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Study on the probiotic effect of oligomannose

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Abstract: *Mannooligosaccharide is a kind of oligosaccharide which is very beneficial to human health. It has obvious intestinal probiotics and immune regulation. As a new prebiotic, the research and application of mannooligosaccharide is less. In order to better develop and utilize mannooligosaccharide and provide theoretical and experimental basis for the development of mannooligosaccharide products. This paper studies the probiotic effect of mannooligosaccharide by fermentation culture of two probiotics, using single factor test and response surface test. The effects of mannooligosaccharides on the probiotics were studied. Main results: (1) the growth promoting effects of mannooligosaccharides and other oligosaccharides on Lactobacillus plantarum and Streptococcus thermophilus were compared by single factor experiment. (2)Through the central composite response surface design and analysis, the optimal initial conditions of Lactobacillus plantarum fermentation were determined as follows: initial pH value was 6.835, yeast extract dosage was 1.6%, mannooligosaccharide dosage was 0.16%, vitamin B1 dosage was 0.122%. At this time, the biomass of Lactobacillus plantarum predicted by the model was 0.960, which was 1.72 times higher than that before optimization. (3) According to the central composite response surface design and analysis, the optimal fermentation conditions of Streptococcus thermophilus were determined as follows: initial pH value was 7.527, peptone dosage was 0.909%, mannooligosaccharide dosage was 0.208%, vitamin C dosage was 0.098%. At this time, the biomass of Streptococcus thermophilus predicted by the model was 0.450, which was 1.77 times higher than that before optimization.*

Key words: *Mannooligosaccharides (MOS), Lactobacillus plantarum, Streptococcus thermophilus, response surface methodology, probiotic effect*

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I. Introduction:

Mannooligosaccharides (MOS) is a kind of oligosaccharide formed by the polymerization of monosaccharide D-mannose. The monosaccharides are connected by β-1,4-glycosidic bonds, and the degree of polymerization is 2~10. Known as mannose oligosaccharides. Most oligomannose is obtained from konjac flour as raw material through hemicellulase enzymatic hydrolysis, separation and purification. Oligomannose not only has good physical and chemical properties such as low calories, strong stability, safety and non-toxicity, but also has the ability to not be digested when passing through the gastrointestinal tract of organisms, but it can effectively promote the intestines represented by bifidobacteria in organisms. The specific proliferation of probiotics can inhibit the growth of harmful bacteria, reduce the production of toxic metabolites, improve the human gastrointestinal tract and improve immunity [1, 2].

Functional oligosaccharides mainly include mannose oligosaccharides, isomalt oligosaccharides, fructooligosaccharides, xylo-oligosaccharides, galacto-oligosaccharides, etc. [3]. As an emerging prebiotic, oligomannose was officially approved as a new food raw material by the National Health and Family Planning Commission in 2013. Compared with other oligosaccharides, the related research and application of oligomannose are relatively lacking.

At present, in the research on probiotic mechanism, it has been fully confirmed that oligomannose can selectively reduce the production rate and quantity of harmful bacteria such as *E. coli* in the intestine, promote the proliferation of specific probiotics such as lactic acid bacteria, and greatly accumulation of short-chain fatty acids and other chemicals that are beneficial to the human body [4]. A large number of studies have shown that oligosaccharides can promote the proliferation of *Lactobacillus plantarum*. Chen Yunhui and others selected 5 strains of *Lactobacillus plantarum* with different physiological characteristics to study fructo-oligosaccharides, xyloligosaccharides, galacto-oligosaccharides, isomalt-oligosaccharides and chrysanthemums. Powder promotes its proliferation under different conditions. The results show that when oligosaccharides are substituted for glucose in the MRS medium as a carbon source, compared with the control, oligosaccharides can significantly promote the growth of *Lactobacillus plantarum* [5]. Yang Yu et al. used several oligosaccharides to ferment and culture *Lactobacillus plantarum* used for fermented milk production. The results showed that compared with the same concentration of glucose, oligomerization had a significant effect on the number of viable *Lactobacillus plantarum* [39].

This paper studies the probiotic effect of mannooligosaccharide by fermentation culture of two probiotics, using single factor test and response surface test, in order to obtain the optimal condition of mannooligosaccharide on the probiotics.

II. Material And Methods:

1. Strains and cultivation

The strains of *Lactobacillus plantarum* and *Streptococcus thermophilus* were cultured in the MRS medium. MRS medium: glucose 2%, beef extract 1%, yeast extract 0.5%, tryptone 1%, dipotassium hydrogen phosphate 0.2%, ammonium citrate 0.2%, the mass fraction of magnesium sulfate hydrate is 0.02%, the mass fraction of manganese sulfate tetrahydrate is 0.005%, the volume ratio of Tween-80 is 1 mL/L, and the volume of distilled water is 1 L. The strains were directly inoculated into a test tube containing 5ml of liquid MRS medium, the test tube was stoppered and sealed, and cultured in a constant temperature incubator at 37°C for 18-24 hours.

