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Effect of the Extract of *Fructus tribuli* on Growth of *Lactobacillus Acidophilus*

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Abstract

Effect of $CaCO_3$, $ZnSO_4$ and $FeCl_3$ on growth of *Bifidobacterium Bifid* BB03 was studied by measuring optical density at 600nm (OD_{600}) and pH using de Man nd sharpe (MRS) broth as the control. The addition of each substance was 0.02, 0.04, 0.06, 0.08 and 0.10 Re ere as follows: Addition of $CaCO_3$ has the significant promotion on growth of *Bifidobacterium Bifidu* BB and 03. The optimum concentration of $CaCO_3$ in MRS broth was 0.10g/L for the two strains itions of $_4$ or $FeCl_3$ all have significant inhibition on growth of *Bifidobacterium Bifidum* BB01 and BB0

Keywords: Bifidobacterium Bifidu aCO$_3$; ZnSO Cl$_3$ probiotics

1. Introduction

The genus dob rium known to be among the first and most dominant gut inhabitants in our early life are an c, gram positive, non-motile, non-sporulating irregular or branched fermentative rod-sha teria l. Bifidobacterium species could locate in intestinal tract of healthy people, and was nfirm to to e health promoting effects in humans, such as reduction of serum cholesterol le incr f the immune response the inhibition of pathogenic microorganisms, anti-mutagenic and anti-c genic ac vity, prevention of diarrhea[4-6].

Beca of bifidobacteria showing poor growth in media, several substances, such as bovine lactoferrin, ey protein concentrate, Caseinomacropeptide, oligosaccharides, amino acid and some

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metal ions have been studied for their potential growth-stimulating activity, with the aim of finding a suitable nutrient supplement to incorporate into culture media [7-14]. Among them, much attention has been paid to metal ions, such as Ca^{2+}, Mg^{2+}, and Fe^{3+}, which can enhance enzyme activity in bifidobacteria [15]. In this study we have analysed the effect of the addition of $CaCO_3$, $ZnSO_4$ and $FeCl_3$ on the growth and acid production of *Bifidobacterium Bifidum BB01* and *BB03* in de Man, Rogosa and sharpe (MRS) broth.

2. Materials and methods

2.1. Materials

Two probiotic strains, *Bifidobacterium Bifidum BB01* and *BB03*, obtained from Coll[illegible] of Life Science & Engineering, Shaanxi University of Science & Technology were used. Al[illegible]hemic[illegible] used were of analytical grade unless otherwise specified

To obtain a fresh culture, both of the strains were grown three successiv[illegible]mes i[illegible] MRS br[illegible] (Hopebio, Qingdao, China) in anaerobic condition. The transfer volume was 2% (v/[illegible]he incu[illegible]ation was at 37 ℃for 18 h.

2.2. Growth condition

The normal MRS broth with the carbon source replaced by lactose (20g/L) a[illegible]d $CaCO_3$, $ZnSO_4$ and $FeCl_3$ in anaerobic tube at 0.02, 0.04, 0.06, 0.08 and 0.10g/L r[illegible]vely. MRS [illegible]th without $CaCO_3$, $ZnSO_4$ or $FeCl_3$ was included in this experiment as a contro[illegible]he sterilized MRS broth was used as a growth medium after inoculation with 4 %(v/v) of a c[illegible]ure in ex[illegible]ential phase. The growth temperature was kept at 37 ℃.

2.3. Measurement of pH

The pH of culture medium was measured through [illegible]H[illegible]eter (pHS-3C Shanghai Precision Scientific Instrument Co., Ltd, Shanghai, China)

2.4. Growth determination

The growth of each strain wa[illegible] mo[illegible]r[illegible]y [illegible]ring the optical density at 600nm (OD_{600}) of the cultures through a spectropho[illegible]eter (S[illegible]56PC, Shanghai Spectrum Instruments Co., Ltd., Shanghai, China).

