frontiers | Frontiers in Sustainable Food Systems

TYPE Original Research
PUBLISHED 18 December 2024
DOI 10.3389/fsufs.2024.1470426

OPEN ACCESS

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RECEIVED 25 July 2024
ACCEPTED 29 November 2024
PUBLISHED 18 December 2024

CITATION
Zhu X, Lin S and Wei Q (2024) Evaluation of polysaccharides from *Laetiporus sulphureus* on the growth of gastrointestinal probiotics and *in vitro* digestion.
Front. Sustain. Food Syst. 8:1470426.
doi: 10.3389/fsufs.2024.1470426

Evaluation of polysaccharides from *Laetiporus sulphureus* on the growth of gastrointestinal probiotics and *in vitro* digestion

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Introduction: Probiotics can improve immune responses and regulate the ecosystem of microorganisms in the gastrointestinal tract.

Methods: Three primary models, including the Reparameterized Gompertz, Huang, and Baranyi and Roberts models were evaluated and developed to investigate the effects of *Laetiporus sulphureus polysaccharides* (LSP) on the growth of *Lactobacillus plantarum, Streptococcus thermophilus, Clostridium tyrobutyricum*, and *Bifidobacterium adolescentis*.

Results: The Huang model and Reparameterized Gompertz model were suitable for describing the growth of *C. tyrobutyricum, S. thermophilus, B. adolescentis*, and *L. plantarum*. LSP could increase the population of *B. adolescentis* in the fluid environment of the stomach in vitro.

Discussion: These results may support the further development of LSP as a functional food or food additive that has the ability to preserve digestive tract health.

KEYWORDS

***Laetiporus sulphureus*, polysaccharides, kinetic model, gastrointestinal probiotics, *in vitro* digestion**

1 Introduction

In recent decades, the gastrointestinal tract has been regarded as the human body's biggest immunological organ. The gastrointestinal tract has a diverse microbiota that is emerged as a critical target for the treatment of disease (Xu et al., 2023; Li et al., 2024). Experimental evidence has shown that metabolic disorders including hypertension, insulin resistance, obesity, hyperglycemia, and hyperlipidemia, etc. are closely associated with gut microbiota (Shao et al., 2022; Zhang H. et al., 2023; Ouyang et al., 2024; Li et al., 2024. Makki et al. (2018) found that a high fat diet could reduce microbial diversity and change the gut microbial metabolism, leading to the development of metabolic syndrome. According to Li et al. (2019), the gut microbiota of the high fat diet-induced rats was modulated in order to prevent hyperlipidemia and hypercholesterolemia. It suggested that improving gastrointestinal tract health might be a new strategy for the treatment of diseases.

Probiotics are the dominant microbiota in the gastrointestinal tract and beneficial to improve the digestion of food. *Lactobacillus plantarum*, *Clostridium tyrobutyricum*, *Bifidobacterium adolescentis*, and *Streptococcus thermophilus* are important bacteria in the gastrointestinal tract. *L. plantarum* is a facultative anaerobic gram-positive bacterium. It is reported that *L. plantarum* can improve intestinal inflammation, preventing the occurrence or aggravation of diseases, and it also can be used to treat mental illness and

improve cognitive functions (Toshimitsu et al., 2016; Rudzki et al., 2019; Hu et al., 2020; Zhang Z. et al., 2023; Zeng et al., 2023). *C. tyrobutyricum* is an obligate anaerobic gram-positive bacterium. Luo et al. (2024) suggested that *C. tyrobutyricum* may alleviate obesity symptoms by improving lipid metabolism and intestinal health, as well as regulating intestinal microbiota. *B. adolescentis* is a gram-positive and non-motile bacterium, which has been shown to alleviate anxiety/depression and treat colitis (Kim et al., 2019; Ma et al., 2021; Sharma et al., 2023). *S. thermophilus* is considered as a probiotic, which is safe and can survive in the human gastrointestinal tract and exhibited health benefits on its host (Uriot et al., 2017). It is widely used in the production of dairy products (Che et al., 2019; Demirci et al., 2024).

Several studies have demonstrated that mushroom polysaccharides are the effective components in the regulation of gut microbiota, as they promote the growth of beneficial microorganisms: *Agaricus bisporus* polysaccharides could enhance the abundance of beneficial bacteria during the stimulating gastrointestinal digestion and gut microbiota fermentation (Fu et al., 2023). Lv et al. (2019) found that *Ganoderma lucidum* polysaccharides had the potential to increase total short chain fatty acids and butyric acid while regulating gut microbiota. On the other hand, they decrease the population of pathogens: a study performed in 2018 showed that chitosan obtained from *G. lucidum* spore powder has the antimicrobial activity for *E. coli* and *S. aureus* (Zhu et al., 2018).

Laetiporus sulphureus (*L. sulphureus*) is an edible and medicinal mushroom belonging to the phylum of Basidiomycota. It has already been documented that *L. sulphureus* contains various bioactive compounds such as lectin, polysaccharides, triterpenoids, and phenolic compounds, etc. (Wang et al., 2018; Lu et al., 2023; Jen et al., 2024). Polysaccharides are the vital bioactive substances of *L. sulphureus*. Interestingly, a number of studies have demonstrated the various health promoting properties of *L. sulphureus* polysaccharides (LSP) including anti-inflammatory (Lu et al., 2023), anti-cancer (Jen et al., 2024), hepatoprotective, antioxidant activity (Zhao et al., 2019), and hypoglycemic activity (Hwang and Yun, 2010). Many reports have shown that modeling experimental data using kinetic models is one of the effective ways to study the effects of prebiotic compounds on probiotics (Altieri et al., 2016; Bernal-Castro et al., 2019; Montes et al., 2024; Wang et al., 2023). However, there was limited investigation into the impact of LSP on gut microbiota or application of any mathematical model.