2. Bacterial density determination

After cultivation of the strains, inoculate them into the liquid culture medium designed for the experiment (1% of inoculation amount), culture them at 37°C for 24h, take 10ml of the bacterial solution in a 15ml centrifuge tube, and centrifuge at 6000r/min at 4°C for 10min. Collect the bacterial cells and wash with distilled water. The centrifugation and washing were repeated twice, distilled water was used as a blank, and the absorbance value of the bacterial solution at 600nm wavelength was measured with a microplate reader.

3. Single factor test

Choose different nitrogen sources, oligosaccharides and vitamins as the main factors affecting the growth of the two bacteria, and use OD600 as the measurement index to obtain the optimal nitrogen sources and vitamins for the growth of the two bacteria. Compare the effects of different oligosaccharides on the growth of the two bacteria.

Medium: lactose 1%, yeast extract 0.25%, dipotassium hydrogen phosphate 0.2%, ammonium citrate 0.2%, magnesium sulfate heptahydrate 0.02%, manganese sulfate tetrahydrate 0.005 %, the volume of distilled water is 1L.

4. Nitrogen source

Using 2.5% tryptone, yeast extract, beef extract, and peptone as the nitrogen source components of the culture medium, the inoculum volume of 4% was connected to a 100ml Erlenmeyer flask containing 50ml liquid culture medium at a culture temperature of 37 ℃, the culture time is 24h, after the fermentation, the bacteria density is determined according to the method in section 2. The added amount of lactose in the medium is 2%.

5. Oligosaccharides

0.3% mannose oligosaccharides, isomalt oligosaccharides, fructooligosaccharides, and galacto-oligosaccharides were used as the medium oligosaccharide components to add, and the volume fraction of the inoculum was 4% to the 50ml liquid medium. In a 100ml Erlenmeyer flask, the culture temperature is 37°C, and the culture time is 24h. After the fermentation is completed, the bacteria density is measured according to the method in section 2.

6. Vitamins

0.15% of vitamin C, vitamin B1, vitamin B3, and vitamin B12 were used as the medium vitamin components to add, and the volume fraction of 4% was inoculated into a 100ml Erlenmeyer flask containing 50ml liquid medium, and the culture temperature was 37°C. The culture time is 24h, after the fermentation, the bacteria density is determined according to the method in section 2.

7. Response surface test

According to the results of the single factor test, by consulting related literature, the initial pH, nitrogen source, oligosaccharide and vitamin addition amount are selected as the influencing factors of the test, and the absorbance of the bacterial liquid is the response value, and 4 factors and 5 levels are designed by Design-Expert 11 software Central Composite response surface test, as shown in Table 1 and Table 2. The absorbance of the bacterial liquid was fitted with a quadratic multiple regression equation, and the regression equation of the functional relationship between each factor and the response value was obtained, and the optimal medium formula was determined according to the contour line and the response surface generated by the experiment.

Table1. *Lactobacillus plantarum* Central Composite test design factor level table

Factors	Levels				
	-2	-1	0	1	2
PH	5.5	6	6.5	7	7.5
Addition of yeast extract powder/%	0.1	0.6	1.1	1.6	2.1
Addition of oligomannose/%	0.04	0.16	0.28	0.40	0.52
Vitamin B1 added amount/%	0.03	0.08	0.13	0.18	0.23

Table 2. *Streptococcus thermophilus* Central Composite test design factor level

Factors	Level				
	-2	-1	0	1	2
PH	5	6	7	8	9
Addition of yeast extract powder/%	0.1	0.6	1.1	1.6	2.1
Addition of oligomannose/%	0.04	0.16	0.28	0.40	0.52
Vitamin B1 added amount/%	0.01	0.06	0.11	0.16	0.21

8. Statistical analysis of data

The single factor test data are all three repeated averages, and Excel is used for data statistical processing; Design-Expert 11 is used for Central Composite response surface test data processing.

III. Results And Discussion:

3.1 Single factor test results

3.1.1 The effect of nitrogen source on the growth of probiotics

Nitrogen sources are divided into organic nitrogen sources and inorganic nitrogen sources. Microorganisms can enzymatically decompose organic nitrogen sources into small molecular substances, which are absorbed and transformed by bacteria to further participate in metabolism, and finally form nutrients necessary for microorganisms; inorganic nitrogen sources can adjust the pH value of the external environment and the metabolism of bacteria, but its utilization rate is faster than that of organic nitrogen sources [7, 8]. The influence of different nitrogen sources on the growth of *Lactobacillus plantarum* is shown in Figure 1. From the bar graph, it can be seen that when yeast extract is the main nitrogen source of the medium, the growth of *Lactobacillus plantarum* is significantly higher than that of other groups, and Peptone D comes next.

