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Preparation of *Dendrobium officinale* flower oligosaccharides and the value-added effects on three types of probiotics

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Abstract

The present study prepared *Dendrobium officinale* flower oligosaccharides and investigated their added value *in vitro*. The crude oligosaccharides obtained by water extraction and alcohol precipitation were purified by ultrafiltration, and the value-added effects of different concentrations of *D. officinale* flower oligosaccharides on Saccharomycetes, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* were examined. The *in vitro* growth kinetic models for the three types of probiotics were established according to the logistic equation. Our results showed that a concentration of 8 g/L *D. officinale* flower oligosaccharides was most suitable for promoting the growth of the three types of probiotics, and the growth rates were significantly improved compared to the control group. Nonlinear fitting was performed for the *in vitro* growth kinetic models of the three bacteria according to the logistic equation, and the degrees of fit reached 0.9895, 0.9899, and 0.99, respectively, indicating a good fit. These kinetic models suitably depicted the *in vitro* growth processes of the three bacteria. *D. officinale* flower oligosaccharides promote the growth of Saccharomycetes, *L. bulgaricus* and *S. thermophilus* and improve the physiological activities of these microorganisms. This study provides a theoretical basis for the application of *D. officinale* flower oligosaccharides in the development of probiotic products.

Keywords: *Dendrobium officinale* flower; oligosaccharide; probiotics; value added.

Practical Application: Preparation dendrobium officinale flower soft candy.

1. INTRODUCTION

Probiotics are live microorganisms that colonize the human body and are beneficial to the host. They possess functions such as promoting the absorption of nutrients (Pandey, Naik, & Vakil, 2015), improving immunity (Cunningham-Rundles et al., 2000), increasing antioxidant levels (Amarettia et al., 2013), and suppressing intestinal inflammation (Kim et al., 2019), all of which are important for human health.

In recent years, several studies in China have evaluated the aqueous extracts of *Dendrobium officinale* Kimura et Migo flowers (Chen et al., 2014; Li et al., 2012; Pan et al., 2014; Rungwichaniwat et al., 2014; Yang et al., 2015; Zhang et al., 2020). For instance, He et al. (2016) studied the antihypertensive effect and mechanisms of *Dendrobium* flowers in spontaneously hypertensive rats, whose results indicated that aqueous extracts of *D. officinale* flowers could effectively lower blood pressure in rats. Gong et al. (2014) investigated the chemical composition and antioxidant activities of *D. officinale* flowers, and their results showed that the flowers of *D. officinale* had stronger reducing capacity than the stems of *D. officinale*. Lei et al. (2015) reported the effect of *D. officinale* flower extracts on hyperthyroid yin-deficiency mice. Their results suggested that *D. officinale* flowers could not only improve the yin-deficiency symptoms of hectic cheek and palpitation in hyperthyroid yin-deficient mice but could also ameliorate liver damage resulting from hyperfunction of the thyroid axis. However, the oligosaccharides present in the aqueous extracts of *D. officinale* flowers have not been reported. Oligosaccharides are also among the active ingredients of *D. officinale* flowers. Contemporary pharmacological studies have shown that oligosaccharides have certain functionalities (Nobre et al., 2015), which include promoting the growth of intestinal probiotics (Feng et al., 2022), improving the internal environment of the human body (Sivamaruthi et al., 2019), and enhancing immunity and anti-oxidative activities, thereby possessing excellent prospects for development.

This article prepared oligosaccharides in aqueous extracts of *D. officinale* flowers through purification, investigated the *in vitro* value-added effects of the *D. officinale* flower oligosaccharide extract solution on three types of probiotics, including Saccharomycetes, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus*, and established the *in vitro* growth kinetic models for the three bacteria with the aid of the logistic function. Our findings should provide a greater theoretical basis for extended research on *D. officinale* flowers.