In general, in order to further develop the application of LSP in promoting microbial growth, the aim of this study was (1) to investigate the effects of LSP on the growth of *C. tyrobutyricum*, *S. thermophilus*, *B. adolescentis*, and *L. plantarum*, (2) combining with kinetic models to select the accurate mathematical models for describing the growth of *C. tyrobutyricum*, *S. thermophilus*, *B. adolescentis*, and *L. plantarum*, (3) to evaluate the efficiency of LSP in increasing the survival of *C. tyrobutyricum*, *S. thermophilus*, *B. adolescentis*, and *L. plantarum* at simulated gastric and intestinal condition. These researches provide a theoretical basis for the development of functional food, health products and other related fields. In addition, they provided a new idea for the exploitation and utilization of LSP.

2 Materials and methods

2.1 Materials and media

The fruiting body of *L. sulphureus* (dried mushroom) was obtained from Changbaishan Co., Ltd. (Jilin, China). Reinforced *Clostridium* medium (RCM) broth, RCM agar medium, tryptone peptone yeast extract (TPY) broth, TPY agar medium, modified Chalmers (MC) broth, MC agar medium, and De-Man Rogosa Sharpe (MRS) broth, MRS agar medium were purchased from Hopebio Co., Ltd. (Qingdao, China).

2.2 Preparation of LSP

The dried powder of *L. sulphureus* was mixed with distilled water (1:20, w/v) at 80°C for 3 h. The supernatant was collected by filtration through a Buchner funnel and precipitated by two volumes of ethanol (95%, v/v) at 4°C for 12 h. The precipitate was obtained by centrifugation (4,000 rpm, 20 min), and then deproteinated with Sevag reagent (n-butanol: chloroform = 1:4, v/v). LSP was harvested after freeze-drying (FDU-2110, Eyela Co., Ltd., Tokyo Japan), which was used for further research.

2.3 Bacterial culture and preparation

Clostridium tyrobutyricum (ATCC25755), *Bifidobacterium adolescentis* (ATCC15703), *Streptococcus thermophilus* (ATCC14485), and *Lactobacillus plantarum* (ATCC8014) were purchased from Guangdong Microbial Culture Collection Center. The bacterial cultures were harvested after 3 consecutive transfers. One day before the experiment, 100 μL of each culture (*C. tyrobutyricum*, *B. adolescentis*, *S. thermophilus*, and *L. plantarum*) was individually transferred to 10 mL of RCM, TPY, MC, and MRS broth and held at 37°C in an anaerobic box (Mitsubishi Gas Chemical Company Incorporated; Tokyo, Japan) for 24 h.

2.4 Bacterial growth

The 100 μL of each overnight culture (*C. tyrobutyricum*, *B. adolescentis*, *S. thermophilus*, and *L. plantarum*) was separately added into 10 mL of RCM, TPY, MC, and MRS broth containing 0% (Control), or 2% of LSP in sterile centrifuge tubes. Each tube was mixed evenly and anaerobically kept for 24 h at 37°C. Each tube was serially diluted and enumerated. Additionally, the bacteria's concentrations were transformed to the natural base, or logarithm of base 10, and stated as Ln CFU/mL or lg CFU/mL.

2.5 Primary models

To select the most suitable kinetic model to describe the growth of *C. tyrobutyricum*, *B. adolescentis*, *S. thermophilus*, and *L. plantarum*, three primary models were chosen. Huang model (Equations 1, 2, Huang, 2013), Baranyi and Roberts model

(Equations 3, 4, Baranyi and Roberts, 1995), and Reparameterized Gompertz model (Equation 5, Zwietering et al., 1990) were used in this research. The Huang model is expressed as

$$Y(t) = Y_0 + Y_{\max} - ln\left\{e^{Y_0} + \left[e^{Y_{\max}} - e^{Y_0}\right]e^{-\mu_{\max}B(t)}\right\} \quad (1)$$

$$B(t) = t + \frac{1}{\alpha}\ln\frac{1+e^{-\alpha(t-\lambda)}}{1+e^{\alpha\lambda}} \quad (2)$$

The variables in this equation are: t presents the time point (h), Y(t) presents the natural logarithm of microorganism count (ln CFU/g), λ presents the lag phase duration (h), Y_{max} presents the bacterial count (ln CFU/g) at the stationary phase, Y_0 presents the initial microorganisms count (ln CFU/g), μ_{max} presents the maximum specific growth rate (h^{-1}), $B(t)$ is the transfer function and α is a constant (α = 4) that defines the transition from the lag phase to the exponential phase of a growth curve (Huang, 2013).