3. Results and discu[illegible]n

3.1. Effect of C[illegible]$_3$ on [illegible]wth of Bifidobacterium bifidum

The g[illegible]h of [illegible]trol g[illegible]p and effect of different concentrations of $CaCO_3$ on the growth of *Bifidob[illegible]ter[illegible] Bifidu[illegible]*01 and BB03 showed in Figure 1-4. The control group was rapidly growing in [illegible]h, OD[illegible] of BB0[illegible] and BB03 were rapidly increased from 0.093 and 0.090 to 0.774 and 0.912 respe[illegible]el[illegible]th rate of the cell was gradually slowed down in 10-18h and in 18-24 OD value almost [illegible]anged which indicated that the cell growth into the stationary phase.

With t[illegible]ncreasing concentration of $CaCO_3$, OD_{600} of BB01 increased gradually and reached maximum [illegible]08, 1.180 and 1.298 at 0.10g/L $CaCO_3$ at incubation 10h, 18h and 24h respectively. Similarly, OD value of BB03 is also increased with the increase of $CaCO_3$ concentration. The OD value in each measured time reached maximum at 0.10g/L $CaCO_3$ which respectively 1.015(10h), 1.320(18h),

1.423 (24h). The pH of culture medium was in contrast with the OD_{600} of both strains, which indicated that $CaCO_3$ on *Bifidobacterium Bifidum* played a significant role in promoting growth. The optimum concentration of $CaCO_3$ in MRS broth for *Bifidobacterium Bifidum* BB01 and BB03 was 0.10g/L.