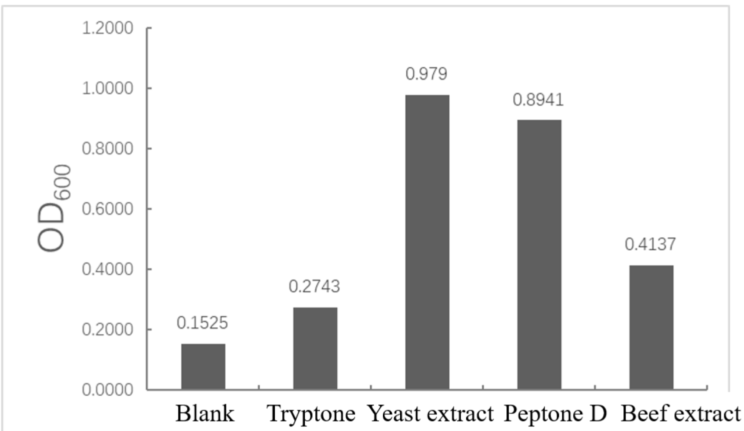

Figure 1. Effect of different nitrogen sources on the biomass of *Lactobacillus plantarum*

Figure 2 is a bar graph showing the effect of different nitrogen sources on the growth of *Streptococcus thermophilus*. It can be seen from the figure that when peptone D is the main nitrogen source of the medium, the growth of *Streptococcus thermophilus* is the highest among all groups, followed by yeast extract.

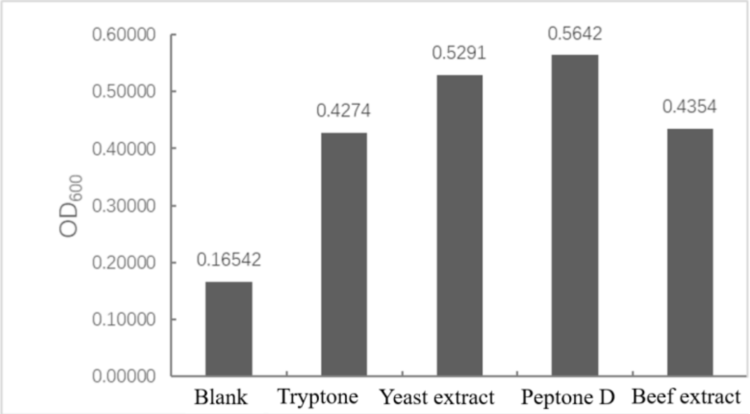

Figure 2. Effect of different nitrogen sources on the biomass of *Streptococcus thermophilus*

In summary, *Lactobacillus plantarum* and *Streptococcus thermophilus* have a preference for the utilization of microbial peptone.

3.1.2 The effect of oligosaccharides on the growth of probiotics

The effect of oligosaccharides on the growth of probiotics has been proved by a large number of studies, but adding oligosaccharides directly to the MRS medium cannot promote the growth of the two probiotics, but will inhibit the growth of *Lactobacillus plantarum* [9]. This phenomenon may be due to the catabolism suppression effect [10-11]. After the culture medium is depleted and oligosaccharides are added, the biomass of the two probiotic experimental groups and the control group is more obvious. Figure 3 is a bar graph showing the effect of adding different oligosaccharides on the biomass of *Lactobacillus plantarum*. It can be seen from the figure that in addition to fructo-oligosaccharides, other oligosaccharides have a promoting effect on the growth of *Lactobacillus plantarum*. Isomaltose has the best growth-promoting effect on *Lactobacillus plantarum*, followed by mannose oligosaccharide, and galacto-oligosaccharide is the worst. Fructooligosaccharide has an inhibitory effect on the growth of Lactobacillus plantarum, which may be due to the problem of the added amount or other components of the culture medium.

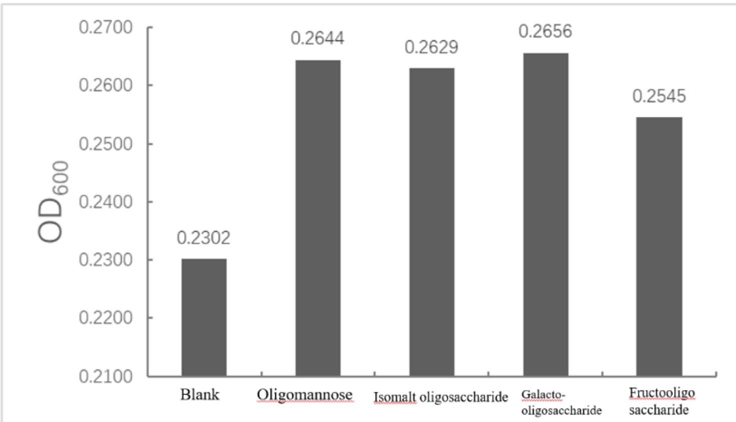

Figure 3. Effect of different oligosaccharides on the growth of *Lactobacillus plantarum*

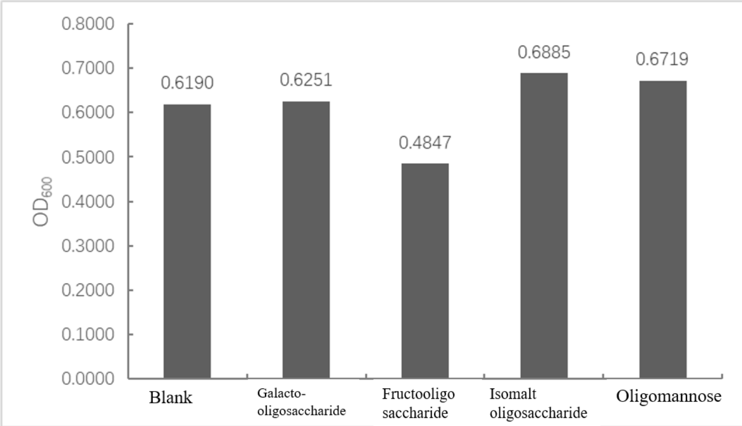

Figure 4． Effect of different oligosaccharides on the growth of *Streptococcus thermophilus*