The Baranyi and Roberts model is expressed as

$$Y(t) = Y_0 + \mu_{\max}A(t) - ln\left[1 + \frac{e^{\mu_{\max}A(t)} - 1}{e^{Y_{\max}-Y_0}}\right] \quad (3)$$

$$A(t) = t + \frac{1}{\mu_{\max}}\ln(e^{-\mu_{\max}t} + e^{-h_0} - e^{-\mu_{\max}t-h_0}) \quad (4)$$

The physiological state of the microorganism, h_0, is equals to $\lambda\times\mu_{max}$. Y_{max}, Y_0, Y(t), and μ_{max}, are specified as in the Huang model. Establishing the h_0 averaged value. $A(t)$ is the transfer function. Next, use a fixed h_0 value to estimate Y_0, μ_{max}, and Y_{max}.

The Reparameterized Gompertz model is expressed as

$$Y(t) = Y_0 + (Y_{\max} - Y_0)\exp\left\{-exp\left[\frac{\mu_{\max}e}{Y_{\max}-Y_0}(\lambda - t) + 1\right]\right\} \quad (5)$$

t, λ, Y(t), Y_{max}, μ_{max}, and Y_0 are specified as in the Huang model.

2.6 Simulated gastrointestinal condition

2.6.1 Effect of LSP on simulated gastric fluids *in vitro*

Simulated gastric fluids were prepared as described by Kenari and Razavi (2022) with slight modification. Briefly, the phosphate buffered saline (0.1 mol/L, pH = 7.4) was used as solvent to obtain RCM broth. The pH values of the RCM broth were adjusted to 3 using hydrochloric acid (8 mol/L). After sterilization, 0.5 g of pepsin (Solarbio Life Sciences Co., Ltd. Bejing, China) was added into 100 mL of RCM broth to obtained the simulated gastric fluid of RCM broth. The simulated gastric fluids of TPY, MC and MRS broths were obtained by using the method as described as the simulated gastric fluid of RCM broth. The 100 μL of each overnight culture (*C. tyrobutyricum*, *B. adolescentis*, *S. thermophilus*, and *L. plantarum*) was separately added into 10 mL of simulated gastric fluids containing 0% (Control) or 2% of LSP. Each tube was collected at 0 and 3 h of digestion. After digestion, the samples

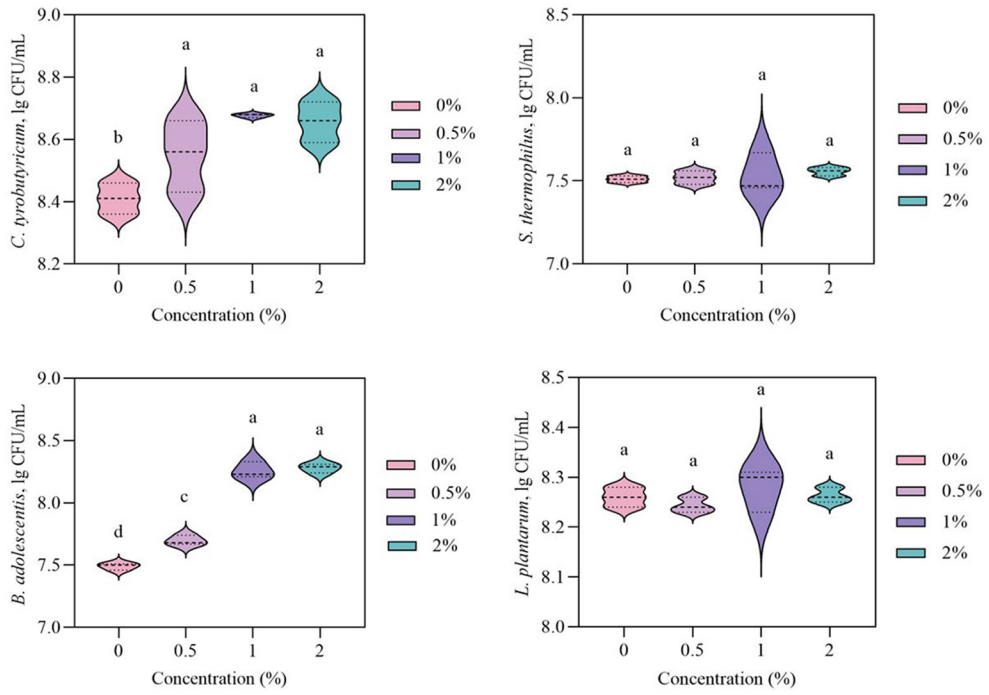

FIGURE 1
Effects of polysaccharides from *Laetiporus sulphureus* on the growth of *Lactobacillus plantarum*, *Streptococcus thermophilus*, *Clostridium tyrobutyricum*, and *Bifidobacterium adolescentis*. Values marked by different letters are significantly different at $p < 0.05$; values marked by the same letter are not significantly different at $p > 0.05$.