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2. MATERIALS AND METHODS

2.1. Materials

The *D. officinale* flowers were provided by the Anhui Engineering Center for Conservation and Sustainable Utilization of Traditional Chinese Medicine Resources of West Anhui University. Yogurt was obtained from Junlebao Dairy, and saccharomycetes was obtained from Angel Yeast; it's a single-celled fungus. Potato dextrose agar (PDA) culture medium and De Man, Rogosa and Sharpe agar (MRS) culture medium were both obtained from Qingdao Hope Bio-Technology.

2.2. Instruments

Tissue ultrafine pulverizer: FTT-2500T model, from Dongguan Fangtai Electrical Appliance Co., Ltd. Far infrared blast drying oven: DGT-G250 model, from HeFei Huadeli Scientific Equipment Co., Ltd. Freeze dryer: LC-18N-50A model, from Shanghai Keluan Instrument. Ultraviolet (UV) spectrophotometer: UV2100 model, from Unico Shanghai Instrument.

2.3. Experimental methods

2.3.1. Preparation of D. officinale flower oligosaccharide samples

Fresh flowers of *D. officinale* were sorted, rinsed, and laid out to dry. They were placed in an 80°C oven for 5 min before crushing. They were then filtered through a 200-mesh strainer. The sample, with a water content of 8.02%, was extracted with distilled water (the ratio of material to liquid is 1:15 g/mL), and the extraction was assisted by ultrasound. The extraction conditions were as follows: time 1 h, temperature 75°C, and power 100 W. The extraction was performed 3 times. Centrifugation was used to obtain the supernatant, which was concentrated via rotary evaporation. The rotary evaporation conditions were as follows: time 10 min, temperature 40°C, and power 180 W. The concentrated solution was precipitated with alcohol (95% ethanol for 8 h), filtered, and concentrated by rotary evaporation again to obtain the crude *D. officinale* oligosaccharide. The *D. officinale* flower oligosaccharide crude product was degreased with petroleum ether (material to liquid ratio was 1:4 g/mL) before deproteinization via polyamide (material to liquid ratio was 1:4 g/mL) and depigmentation via activated charcoal (with activated charcoal at 5% of the current volume of the sample, adsorbing for 20 min, and repeated three times). Finally, distilled water elution was performed in a 300-mm Büchner funnel for 24 h, and the middle stage eluate was collected for rotary evaporation concentration, followed by freeze-drying for 48 h to obtain the purified product of *D. officinale* oligosaccharides (Equation 1).

$$\text{Oligosaccharide yield}/\% = \frac{\text{Mass of } D.officinale\ flower \text{ oligosaccharides}}{\text{Mass of dried } D.officinale \text{ flowers}} \times 100 \quad (1)$$

2.3.2. Preparation of test bacteria

Preparation of Saccharomycetes: In a UV clean bench, sterile PDA solid culture medium was poured into the prepared Petri dishes, which were cooled and solidified into culture plates. An aliquot of 0.1 mL Saccharomycetes solution (powder to liquid ratio of 1:100 g/mL) was aspirated from the test tube using a sterile pipette and inoculated onto the PDA culture plate, and then evenly coated, spread, and left at rest for 5 min before being placed upside down in a thermostatic incubator at 28°C to culture for 48 h. Purification was repeated four times to derive Saccharomycetes.

Preparation of lactic acid bacteria mixed strains: In a UV clean bench, yogurt samples were diluted with normal saline solution to 10^{-5}, 10^{-6}, and 10^{-7}. A volume of 0.1 mL of the diluents at each level was transferred into the prepared Petri dishes, to which 15 mL sterile MRS solid culture medium at 50°C was added. After mixing well, cooling, and solidifying, the dishes were placed upside down in a thermostatic incubator at 36°C to culture for 48 h. Purification was repeated four times to derive the lactic acid bacteria mixed strains.

Preparation of *L. bulgaricus*: In a UV clean bench, the sterile MRS solid culture medium (pH was adjusted to 5.0 with 1% hydrochloric acid) was poured into the prepared Petri dishes, which were cooled and solidified into culture plates. The plates were streaked with lactic acid bacteria mixed strains, after which the plates were placed upside down in a thermostatic incubator at 40°C to culture for 48 h. Purification was repeated four times to derive *L. bulgaricus*.