From Figure 4, it can be seen that compared with the control group, the four oligosaccharides have a growth-promoting effect on *Streptococcus thermophilus*. Galacto-oligosaccharides are the best, followed by mannose, and then Isomaltose and oligofructose are the worst. In summary, when the carbon source is lactose and the medium is poorer than the MRS medium, adding a small amount of oligosaccharides can promote the growth of the two probiotics. Compared with other oligosaccharides, the oligomannose has the second beneficial effect on the two probiotics.

3.1.3 Vitamins

Vitamins, as growth-promoting factors in the culture medium, are substances that are necessary for the growth of some bacteria but cannot be synthesized by themselves. Adding a small amount of vitamins to the culture medium can meet the needs of the two probiotics and promote their growth.

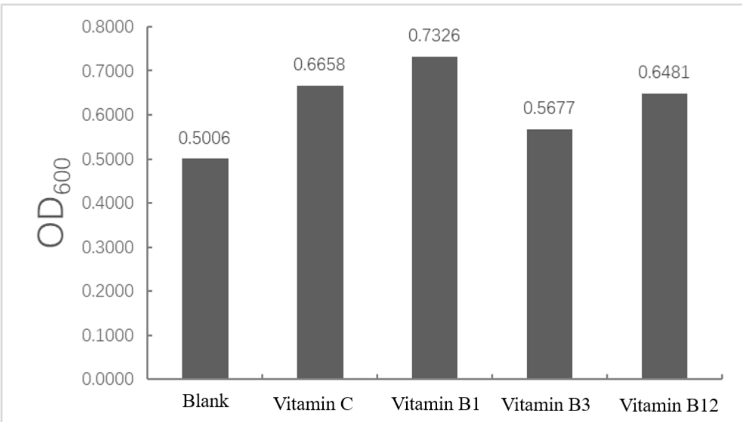

Figure 5． Effect of different vitamins on the biomass of *Lactobacillus plantarum*

It can be seen from Figure 5 that the four vitamins added in the experimental group have a growth-promoting effect on *Lactobacillus plantarum*. Among them, vitamin B1 has the best effect, followed by vitamin C, then vitamin B12, and finally vitamin B3.

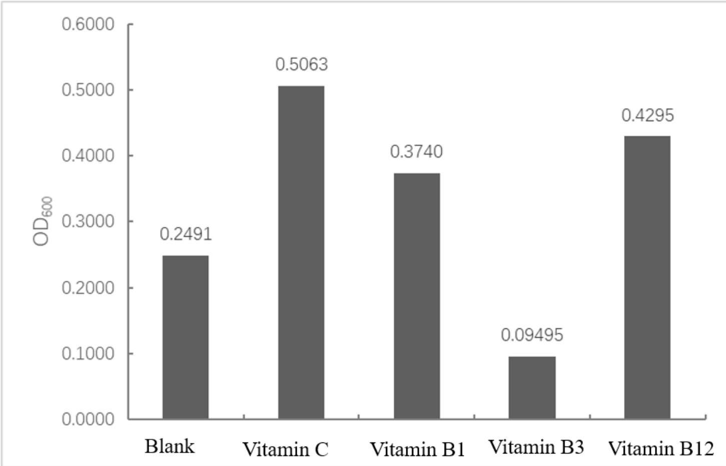

Figure 6. Effect of different vitamins on the biomass of *Streptococcus thermophilus*

Figure 6 is a bar graph showing the effect of different vitamins on the biomass of *Streptococcus thermophilus*. It can be seen from the figure that in addition to vitamin B3, other vitamins have a growth-promoting effect on Streptococcus thermophilus. Among them, vitamin C is the most effective excellent, followed by vitamin B12, and vitamin B1 has the worst effect.

3.2 Response surface test results

3.2.1 Lactobacillus plantarum

Design-Expert 11 software was used to design a Central Composite test of 4 factors and 5 levels for the initial pH value of *Lactobacillus plantarum*, the added amount of yeast extract, the added amount of oligomannose, and the added amount of vitamin B1. After multiple regression fitting, the vegetable milk was obtained. The quadratic regression model equation of the predicted value of bacillus biomass to the coded value of A, B, C, D.

OD_{600}=0.8236+0.0232A+0.0863B-0.1307C+0.0095D-0.0134AB-0.0125AC-0.0048AD-0.0063BC-0.0180BD-0.0073CD-0.0171A^2-0.0345B^2-0.0602C^2- 0.0134D^2

The response surface results are shown in Table 3, and the analysis of variance is shown in Table 4. The selected regression model has a $P<0.05$, indicating that the overall model has a significant impact on the test results and has credibility; the regression equation correlation coefficient R2=0.8874, correction determination coefficient R2Adj=0.7630, indicating that the model can explain 88.74% of the change, Has a certain predictive ability for the test results. The primary terms B and C of the model are highly significant, and the quadratic terms B2 and C2 are significant, indicating that the influence of various experimental factors on the biomass of Lactobacillus plantarum is complicated and not a simple linear relationship.