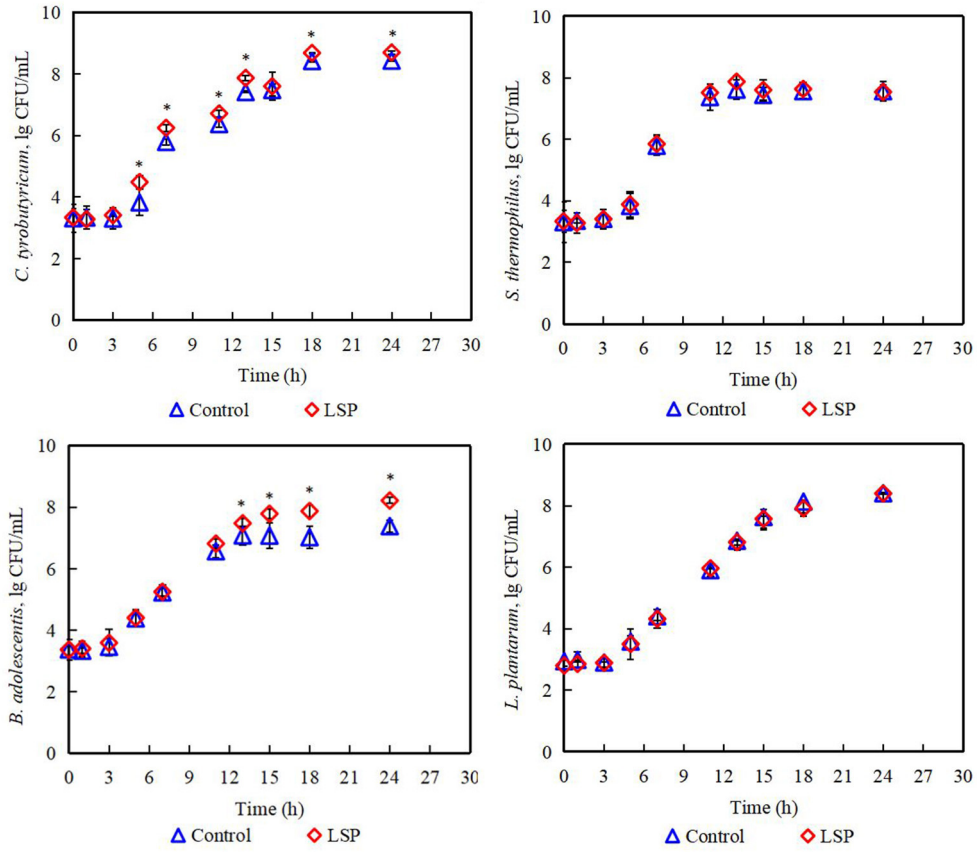

FIGURE 2
Population of *Lactobacillus plantarum*, *Streptococcus thermophilus*, *Clostridium tyrobutyricum*, and *Bifidobacterium adolescentis*. Control: 0% of *Laetiporus sulphureus* polysaccharides, LSP: 2% of *Laetiporus sulphureus* polysaccharides. *Significant difference at $p < 0.05$.

were serially diluted and enumerated on RCM, TPY, MC and MRS agar media to enumerate *C. tyrobutyricum*, *B. adolescentis*, *S. thermophilus*, and *L. plantarum*. The bacterial concentrations were also converted to the logarithm of base 10 or natural base, recorded as Log_{10} CFU/mL or Ln CFU/mL.

2.6.2 Effect of LSP on simulated intestinal fluids *in vitro*

Simulated intestinal fluids were prepared by the method of Razavi et al. (2020). For the intestinal digestion, the phosphate buffered saline (0.1 mol/L, pH = 7.4) was used as a solvent to obtain RCM broth. The pH values of the RCM broth were adjusted to 7 using hydrochloric acid (8 mol/L). After sterilization, 1 g of trypsin (Solarbio Life Sciences Co., Ltd. Bejing, China) was added into 100 mL of RCM broth to obtained the simulated intestinal fluid of RCM broth. The simulated intestinal fluids of TPY, MC and MRS broths were obtained by using the method as described as the simulated intestinal fluid of RCM broth. The 100 μL of each overnight culture (*C. tyrobutyricum*, *B. adolescentis*, *S. thermophilus*, and *L. plantarum*) was separately added into 10 mL of simulated intestinal fluids containing 0% (Control) or 2% of LSP. Each tube was collected at 0 and 3 h of digestion. After digestion, the samples were serially diluted and enumerated on RCM, TPY, MC, and MRS agar media to enumerate *C. tyrobutyricum*, *B. adolescentis*, *S. thermophilus*, and *L. plantarum*. The bacterial concentrations were also converted to the logarithm of base 10 or natural base, recorded as Log_{10} CFU/mL or Ln CFU/mL.

2.7 Statistical analysis

The growth curves of *C. tyrobutyricum*, *B. adolescentis*, *S. thermophilus*, and *L. plantarum* were investigated by the Integrated Pathogen Modeling Program (IPMP) established by the United States Department of Agriculture (USDA, Huang, 2013). The specific growth rates, maximum bacterial concentration, confidence intervals, lag phase duration, and data analysis were acquired from IPMP analysis. Three replication of each experiments were conducted. One-way ANOVA was used to examine the acquired data. Graphpad Prism 9 was used for the statistical analyses.