Preparation of *S. thermophilus*: In a UV clean bench, sterile MRS solid culture medium (pH was adjusted to 9.0 with 1% NaOH) was poured into the prepared Petri dishes, which were cooled and solidified into culture plates. The plates were streaked with lactic acid bacteria mixed strains, and the obtained plates were placed upside down in a thermostatic incubator at 36°C to culture for 48 h. Purification was repeated four times to derive *S. thermophilus*.

2.4. Determination of the appropriate additive concentrations of D. officinale *flower oligosaccharides*

Dendrobium officinale flower oligosaccharide concentrations of 0, 2, 4, 8, and 16 g/L were prepared in PDA culture medium. After adding 0.1 mL Saccharomycetes suspension, the samples were placed in a shaker at 28°C and 120 r/min for 48 h. After three repetitions in parallel, the absorbance values (optical density – OD values) of the samples were measured at 600 nm to determine the appropriate concentration for addition. Based on the suitable additive concentration, the effects of the culture medium with *D. officinale* flower oligosaccharides on Saccharomycetes growth status over time were compared (the culture medium without bacterial solution was used as a reference).

Dendrobium officinale flower oligosaccharide concentrations of 0, 2, 4, 8, and 16 g/L were prepared in MRS culture medium. After adding 0.1 mL *L. bulgaricus* suspension, they were placed in a shaker at 40°C and 120 r/min for 48 h. After three repetitions in parallel, the absorbance values of the samples were measured at 600 nm to determine the appropriate concentration for addition. Based on the suitable additive concentration, the effects of the culture medium with *D. officinale* flower oligosaccharides on Saccharomycetes growth status over time were compared (the culture medium without bacterial solution was used as a reference).

Dendrobium officinale flower oligosaccharide concentrations of 0, 2, 4, 8, and 16 g/L were prepared with MRS medium. After adding 0.1 mL *S. thermophilus* suspension, the samples were placed in a shaker at 36°C and 120 r/min for 48 h. After three repetitions in parallel, the absorbance values of the samples were measured at 600 nm to determine the appropriate concentration for addition. Based on the suitable additive concentration, the effects of the culture medium with *D. officinale* flower oligosaccharides on Saccharomycetes growth status over time were compared (the culture medium without bacterial solution was used as a reference).

2.5. Growth kinetics analysis of the three bacteria after the addition of D. officinale *flower oligosaccharides*

The logistic equation (Chen et al., 2020) can depict the basic law of population growth within a limited space. It is characterized by slow initial population growth, a rapid increase within a certain period of time thereafter, and then a slowing down of growth once the limit of growth is reached, thereby exhibiting an S-shaped curve. Since the growth curves of the three bacteria cultivated in this experiment were relatively standard S-shaped growth curves, the logistic equation (Liu et al., 2002) (Equation 2) was chosen to fit the bacterial growth patterns and establish the mathematical models of the growth changes for the three bacteria after oligosaccharide addition.

$$\frac{dN}{dt} = \mu_m N\left(1 - \frac{N}{N_m}\right) \quad (2)$$

In Equation 2:

dN/dt: the growth and proliferation rate for the three bacteria, g/(L•h);

μ_m: the maximum specific growth rate for the three bacteria, h^{-1};

N: the OD value for the three bacteria, g/L;

N_m: the maximum OD value for the three bacteria, g/L;

t: is the corresponding fermentation time, h.

Through integral processing of the logistic equation, the corresponding algebraic equation was attained, as shown in Equation 3.

$$N = \frac{N_0 N_m e^{\mu_m t}}{N_m - N_0 + N_0 e^{\mu_m t}} \quad (3)$$

In the equation:

N_0: the initial bacterial OD value for the three bacteria, g/L.

2.6. Statistical analysis

SPSS 22.0 (IBM Corp., Armonk, NY, USA) was used to analyze the data for significance at the $\alpha = 0.05$ level. Plots were produced in Excel 2003 (Microsoft Corp., Redmond, WA, USA). The impact of *D. officinale* flower oligosaccharides on the three bacteria was comprehensively evaluated.