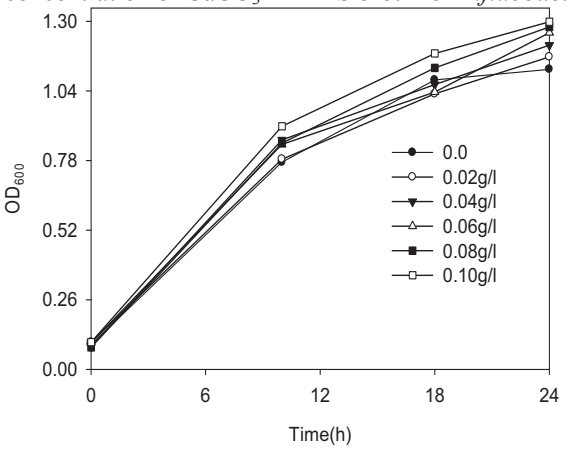


Fig1. Effect of $CaCO_3$ on growth of *B.bifidum BB01*

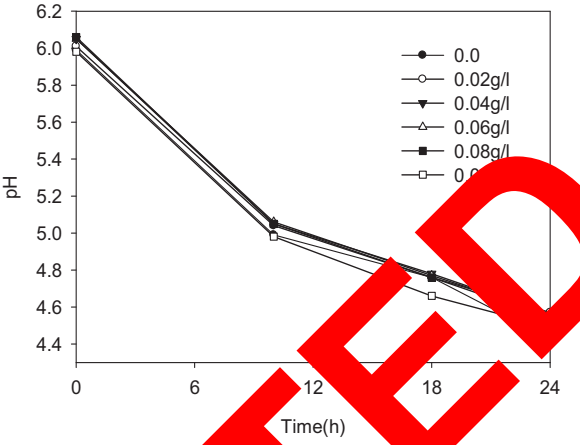


Fig2. Effect of $CaCO_3$ on pH of *B.bifidum BB01*

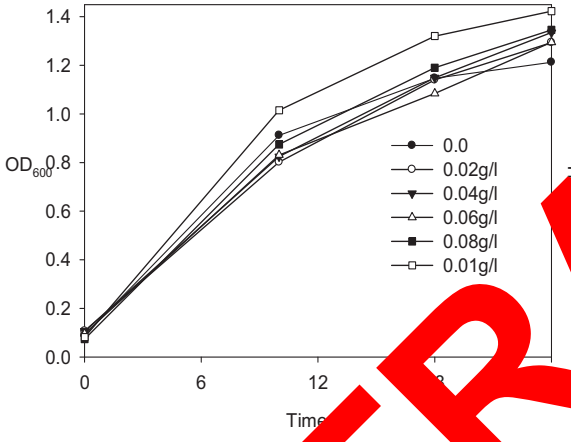


Fig3. Effect of $CaCO_3$ on growth of *B.bifidum BB03*

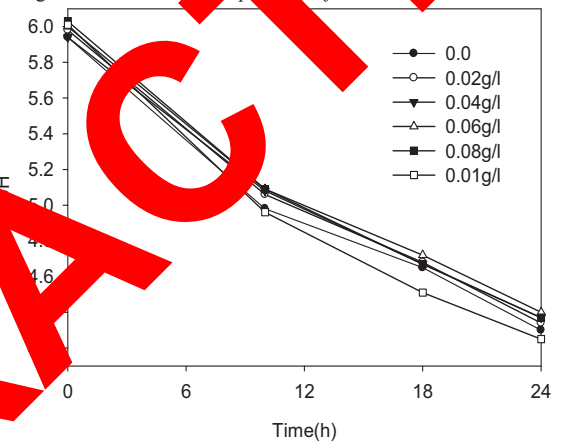


Fig4. Effect of $CaCO_3$ on pH of *B.bifidum BB03*

3.2. Effect of $ZnSO_4$ on growth of Bifidobacterium bifidum

Eeffect of different concentrations of $ZnSO_4$ on the growth of *Bifidobacterium Bifidum* BB01 and BB03 was shown in Figure 5.8. After having added $ZnSO_4$, the OD_{600} of BB01 and BB03 without $ZnSO_4$ was 0.46 and 0.332(6h), 1.065 and 0.986(16h), 1.206 and 1.12(24h), the OD_{600} of BB01 and BB03 0.1g/l ZnSO4 was 0.255 and 0.299(6h), 0.945 and 1.012(16h), 1.069 and 1.084(24h), respectively. The pH of BB01 and BB03 without $ZnSO_4$ was 5.43 and 5.58(6h), 4.79 and 4.81(16h), 4.65 and 4.67(24h). The pH of BB01 and BB03 at 0.10g/l $ZnSO_4$ was 5.70 and 5.60(6h), 4.76 and 4.63(16h), 4.58 and 4.43(24h), respectively. However, the differences between different concentrations of added $ZnSO_4$ on growth of both strains were not obvious. This indicated that ZnSO4 inhibited the growth of Bifidobacterium Bifidum BB01 and BB03 and promoted acid production of the two strains.