The optimal medium parameters optimized by the model: the initial pH value is 6.835, the addition amount of yeast extract is 1.6%, the addition amount of oligomannose is 0.16%, and the addition amount of vitamin B1 is 0.122%. At this time, the model predicts the organism of Lactobacillus plantarum. The amount is 0.960. In the actual test, the initial pH value is 7, the addition amount of yeast extract is 1.6%, the addition amount of oligomannose is 0.16%, and the addition amount of vitamin B1 is 0.18%. Under these conditions, the highest biomass of *Lactobacillus plantarum* can reach 0.9608.

Table 3. Experimental design and results of *Lactobacillus plantarum* Central Composite

Experiment No.	A Initial pH value	B Yeast extract/%	C Oligomannose/%	D Vitamin B1/%	OD_{600}
1	6.0	1.6	0.16	0.18	0.8873
2	6.5	1.1	0.28	0.23	0.8475
3	7.0	0.6	0.40	0.18	0.5305
4	7.0	0.6	0.40	0.08	0.4498
5	6.0	0.6	0.16	0.08	0.716
6	6.5	1.1	0.28	0.13	0.8482
7	7.5	1.1	0.28	0.13	0.8708
8	6.5	0.1	0.28	0.13	0.5152
9	5.5	1.1	0.28	0.13	0.7474

10	6.5	1.1	0.28	0.13	0.8056
11	6.0	1.6	0.40	0.18	0.5980
12	7.0	0.6	0.16	0.18	0.7924
13	6.0	0.6	0.40	0.18	0.4291
14	6.5	1.1	0.28	0.13	0.8100
15	7.0	1.6	0.16	0.08	0.9474
16	6.5	1.1	0.04	0.13	0.7826
17	6.0	0.6	0.40	0.08	0.3698
18	6.5	1.1	0.28	0.13	0.8254
19	7.0	1.6	0.40	0.18	0.4938
20	7.0	1.6	0.40	0.08	0.6008
21	6.5	2.1	0.28	0.13	0.9640
22	6.5	1.1	0.28	0.13	0.8578
23	7.0	0.6	0.16	0.08	0.7509
24	6.0	1.6	0.16	0.08	0.8467
25	7.0	1.6	0.16	0.18	0.9608
26	6.5	1.1	0.28	0.13	0.7946
27	6.5	1.1	0.28	0.03	0.8001
28	6.0	0.6	0.16	0.18	0.7456
29	6.0	1.6	0.4	0.08	0.6227
30	6.5	1.1	0.52	0.13	0.4911

Table 4. Analysis of variance of regression model of *Lactobacillus plantarum Central Composite test*

Items	Sum of square	Freedom	Mean square（MS）	F value	P value	Significance
Model	0.7350	14	0.0525	7.67	0.0002	*
A Initial pH	0.0130	1	0.0130	1.89	0.1888	
B Yeast extract %	0.1787	1	0.1787	26.10	0.0001	**
C Oligomannose %	0.4097	1	0.4097	59.84	< 0.0001	**
D Vitamin B1 %	0.0022	1	0.0022	0.3169	0.5818	
AB	0.0029	1	0.0029	0.4220	0.5258	
AC	0.0025	1	0.0025	0.3674	0.5535	
AD	0.0004	1	0.0004	0.0530	0.8210	
BC	0.0006	1	0.0006	0.0935	0.7640	
BD	0.0052	1	0.0052	0.7614	0.3966	
CD	0.0009	1	0.0009	0.1245	0.7291	
A^2	0.0080	1	0.0080	1.17	0.2956	
B^2	0.0326	1	0.0326	4.77	0.0453	*
C^2	0.0994	1	0.0994	14.51	0.0017	*
D^2	0.0050	1	0.0050	0.7245	0.4080	
Residue	0.1027	15	0.0068			
Lack of fit	0.0996	10	0.0100	15.92	0.0035	*
Pure error	0.0031	5	0.0006			
Corrected sum	0.8376	29				
R^2	0.8874					
R^2_{Adj}	0.7630					

Note: P>0.05 is not significant; P≤0.05 is significant, represented by *; P≤0.0001 is highly significant, represented by **.

The response surface three-dimensional map and contour map made by the regression equation are shown in Figure 7, which mainly reflects the interaction between the initial pH value, the added amount of yeast extract, the added amount of oligomannose and the added amount of vitamin B1. The magnitude of the interaction between the two factors is: the amount of yeast extract and the amount of vitamin B1 added> the initial pH value and the amount of yeast extract powder> the initial pH value and the amount of oligomannose added> the amount of oligomannose added and the amount of vitamin B1 added ＞Addition of yeast extract powder and addition of oligomannose ＞initial pH value and addition of vitamin B1. By analyzing the F value, we can see that the influence of each factor on the biomass of Lactobacillus plantarum is in order: oligomannose addition amount (C)> yeast extract powder addition amount (B)> initial pH value (A)> vitamin B1 addition amount (D).