3. RESULTS AND ANALYSIS

3.1. Purification of the D. officinale *flower oligosaccharides*

The *D. officinale* flowers were extracted according to the extraction scheme in the section "Preparation of *D. officinale* flower oligosaccharide samples", and the derived filtrates were combined and subsequently concentrated. Impurities were removed by ethanol precipitation. After freeze drying, 2.8 g crude oligosaccharides were obtained, and the yield rate of crude oligosaccharides was 28%, according to the calculation from Equation 1. After purification, a total of 623 mg of pure oligosaccharides were obtained, with a yield rate of 22.25% according to the calculation from Equation 1.

3.2. Suitable additive concentration of D. officinale *flower oligosaccharides*

According to the OD values in Table 1, it can be preliminarily determined that an additive concentration of 8 g/L oligosaccharides was more appropriate.

3.3. In vitro *value-added outcomes of* D. officinale *flower oligosaccharides on the three probiotics*

3.3.1. Value-added results of Saccharomycetes *by* D. officinale *flower oligosaccharides*

The growth curve of Saccharomycetes was measured with the addition of 8 g/L *D. officinale* flower oligosaccharides.

It can be seen in Figure 1 that, compared to the control, the *D. officinale* flower oligosaccharides could evidently shorten the adjustment period of the Saccharomycetes's culture, thereby making it enter the logarithmic phase in advance and increasing the speed of microbial growth. After 36 h, bacterial growth velocity slowed down, which may be due to the altered microbial growth environment caused by the depletion of nutrients in the culture medium and the production

Table 1. Absorbance values (OD values) when cultured for 48 h at different concentrations (g/L) of *Dendrobium officinale* flower oligosaccharides[@].

Bacteria	OD values at different concentrations (g/L) of *D. officinale* flower oligosaccharides				
	0	2	4	8	16
Saccharomycetes	1.021±0.066	1.146±0.042*	1.431±0.034*	1.694±0.017	1.336±0.017
L. bulgaricus	0.147±0.034	0.615±0.021*	0.983±0.014*	1.496±0.023	1.620±0.038*
S. thermophilus	0.518±0.045	0.505±0.019	0.512±0.017*	0.526±0.015	0.494±0.010*

[@]The OD values in this table are the means of three repeated experiments; *significance at the level of α=0.05.

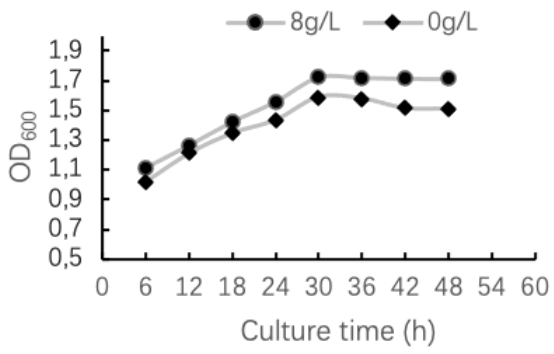

Figure 1. The growth curve of Saccharomycetes.

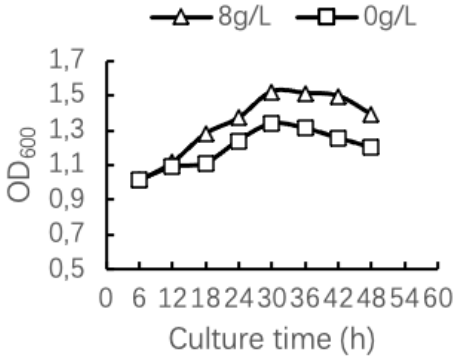

Figure 2. The growth curve of *Lactobacillus bulgaricus*.

of metabolic substances, leading to a reduced bacterial proliferation rate. However, at each growth stage, the microbial concentrations of the experimental group were all higher than those of the control group, indicating that *D. officinale* flower oligosaccharides could effectively promote the growth of Saccharomycetes.

3.3.2. Value-added results of L. bulgaricus *by* D. officinale *flower oligosaccharides*

The growth curve of L. bulgaricus was measured with the addition of 8 g/L *D. officinale* flower oligosaccharides.

