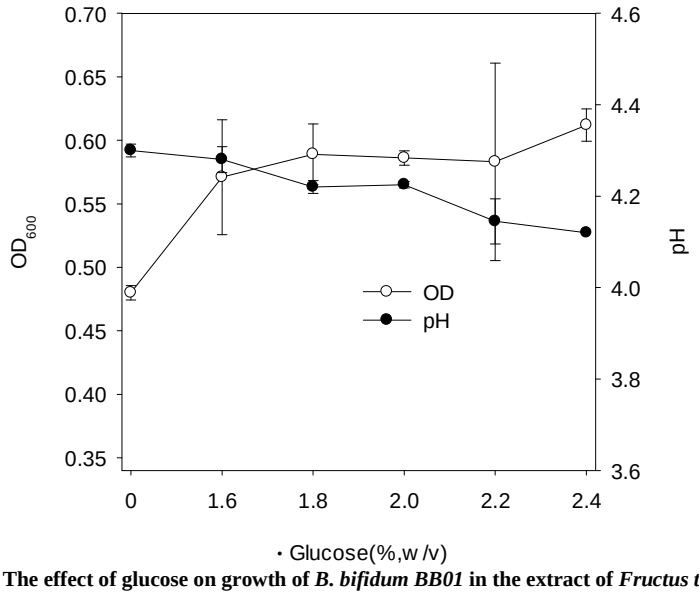

Fig.1 The effect of glucose on growth of *B. bifidum BB01* in the extract of *Fructus tribuli*

The effect of glucose on growth of B. *bifidum BB28* showed in Figure2. With the increasing concentration of glucose, OD value increased gradually from 0.476 at Glucose 0% to 0.647 at glucose 2.20%, then decreased to 0.600 at glucose 2.40%, the pH decreased gradually from 4.35 at glucose 0% to 4.24 at glucose 2.20%, then increased to 4.53 at glucose 2.40%, which indicated that glucose could significantly promote the growth of *Bifidobacterium bifidum BB28* in the extract of *Fructus tribuli*（$p<0.05$）.

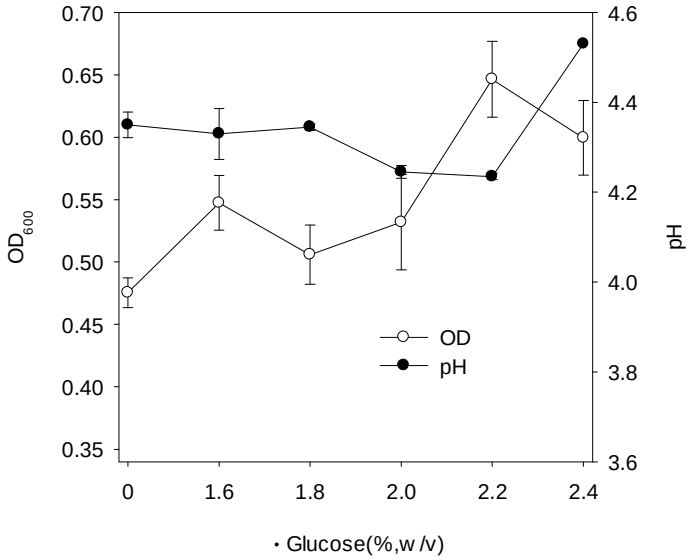

Fig.2 The effect of glucose on growth of *B. bifidum BB28* in the extract of *Fructus tribuli*

Effect of Maltose on growth of *B. bifidum BB01* and *BB28*

The effect of maltose on growth of *B. bifidum BB01* showed in Figure4. With the increasing concentration of maltose, OD value increased gradually from 0.413 at maltose 0% to 0.442 at maltose 1.80%, then decreased to 0.431 at maltose 2.0%, then increased to 0.480 at maltose 2.20%, and then decreased to 0.409 at 2.40%, the pH decreased gradually from 4.19 at maltose 0% to 4.01 at maltose 1.80%, then increased to 4.18 at maltose2.4%, which indicated that maltose could significantly promote the growth of *Bifidobacterium bifidum BB01* in the extract of *Fructus tribuli*（$p<0.05$）.