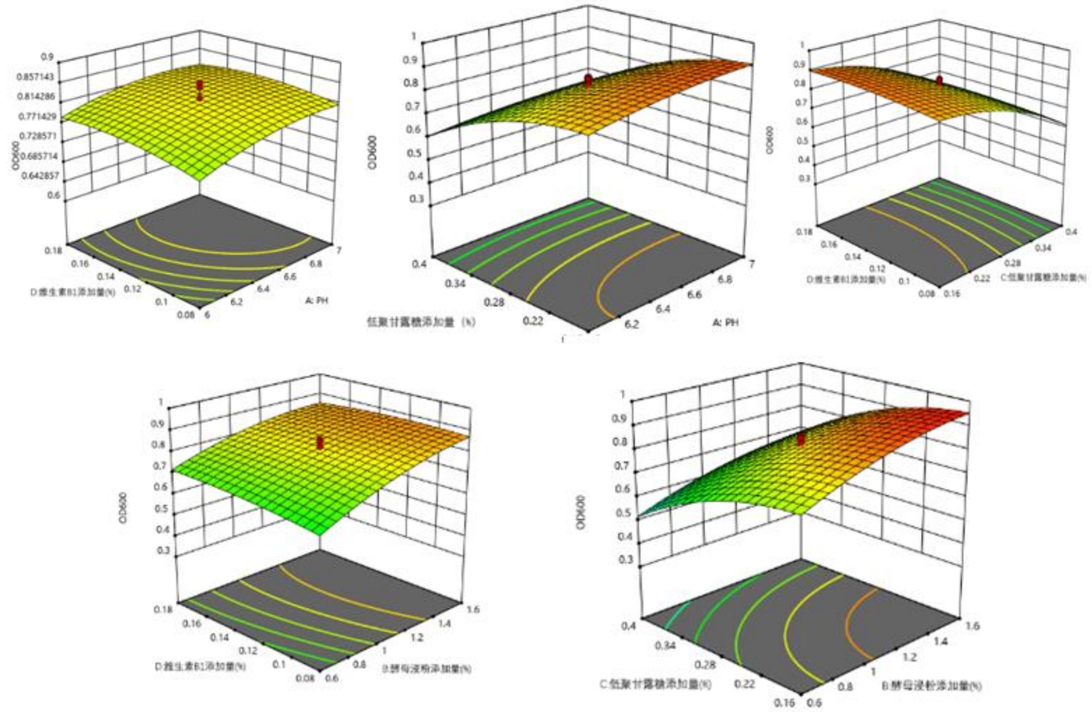

Figure 7. Response surface of the interaction of various factors on *Lactobacillus plantarum*

3.2.2 Streptococcus thermophilus

Design-Expert 11 software was used to design a Central Composite test of 4 factors and 5 levels for the initial pH value of Streptococcus thermophilus, the amount of peptone D, the amount of oligomannose, and the amount of vitamin C. After multiple regression fitting, the thermophilic was obtained. The quadratic regression model equation of the predicted value of streptococcal biomass to the coded value of A, B, C, D

$$OD600=0.4114+0.1221A+0.0040B-0.0135C-0.0405D+0.0095AB-0.0069AC+0.0086AD+0.0079BC+0.0018BD-0.0053CD-0.0558A^2-0.0464B^2-0.0506C^2-0.0341D^2$$

The response surface results of *Streptococcus thermophilus* are shown in Table 5, and the analysis of variance is shown in Table 6. The selected regression model has a $P<0.05$, indicating that the overall model has a significant impact on the test results and has credibility; the regression equation correlation coefficient R2=0.8018, correction determination coefficient R2Adj=0.6168, indicating that the model can explain 80.18% of the change, and has a certain predictive ability for the test results. The first term A of the model is significant, and the quadratic terms A2, B2, and C2 are significant, indicating that the influence of various experimental factors on the biomass of *Streptococcus thermophilus* is complicated and not a simple linear relationship.

The optimal medium parameters optimized by the model: the initial pH value is 7.527, the addition of peptone D is 0.909%, the addition of oligomannose is 0.208%, and the addition of vitamin C is 0.098%. At this time, the model predicts the organism of *Streptococcus thermophilus* biomass is 0.450. In the actual test, the initial pH value is 7, the addition amount of peptone D is 1.1%, the addition amount of oligomannose is 0.28%, and the addition amount of vitamin C is 0.11%. Under these conditions, the highest biomass of *Streptococcus thermophilus* can reach 0.4237. The initial pH value in the model optimization results is different from previous studies, which may be due to the decrease of the pH value after the medium is sterilized by high-pressure and high-temperature steam [51].