It can be seen in Figure 2 that, compared to the control, the *D. officinale* flower oligosaccharides did not significantly alter the culture of *L. bulgaricus* in the lag phase, but the value-adding speed of the bacteria accelerated after entering the logarithmic phase. *Lactobacillus bulgaricus* has a potent acid production ability, and acid production capacity is a key physiological property of lactic acid bacteria. The lactic acid content in the fermented broth increased and the bacteria grew faster. These findings indicate that the *D. officinale* flower oligosaccharides effectively promoted the growth of *L. bulgaricus*, and the bacterial concentrations of the experimental group were all greater than those of the control group in the later stage.

3.3.3. Value-added results of S. thermophilus *by* D. officinale *flower oligosaccharides*

The growth curve of *S. thermophilus* was measured with the addition of 8 g/L *D. officinale* flower oligosaccharides.

It can be seen in Figure 3 that, compared to the control, the *D. officinale* flower oligosaccharides did not affect the culture of *S. thermophilus* significantly in the lag phase, and the value-added speed of the bacteria accelerated after entering the logarithmic phase. *Streptococcus thermophilus* can digest lactose, and lactose breaks down to generate glucose. This increased the carbon source availability in the culture medium and accelerated bacterial growth. These results suggest that *D. officinale* flower oligosaccharides effectively promoted the growth of *S. thermophilus*, and the bacterial concentrations of the experimental group were all higher than those of the control group in the later stage.

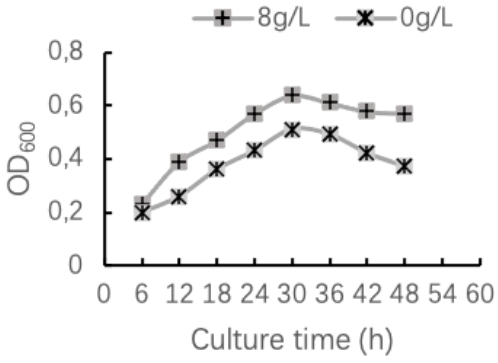

Figure 3. The growth curve of *Streptococcus thermophilus*.

3.4. Growth kinetics analysis results of the three bacteria after the addition of D. officinale *flower oligosaccharides*

Experimental data in Figures 1–3 were substituted into Equation 3, and nonlinear fitting of bacterial growth models was performed using Excel. Results are shown in Figures 4, 5, and 6.

The equations used experimental data for the bacterial growth kinetic parameters N_0, N_m, and μ_m, and the growth kinetics equations for the three bacteria were derived as follows (Equations 4–6):

$$N_{Saccharomycetes} = \frac{1.9148e^{0.21t}}{0.61 + 1.112e^{0.21t}}, \quad (4)$$

$$N_{L.bulgaricus} = \frac{1.5392e^{0.305t}}{0.509 + 1.012e^{0.305t}}, \quad (5)$$

$$N_{S.thermophilus} = \frac{0.1467e^{0.312t}}{0.408 + 0.23e^{0.312t}}. \quad (6)$$

Nonlinear fitting was performed in Excel for the predicted growth curves and experimental values. It can be seen from Figures 4–6 that the correlation coefficient R^2 values of the growth models for the three bacteria were 0.9895, 0.9899, and 0.99, respectively. These values indicate that the theoretical growth curves fit well with the actual growth status models for

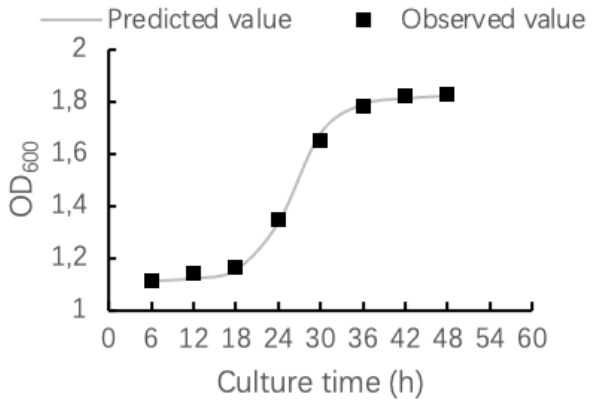

Figure 4. The growth model fitting curve of Saccharomycetes.