3 Results and discussion

3.1 Effects of LSP on the growth of microbial

Recent research has demonstrated that polysaccharides have the capacity to modulate gut microbiota and the balance of

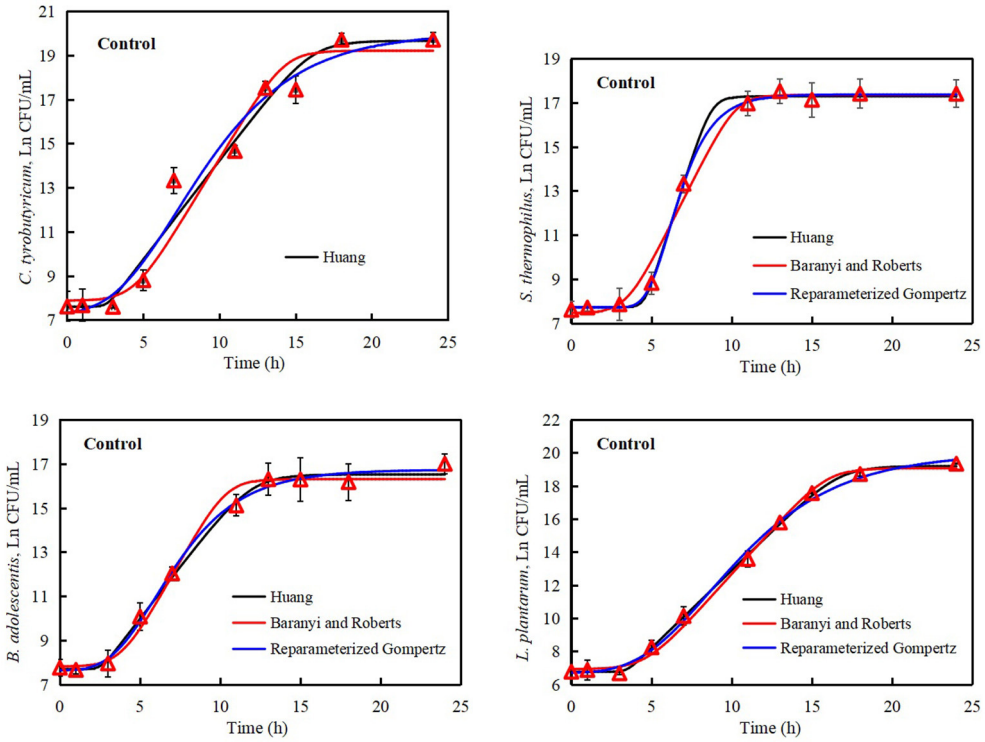

FIGURE 3
Growth curves of *Lactobacillus plantarum*, *Streptococcus thermophilus*, *Clostridium tyrobutyricum*, and *Bifidobacterium adolescentis*. Solid black line: Huang model, solid red line: Baranyi and Roberts model, solid blue line: Reparameterized Gompertz model, triangle: observed growth data.

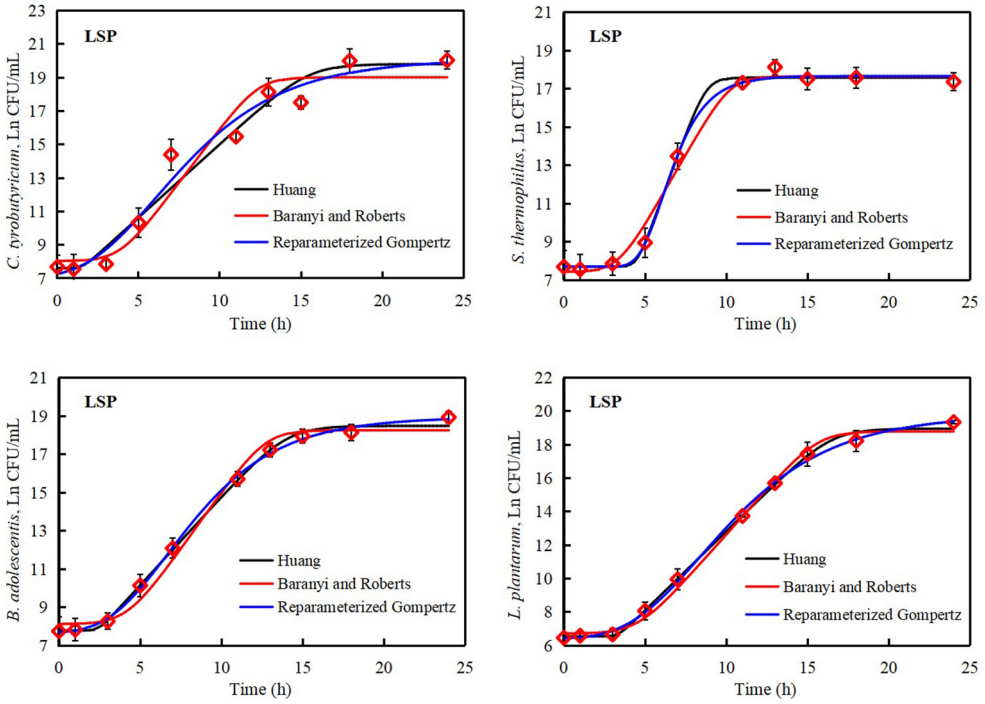

FIGURE 4
Growth curves of *Lactobacillus plantarum*, *Streptococcus thermophilus*, *Clostridium tyrobutyricum*, and *Bifidobacterium adolescentis* treated with polysaccharides of *Laetiporus sulphureus*. Solid black line: Huang model, solid red line: Baranyi and Roberts model, solid blue line: Reparameterized Gompertz model, diamond: observed growth data.