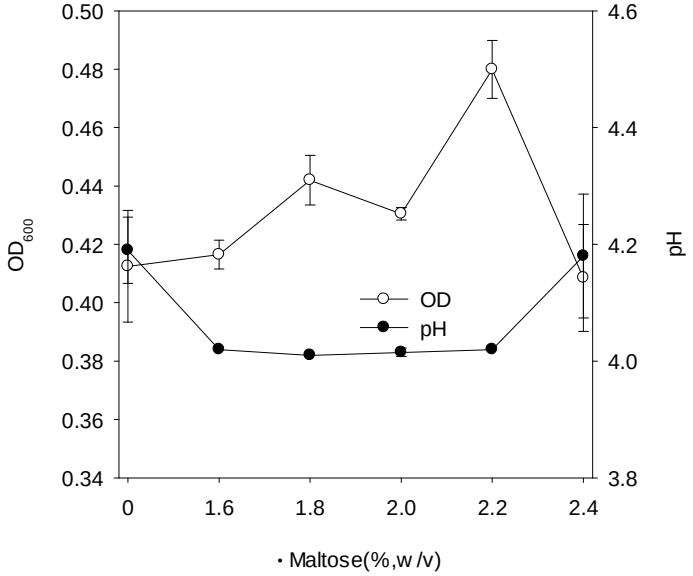

Fig.3 The effect of maltose on growth of *B. bifidum BB01* in the extract of *Fructus tribuli*

The effect of maltose on growth of *B. bifidum BB28* showed in Figure3. With the increasing concentration of maltose, OD value increased gradually from 0.539 at maltose 0% to 0.550 at maltose 1.60%, then decreased to 0.537 at maltose 1.80%, then increased to 0.555 at maltose 2.0%, then decreased to 0.537 at Maltose 2.20%,and then decreased to 0.559 at maltose 2.40%, the pH decreased gradually from 3.97 at maltose 0% to 3.75 at maltose 2.0%, then increased to 3.79 maltose 2.4%, which indicated that maltose could not promote the growth of *Bifidobacterium bifidum BB28* in the extract of *Fructus tribuli*（$p>0.05$）.

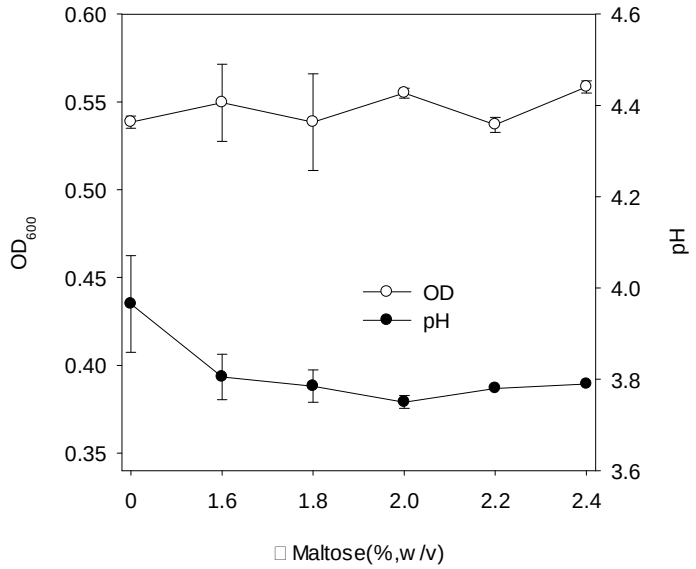

Fig.4 The effect of maltose on growth of *B. bifidum BB28* in the extract of *Fructus tribuli*

Effect of Lactose on growth of *B. bifidum BB01* and *BB28*

The effect of Lactose on growth of *B. bifidum BB01* showed in Figure5. When the lactose act as the carbon source, with the increasing concentration of lactose, OD value decreased gradually from 0.525 at lactose 0% to 0.365 at lactose 1.60%, then increased to 0.478, the pH increased gradually from 4.35 at lactose 0% to 4.37 at lactose 1.60%, then decreased to 4.19 at lactose 2.0%, and then increased to 4.29 at lactose 2.40%, which indicated that lactose significantly promote the growth of *Bifidobacterium bifidum BB01* in the extract of *Fructus tribuli9*（$p<0.05$）.