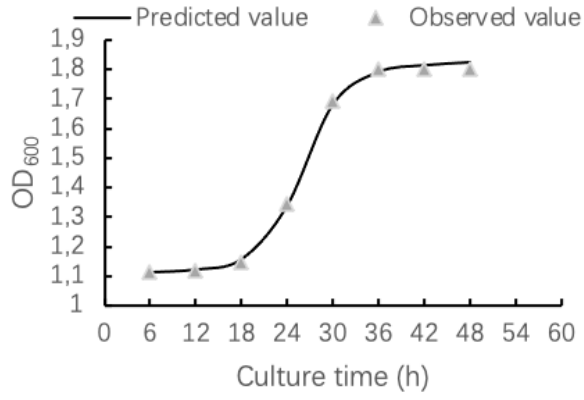

Figure 5. The growth model fitting curve of *Lactobacillus bulgaricus*.

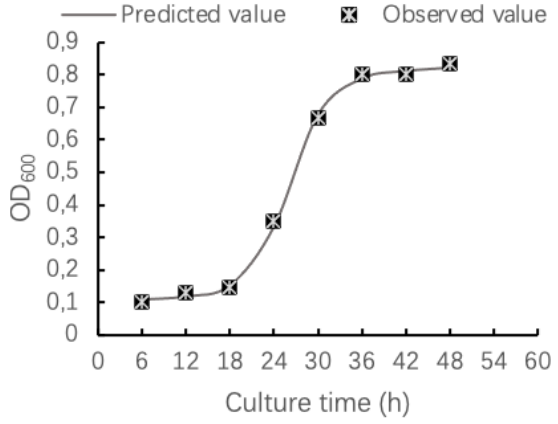

Figure 6. The growth model fitting curve of *Streptococcus thermophilus*.

Saccharomycetes, *L. bulgaricus*, and *S. thermophilus* in the culture medium with 8 g/L *D. officinale* flower oligosaccharides, and these three models could well simulate the growth states of the three strains under this environmental condition. These findings provide a reference for the fermentation and production of Saccharomycetes, *L. bulgaricus*, and *S. thermophilus* under oligosaccharide addition.

4. CONCLUSION

Through ultrafiltration of the *D. officinale* flower oligosaccharides obtained by water extraction and alcohol precipitation, 623 mg of product was obtained with a purity of around 80%. By comparing the levels of absorbance at 600 nm over 48 h for Saccharomycetes, *L. bulgaricus*, and *S. thermophilus*, 8 g/L was selected as the suitable concentration of *D. officinale* flower oligosaccharides to influence the growth of Saccharomycetes, *L. bulgaricus*, and *S. thermophilus*. Our findings revealed that the addition of 8 g/L *D. officinale* flower oligosaccharides could obviously shorten the growth lag phases of Saccharomycetes, *L. bulgaricus*, and *S. thermophilus*, enhance the bacterial growth rates in the logarithmic phase, and elevate the absorbance values of Saccharomycetes, *L. bulgaricus*, and *S. thermophilus*.

Growth kinetics were analyzed for Saccharomycetes, *L. bulgaricus*, and *S. thermophilus* after adding *D. officinale* flower oligosaccharides, and bacterial growth equations were derived (as shown in Equations 4–6). The fitting results showed that the R^2 values were 0.9895, 0.9899, and 0.99, respectively, thereby exhibiting a good fit.

Our results indicate that *D. officinale* flower oligosaccharides can facilitate the growth of Saccharomycetes, *L. bulgaricus*, and *S. thermophilus* and are conducive to improving the physiological activities of these bacteria. These research findings provide a theoretical basis and scientific foundation for broadening the applications of *D. officinale* flower oligosaccharides as well as Saccharomycetes, *L. bulgaricus*, and *S. thermophilus* in the food field and for developing *D. officinale* flower oligosaccharide probiotic complex products.

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