microbial metabolites (Yin et al., 2020; Xue et al., 2020). Certain species of gut microbiota might acquire energy and carbon sources via the breakdown of polysaccharides for their own proliferation (Ze et al., 2012). Tong et al. (2020) reported that *Ganoderma lucidum* polysaccharides could increase the relative abundances of beneficial bacteria (*Ruminococcus*, *Oscillibacter*, *Bifidobacterium*, *Prevotella*, *Alloprevotella*, *Paraprevotella,* and *Alistipes*). The effects of LSP on the population of *C. tyrobutyricum*, *B. adolescentis*, *S. thermophilus*, and *L. plantarum* were shown in Figure 1. The LSP at 0, 0.5, 1, and 2% concentrations exhibited the population of *C. tyrobutyricum* of 8.41 ± 0.05, 8.55 ± 0.10, 8.68 ± 0.01, and 8.66 ± 0.06 lg CFU/mL, respectively. The LSP showed a higher population of *C. tyrobutyricum* than 0% of LSP. Moreover, there was no significant difference in the population of *C. tyrobutyricum* among 0.5, 1, and 2% of LSP ($p > 0.05$). The LSP at 0, 0.5, 1, and 2% concentrations exhibited the population of *B. adolescentis* of 7.49 ± 0.02, 7.70 ± 0.03, 8.26 ± 0.06, and 8.28 ± 0.03 lg CFU/mL, respectively. The LSP exhibited an increasing population of *B. adolescentis* at higher concentrations. Compared to 0% of LSP, 1% of LSP could significantly increase the population of *B. adolescentis* and reach the maximum population of *B. adolescentis* ($p < 0.05$). There was no significant difference between 1% of LSP and 2% of LSP with regard to their population of *B. adolescentis* ($p > 0.05$). Simultaneously, the population of *S. thermophilus*, and *L. plantarum* was not increased by LSP ($p > 0.05$). The results suggested that 1% of LSP could increase the population of *C. tyrobutyricum* and *B. adolescentis*. Therefore, 1% of LSP was selected for further research.

The effects of LSP on the growth of *C. tyrobutyricum*, *B. adolescentis*, *S. thermophilus*, and *L. plantarum* were displayed in Figure 2. The population of *C. tyrobutyricum*, *B. adolescentis*, *S. thermophilus*, and *L. plantarum* increased with increasing incubation time. Compared to the control group, LSP could increase the population of *B. adolescentis* at 11–24 h, while increasing the population of *C. tyrobutyricum* at 5–24 h. The result suggested that LSP exhibited an impact on increasing the population of *C. tyrobutyricum* and *B. adolescentis in vitro*. A similar phenomenon was found by Li et al. (2019) in which *Ganoderma lucidum* polysaccharides could increase the relative abundance of *Bifidobacterium* in rats. Furthermore, the population of *S. thermophilus* and *L. plantarum* in the control group and LSP group showed no significant difference. It suggests that LSP could not increase the population of *S. thermophilus* and *L. plantarum in vitro*.

3.2 Mathematical modeling

The Huang, Reparameterized Gompertz, and Baranyi and Roberts models were used to evaluate microbial inactivation, survival, and growth in reaction to surrounding environment (Akkermans et al., 2018; Tashiro and Yoshimura, 2019). The commonly reported primary models include the Huang model, Reparameterized Gompertz model, logistic model, and Baranyi and Roberts model (Jia et al., 2020). The growth data of *C. tyrobutyricum*, *B. adolescentis*, *S. thermophilus*, and *L. plantarum* were analyzed by Huang, Reparameterized Gompertz, and Baranyi and Roberts models. The growth curves of *C. tyrobutyricum*, *B. adolescentis*, *S. thermophilus*, and *L. plantarum* all presented lag phase, exponential phase, and stationary phase (Figures 3, 4).

The mean square error (MSE), root mean square error (RMSE), and akaike information criterion (AIC) are important to evaluate the accuracy of the primary models. The smaller value of MSE, RMSE, and AIC, indicated the higher accuracy of the model. For Huang, Baranyi and Roberts, and Reparameterized Gompertz models, the MSE of *C. tyrobutyricum* in control group were 0.969, 1.309, and 0.977 Ln CFU/mL, respectively, while LSP showed the MSE of *C. tyrobutyricum* of 1.292, 1.904, and 1.048 Ln CFU/mL, respectively (Table 1). There were no significant differences in the values of MSE, RSME, and AIC among Huang, Baranyi and Roberts, and Reparameterized Gompertz models, indicating that Huang, Baranyi and Roberts, and Reparameterized Gompertz models were suitable for describing the growth of *C. tyrobutyricum*.

For Huang, Baranyi and Roberts, and Reparameterized Gompertz models, the AIC of *S. thermophilus* in the control group were −12.358, −1.994, and −17.508, respectively, and MSE were 0.040, 0.236, and 0.024 Ln CFU/mL, respectively. Simultaneously, LSP showed the AIC of −5.590, −0.920, and −5.555, respectively, while MSE of 0.078, 0.236, and 0.078 Ln CFU/mL, respectively. Compared to Baranyi and Roberts model, Huang model and Reparameterized Gompertz model had the lower MSE, RMSE, and AIC values in *B. adolescentis*, *S. thermophilus*, and *L. plantarum*, indicating that Huang model and Reparameterized Gompertz model were more suitable for describing the growth of *B. adolescentis*, *S. thermophilus*, and *L. plantarum* than Baranyi and Roberts model. This was consistent with the results of Wei et al. (2021), in which the Reparameterized Gompertz model was suitable for describing the growth of *Lactococcus lactis* in *Flammulina velutipes* fruiting bodies. Therefore, the Reparameterized Gompertz model was suitable for describing the growth of lactic acid bacteria.