Table 5. Experimental design and results of *Streptococcus thermophilus* Central Composite

Experiment No.	A Initial pH	B PeptoneD /%	C Oligomannose /%	D Vitamin C/%	OD_{600}
1	5	1.1	0.28	0.11	0.03125
2	7	1.1	0.52	0.11	0.1678
3	6	1.6	0.40	0.06	0.1761
4	7	1.1	0.04	0.11	0.3108
5	8	0.6	0.40	0.16	0.1993
6	8	0.6	0.16	0.16	0.2779
7	7	1.1	0.28	0.01	0.4404
8	6	1.6	0.16	0.16	0.0375
9	8	1.6	0.16	0.06	0.3779
10	7	1.1	0.28	0.11	0.4094
11	8	1.6	0.40	0.16	0.4143
12	7	1.1	0.28	0.11	0.4053
13	8	0.6	0.40	0.06	0.3301
14	8	0.6	0.16	0.06	0.346
15	9	1.1	0.28	0.11	0.4054
16	6	0.6	0.16	0.16	0.0267
17	7	1.1	0.28	0.11	0.4237
18	8	1.6	0.16	0.16	0.4183
19	7	1.1	0.28	0.11	0.4179
20	8	1.6	0.40	0.06	0.4022
21	6	0.6	0.16	0.06	0.036
22	7	1.1	0.28	0.11	0.4028
23	6	1.6	0.16	0.06	0.1735
24	7	1.1	0.28	0.21	0.1701
25	7	2.1	0.28	0.11	0.0883
26	7	1.1	0.28	0.11	0.4096
27	7	0.1	0.28	0.11	0.4236
28	6	0.6	0.40	0.16	0.0268
29	6	0.6	0.40	0.06	0.0483
30	6	1.6	0.40	0.16	0.0589

Table 6. Analysis of variance of the regression model of the Central Composite test of *Streptococcus thermophilus*

Source of variance	Sum of square	Freedom	Mean square	F value	P value	Significance
Model	0.5828	14	0.0416	4.33	0.0039	*
A Initial pH	0.3578	1	0.3578	37.26	< 0.0001	**
B Yeast extract	0.0004	1	0.0004	0.0408	0.8426	
C Oligomannose	0.0044	1	0.0044	0.4549	0.5103	
D Vitamin C	0.0393	1	0.0393	4.09	0.0613	
AB	0.0014	1	0.0014	0.1488	0.7051	
AC	0.0008	1	0.0008	0.0796	0.7817	
AD	0.0012	1	0.0012	0.1232	0.7304	
BC	0.0010	1	0.0010	0.1040	0.7516	
BD	0.0001	1	0.0001	0.0055	0.9420	
CD	0.0004	1	0.0004	0.0464	0.8324	

A^2	0.0855	1	0.0855	8.91	0.0093	*
B^2	0.0591	1	0.0591	6.16	0.0254	*
C^2	0.0702	1	0.0702	7.31	0.0163	*
D^2	0.0319	1	0.0319	3.32	0.0883	
Residue	0.1440	15	0.0096			
Lack of fit	0.1437	10	0.0144	230.39	< 0.0001	
Pure error	0.0003	5	0.0001			
Corrected sum	0.7268	29				
R^2	0.8018					
R^2_{Adj}	0.6168					

Note: P>0.05 is not significant; P≤0.05 is significant, represented by *; P≤0.0001 is highly significant, represented by **.

The response surface three-dimensional map and contour map made by the regression equation are shown in Figure 8, which mainly reflects the interaction between the initial pH value, the amount of peptone D, the amount of oligomannose and the amount of vitamin C. The interaction of the two factors is as follows: initial PH value and added amount of peptone D>initial PH value and added amount of vitamin C> added amount of peptone D and added amount of oligomannose>initial pH value and added amount of oligomannose>oligomeric The added amount of mannose and the added amount of vitamin C> the added amount of peptone D and the added amount of vitamin C. Through the analysis of the F value, it can be seen that the influence of each factor on the biomass of Streptococcus thermophilus is in order: initial pH value (A)> vitamin C addition (D)> oligomannose addition (C)> peptone D addition (B).