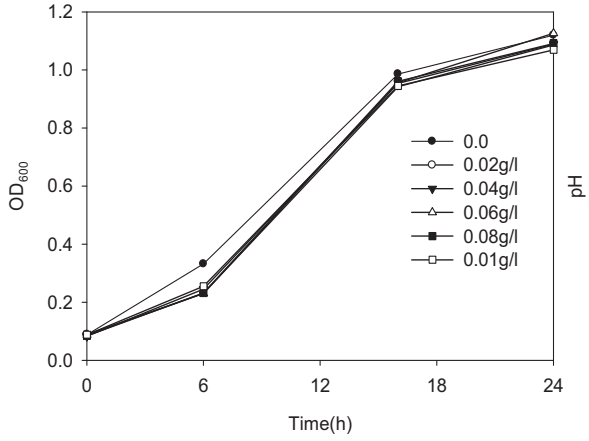


Fig5. Effect of $ZnSO_4$ on growth of *B.bifidum BB01*

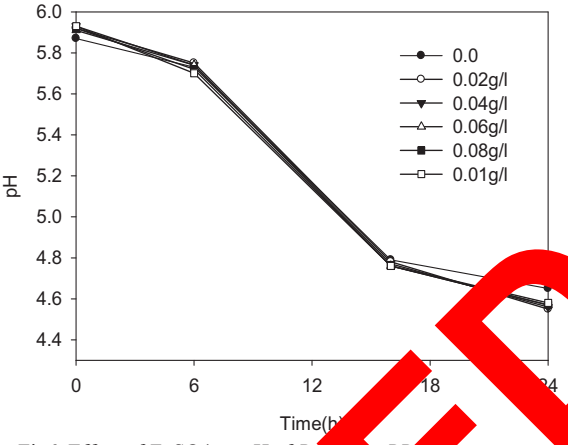


Fig6. Effect of ZnSO4 on pH of *B.bifidum BB01*

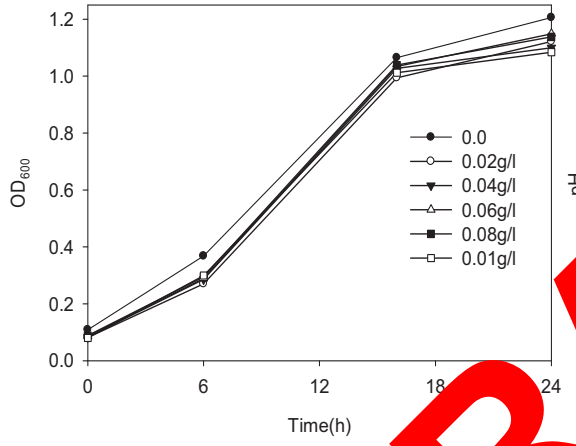


Fig7. Effect of ZnSO4 on growth of *B.bifidum*

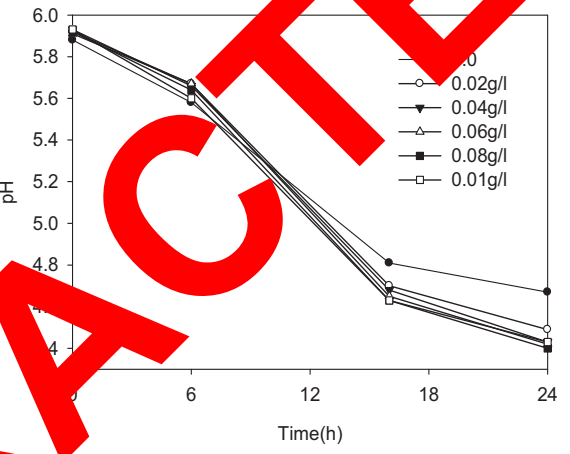


Fig8. Effect of ZnSO4 on pH of *B.bifidum BB03*

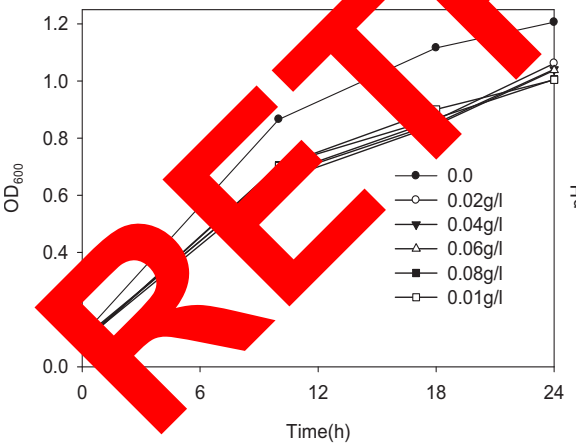


Fig9. Effect of $FeCl_3$ on growth of *B.bifidum BB01*

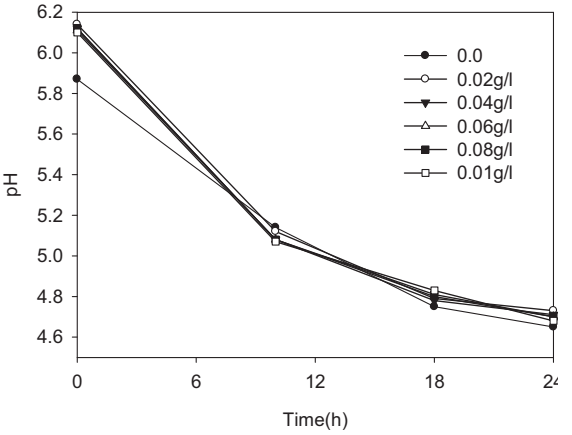


Fig10. Effect of $FeCl_3$ on pH of *B.bifidum BB01*

3.3. Effect of $FeCl_3$ on growth of Bifidobacterium bifidum

Effect of different concentrations of $FeCl_3$ on the growth of *Bifidobacterium Bifidum* BB01 and BB03 were showed in Figure 9-12. After having added $FeCl_3$, the OD_{600} decreased gradually from 0.774, 1.081 and 1.121 of countrol to 0.702, 0.901, 1.004 of BB01 and 0.702, 0.911, 1.040 of BB03 at 0.10g/L $FeCl_3$ at incubation 10h, 18h and 24h, respectively. After 18 hours, pH of the