Compared to control group, the lag time of microbial (*C. tyrobutyricum*, *B. adolescentis*, *S. thermophilus*, and *L. plantarum*) decreased while LSP added. The maximum population of *B. adolescentis* obtained from the Huang model, Baranyi and Roberts model, and Reparameterized Gompertz model of 18.479, 18.252, and 18.940 Ln CFU/mL, respectively, while control exhibited the maximum population of *B. adolescentis* of 16.530, 16.318, and 16.746 Ln CFU/mL, respectively. The results suggested that LSP exhibited a higher maximum population of *B. adolescentis* than the control group.

3.3 Effects of LSP on microbial in simulated gastric fluid

Simulated gastrointestinal conditions can be used to study the composition of intestinal microorganisms and understand the relationship between intestinal health and disease. Experimental evidence has shown that prebiotics have health-promoting

TABLE 1 Estimated parameters for primary models.

Microorganism	*Clostridium tyrobutyricum*						*Streptococcus thermophilus*						*Bifidobacterium adolescentis*						*Lactobacillus plantarum*					
Groups	Control			1% LSP			Control			1% LSP			Control			1% LSP			Control			1% LSP		
Primary models	Huang	Baranyi	Gompertz	Huang	Baranyi	Gompertz	Huang	Baranyi	Gompertz	Huang	Baranyi	Gompertz	Huang	Baranyi	Gompertz	Huang	Baranyi	Gompertz	Huang	Baranyi	Gompertz	Huang	Baranyi	Gompertz
Y_0 (Ln CFU/g)	7.612	7.891	7.292	7.610	8.041	7.040	7.736	7.477	7.733	7.695	7.427	7.703	7.702	7.826	7.648	7.780	8.125	7.688	6.778	6.944	6.710	6.544	6.717	6.422
L95CI	5.915	6.449	5.249	5.544	6.220	4.126	7.452	6.790	7.515	7.300	6.698	7.306	7.204	7.174	7.132	7.354	7.421	7.314	6.511	6.495	6.073	6.132	6.205	5.991
U95CI	9.309	9.333	9.335	9.676	9.863	9.954	8.016	8.165	7.951	8.091	8.156	8.099	8.201	8.479	8.163	8.206	8.828	8.061	7.045	7.393	7.347	6.957	7.230	6.853
Y_{max} (Ln CFU/g)	19.669	19.220	20.048	19.799	19.009	20.094	17.297	17.363	17.384	17.579	17.639	17.661	16.530	16.318	16.746	18.479	18.252	18.940	19.213	19.084	20.016	18.952	18.795	19.806
L95CI	17.815	17.467	17.462	17.748	17.212	17.539	17.078	16.817	17.201	17.272	17.064	17.329	16.131	15.779	16.240	18.083	17.518	18.507	18.854	18.442	18.927	18.421	18.082	19.096
U95CI	21.523	20.973	22.634	21.851	20.805	22.650	17.515	17.909	17.567	17.885	18.214	17.994	16.930	16.856	17.251	18.875	18.987	19.373	19.571	19.726	21.105	19.484	19.508	20.516
μ_{max} (h^{-1})	0.897	1.145	1.092	0.878	1.291	1.110	2.299	1.452	2.446	2.301	1.484	2.513	0.943	1.303	1.160	0.924	1.189	1.114	0.940	1.074	1.103	0.945	1.091	1.100
L95CI	0.623	0.927	0.608	0.561	0.947	0.593	1.895	1.275	2.110	1.753	1.297	1.910	0.791	1.131	0.904	0.839	1.060	0.998	0.883	1.016	0.928	0.859	1.023	0.987
U95CI	1.171	1.363	1.576	1.194	1.635	1.626	2.703	1.629	2.781	2.848	1.671	3.116	1.094	1.474	1.415	1.010	1.318	1.229	0.996	1.133	1.277	1.032	1.159	1.213
λ (h)	2.603	4.367	2.942	1.557	3.873	1.763	4.559	3.444	4.670	4.485	3.369	4.637	2.571	3.837	3.109	2.434	4.205	3.018	3.470	4.655	4.185	3.361	4.583	3.954
MSE (Ln CFU/g)	0.969	1.309	0.977	1.292	1.904	1.048	0.040	0.236	0.024	0.078	0.263	0.078	0.083	0.223	0.087	0.061	0.296	0.038	0.033	0.134	0.138	0.075	0.173	0.059
RMSE [$(Ln CFU/g)^2$]	0.984	1.144	0.989	1.137	1.380	1.024	0.199	0.486	0.154	0.280	0.513	0.280	0.288	0.472	0.295	0.246	0.544	0.196	0.181	0.366	0.372	0.274	0.416	0.243
AIC	19.573	15.126	19.661	22.453	18.871	20.356	−12.358	−1.994	−17.508	−5.590	−0.920	−5.555	−4.974	−2.569	−4.545	−8.147	0.254	−12.699	−14.348	−7.661	0.094	−6.034	−5.114	−8.403

10.3389/fsufs.2024.1470426

TABLE 2 Survival of *Lactobacillus plantarum*, *Streptococcus thermophilus*, *Clostridium tyrobutyricum*, and *Bifidobacterium adolescentis* in simulated gastric fluid treated with polysaccharides of *Laetiporus sulphureus*.