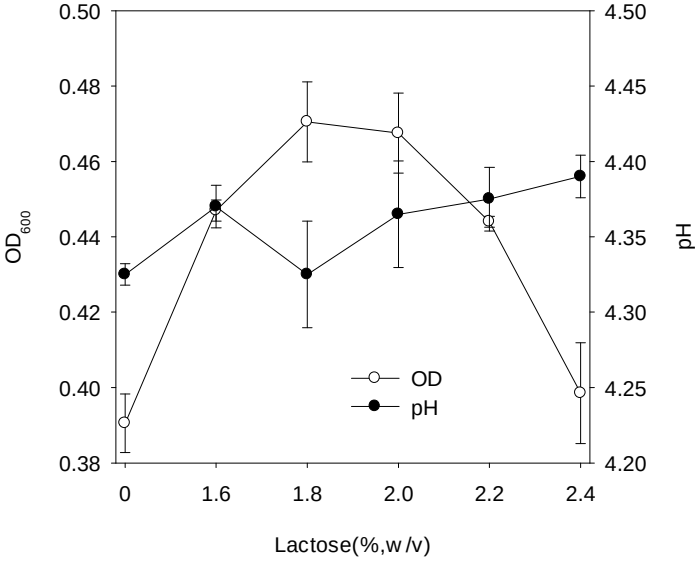

Fig.5 The effect of lactose on growth of *B. bifidum BB01* in the extract of *Fructus tribuli*

The effect of lactose on growth of *B. bifidum BB28* showed in Figure6. With the increasing concentration of lactose, OD value decreased gradually from 0.525 at lactose 0% to 0.365 at lactose 2.20%, then increased to 0.475 at lactose 2.40%, the pH increased gradually from 4.35 at lactose 0% to 4.365 at lactose 1.60%, then decreased to 4.185 at lactose 2.0%, and then increased to 4.29 at lactose 2.40%, which indicated that lactose significantly inhibit the growth of *Bifidobacterium bifidum BB28* in the extract of *Fructus tribuli*（$p<0.05$）.

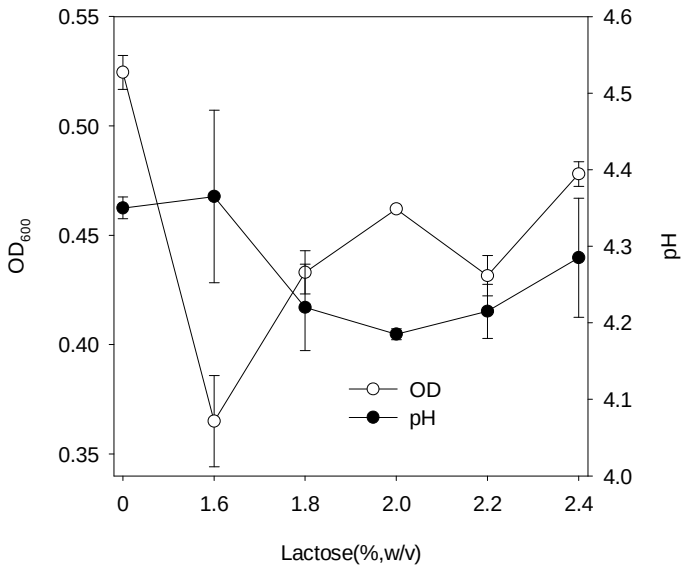

Fig.6 The effect of lactose on growth of *B. bifidum BB28* in the extract of *Fructus tribuli*

The effect of glucose, maltose and lactose on growth of *Bifidobacterium bifidum BB01* and *BB28* in the extract of *Fructus tribuli* showed in Figure1, Figure2, Figure3, Figure4, Figure5 and Figure6.Addition of glucose, maltose and lactose in the extract of *Fructus tribuli* could promote the growth of *Bifidobacterium bifidum BB01*, there was a significant difference among the results of glucose at 2.40%, maltose at 2.20% and lactose at 1.8% for *Bifidobacterium bifidum BB01($p < 0.001$).* Addition of glucose in the extract of *Fructus tribuli* could significantly promote the growth of *Bifidobacterium bifidum BB28.* The optimum carbon source on growth of *Bifidobacterium bifidum BB01* and *BB28* in the extract of *Fructus tribuli* was glucose and the demand of Bifidobacterium on carbon source for strain to strain.

CONCLUSION

Addition of glucose in the extract of *Fructus tribuli* could significantly promote the growth of *Bifidobacterium bifidum BB01*, maltose and lactose added to the extract of *Fructus tribuli* could significantly promote the growth of *Bifidobacterium bifidum BB01* but inhibit the growth of *Bifidobacterium bifidum BB28.* The optimum carbon source on growth of Bifidobacteria in the extract of Fructus tribuli was glucose. The optimum concentration of glucose in the extract of *Fructus tribuli* was 2.40% for *Bifidobacterium bifidum BB01*and 2.20% for *Bifidobacterium bifidum BB28*, respectively.

Acknowledgment
The project was partly supported by the science and technology project of Xi'an city [No.CX12186 (5)] and the Natural Science Foundation of Shaanxi Province (No.2010JM2013), China.

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