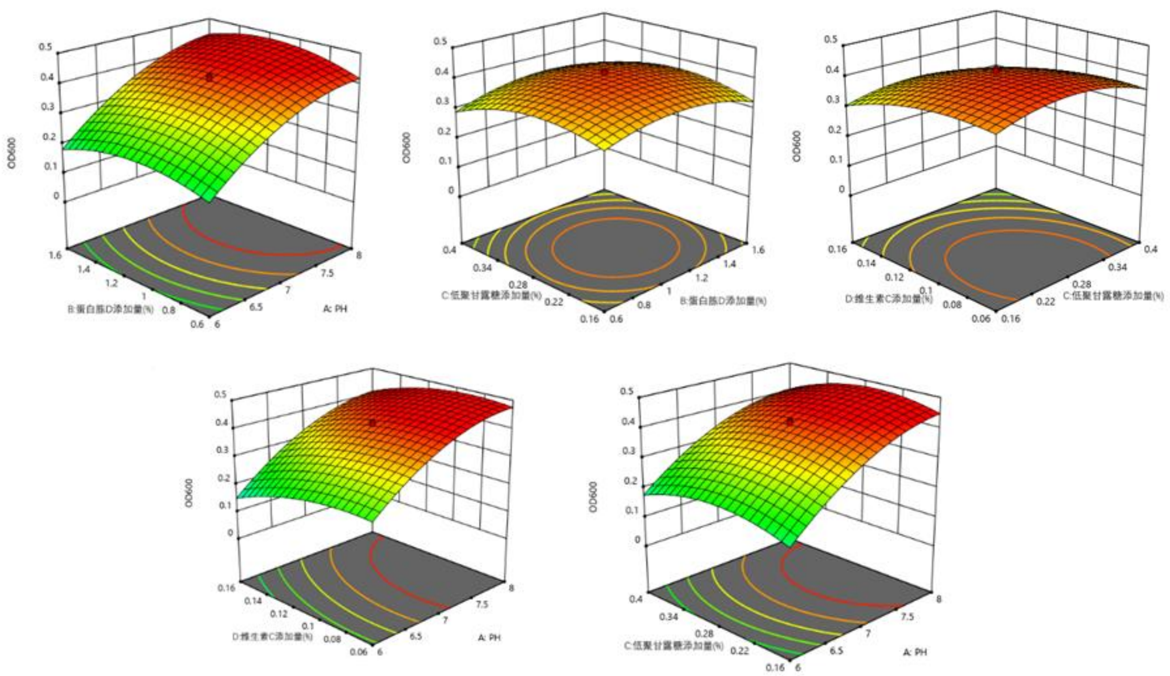

Figure 8 Response surface of the interaction of various factors of *Streptococcus thermophilus*

Through response surface experiment design, the optimal medium composition and initial conditions of *Lactobacillus plantarum* and *Streptococcus thermophilus* containing oligomannose are obtained. From the significance of the model, the experimental design models of the two probiotics are both significant, indicating that the model has credibility. But the lack of fit is significant, indicating that the experimental data is not ideal, which may be related to the design of the experimental factor level, experimental error, etc. Analyze the response surface and contour map of the interaction of the two probiotics. The unsatisfactory experimental data may be due to the small design range of each factor level and the small difference in response value OD600 between different groups, so the response surface Inclined and relatively gentle.

After the optimization of the model, the biomass of *Lactobacillus plantarum* and *Streptococcus thermophilus* both increased nearly twice as compared with that before optimization, indicating that the optimization of the model has a more obvious effect, and it proves that oligomannose and vitamins are effective for both bacteria. Obvious growth promotion effect. Among them, the primary and secondary terms of the factor oligomannose in the response surface test data of Lactobacillus plantarum showed significant status, which proves that oligomannose is used as a prebiotic for *Lactobacillus plantarum*. Probiotics have a significant effect of promoting proliferation. Compared with *Lactobacillus plantarum*, the effect of oligomannose on the proliferation of *Streptococcus thermophilus* is slightly worse, which may be due to the influence of other fermentation conditions, such as fermentation temperature, aerobic environment, etc., or it may be due to the body of *Streptococcus thermophilus*. There is no enzyme system that breaks down large molecular carbohydrates, so the utilization of polysaccharides such as oligomannose is poor [13].

IV. Conclusion

Through a single factor test, the oligomannose and other oligosaccharides were compared for the growth-promoting effects of two probiotics-*Lactobacillus plantarum* and *Streptococcus thermophilus*. The results showed that oligomannose and isomaltose, low Compared with fructose and galactooligosaccharide, the two probiotics have good proliferation effects. The effect of proliferating *Lactobacillus plantarum* is second only to isomaltose; the effect of proliferating *Streptococcus thermophilus* is second only to galacto-oligosaccharide. The single factor test determined that the optimal nitrogen source of Lactobacillus plantarum was yeast extract powder and the optimal vitamin was vitamin B1, and four factors that had an impact on its biomass were selected, including initial pH value, yeast extract powder addition, and low. The amount of polymannose added and the amount of vitamin B1 added, and the response surface Central Composite test was used to establish a quadratic polynomial regression model. The final fermentation initial conditions were the initial pH value of 7, the amount of yeast extract added 1.6%, and the amount of oligomannose added. 0.16% and 0.18% of vitamin B1. Under these conditions, the biomass of Lactobacillus plantarum (0.9608) is 1.72 times higher than that before optimization (0.5598). In the response surface model, the effect of oligomannose on the biomass of Lactobacillus plantarum is highly significant, indicating that oligomannose has a significant effect on the proliferation of Lactobacillus plantarum, and demonstrates that oligomannose has a probiotic effect in the proliferation of probiotics. The single factor experiment determined that the best nitrogen source for Streptococcus thermophilus was peptone D and the best vitamin was vitamin C, and selected 4 factors that have an impact on its biomass, including the initial pH value, the amount of peptone D added, and oligomerization. The addition of mannose and vitamin C were used to establish a quadratic polynomial regression model using the response surface Central Composite experiment. The final fermentation initial conditions were the initial pH of 7, the addition of peptone D was 1.1%, and the addition of oligomannose The maximum biomass of Streptococcus thermophilus (0.4237) was 1.77 times higher than that before optimization (0.2397) under these conditions.

By culturing and fermenting two kinds of probiotics, this paper proves that oligomannose has the probiotic effect of proliferating probiotics, and provides a laboratory basis for the development of compatible health foods with oligomannose and probiotics as the main body. The research and application of polymannose is of great significance.

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