groups added $FeCl_3$ decreased more slowly than the control groups' from 4.76 to 4.43 and 4.65 to 4.30, respectively manifested the decline from 4.83 to 4.68 and 4.76 to 4.58. However, the differences between different concentrations of added $FeCl_3$ on growth of both strains were not obvious. Figure 9-12 indicated that the addition of Fe[illegible] significant inhibition on growth of *Bifidobacterium Bifidum BB01* and *BB03*.

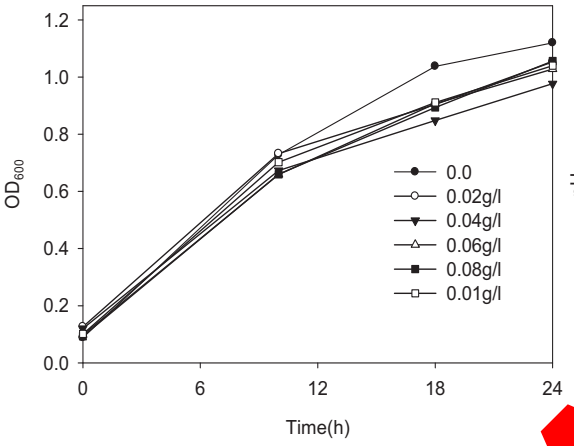
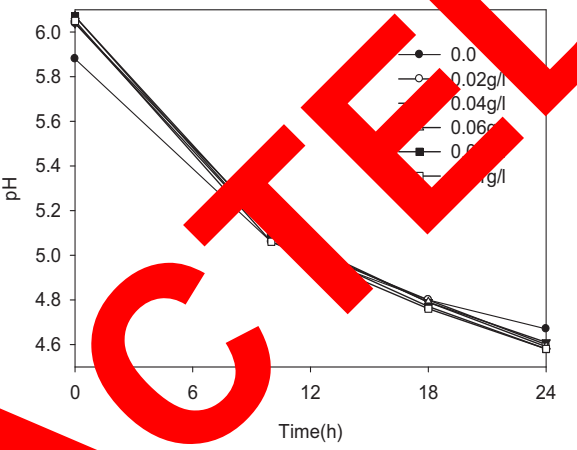


Fig11. Effect of $FeCl_3$ on growth of *B.bifidum BB03*



[illegible]ffect of $FeCl_3$ on pH of *B.bifidum BB03*

4. Conclusions

Addition of $CaCO_3$ has the sig[illegible]ant pr[illegible]otion o[illegible]growth of *Bifidobacterium Bifidum* BB01 and BB03. The optimum concentration [illegible]roth was 0.10g/L. Addition of $ZnSO_4$ and $FeCl_3$ all have significant inhibition [illegible]growth[illegible] *Bifidobacterium Bifidum* BB01 and BB03.

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