Groups	Time (h)	*Bifidobacterium adolescentis* (lg CFU/mL)	*Lactobacillus plantarum* (lg CFU/mL)	*Streptococcus thermophilus* (lg CFU/mL)	*Clostridium tyrobutyricum* (lg CFU/mL)
Control	0	5.34 ± 0.04^{a}	5.81 ± 0.34^{a}	5.25 ± 0.05^{a}	5.18 ± 0.09^{a}
	3	4.70 ± 0.25^{b}	5.86 ± 0.24^{a}	-	4.39 ± 0.17^{c}
LSP	0	5.29 ± 0.03^{a}	5.73 ± 0.1^{a}	5.21 ± 0.15^{a}	5.23 ± 0.21^{a}
	3	5.24 ± 0.04^{a}	5.69 ± 0.07^{a}	-	4.77 ± 0.10^{b}

-: no bacterium was observed. Values marked by different letters are significantly different at $p < 0.05$; values marked by the same letter are not significantly different at $p > 0.05$.

TABLE 3 Survival of *Lactobacillus plantarum*, *Streptococcus thermophilus*, *Clostridium tyrobutyricum*, and *Bifidobacterium adolescentis* in simulated intestinal fluid treated with polysaccharides of *Laetiporus sulphureus*.

Groups	Time (h)	*Bifidobacterium adolescentis* (lg CFU/mL)	*Lactobacillus plantarum* (lg CFU/mL)	*Streptococcus thermophilus* (lg CFU/mL)	*Clostridium tyrobutyricum* (lg CFU/mL)
Control	0	5.30 ± 0.05^{a}	5.69 ± 0.07^{a}	5.11 ± 0.10^{a}	4.99 ± 0.06^{a}
	3	5.01 ± 0.09^{b}	5.77 ± 0.17^{a}	4.98 ± 0.05^{a}	4.95 ± 0.17^{a}
LSP	0	5.27 ± 0.05^{a}	5.73 ± 0.05^{a}	5.01 ± 0.05^{a}	4.96 ± 0.08^{a}
	3	5.24 ± 0.06^{a}	5.69 ± 0.08^{a}	4.97 ± 0.13^{a}	5.02 ± 0.16^{a}

Values marked by different letters are significantly different at $p < 0.05$; values marked by the same letter are not significantly different at $p > 0.05$.

properties by increasing the survivability of bacteria traveling through the gastrointestinal tract (Chow, 2002; Khalf et al., 2010). It has been suggested that prebiotics contribute to improving probiotic proliferation in the intestine and modulate the composition of gut microbiota community structure (Zhao et al., 2019; Xue et al., 2020). It is a vital characteristic for probiotics to have the gastrointestinal tolerance, which is related to the type and the fermentation ability of probiotics (Shi et al., 2019). Table 2 presented the survival of *C. tyrobutyricum*, *B. adolescentis*, *S. thermophilus*, and *L. plantarum* in simulated gastric fluid. In the present study, the decreasing population of *C. tyrobutyricum* was observed in control and LSP groups under simulated gastric fluid condition for 3 h. The population of *C. tyrobutyricum* in control group was 4.39 ± 0.17 lg CFU/mL while that of LSP group was 4.77 ± 0.10 lg CFU/mL under simulated gastric fluid conditions for 3 h. *S. thermophilus* was not found after 3 h of digestion in simulated gastric fluid. It suggested that the *S. thermophilus* might not survive in simulated gastric fluid. Meanwhile, there was no significant difference between control and LSP at the same condition concerning to their population of *L. plantarum*. Over 3 h gastric digestion, the population of *B. adolescentis* in the LSP group (5.24 ± 0.04 lg CFU/mL) was significantly higher than the control group (4.70 ± 0.25 lg CFU/mL, $p < 0.05$). There was no significant difference between 0 and 3 h concerning to the population of *B. adolescentis* in the LSP group ($p > 0.05$). The results indicated that LSP could prevent the decrease of the population of *B. adolescentis* in simulated intestinal fluid. It can also be seen from the different performances of *L. plantarum* and *B. adolescentis* in simulated gastric fluid that the effects of LSP on probiotics may be strain specific rather than species or genera based, as suggested by Nobre et al. (2018). Therefore, the results showed evidence that LSP could enhance the population of *B. adolescentis* and *C. tyrobutyricum* in simulated gastric fluid conditions.

3.4 Effects of LSP on microbial in simulated intestinal fluid

Survival of *C. tyrobutyricum*, *B. adolescentis*, *S. thermophilus*, and *L. plantarum* in simulated intestinal fluid was illustrated in Table 3. The population of *B. adolescentis* in the LSP group was 5.24 ± 0.06 lg CFU/mL, which was significantly higher than the control group (5.01 ± 0.09 lg CFU/mL) under simulated intestinal fluid condition for 3 h ($p < 0.05$). Compared to 0 h, the population of *B. adolescentis* in the control group significantly decreased after 3 h digestion in simulated intestinal fluid condition ($p < 0.05$). Consequently, there was no significant difference between 0 and 3 h with regard the population of *B. adolescentis* in the LSP group ($p > 0.05$). In the meantime, there was no significant difference in the population of *C. tyrobutyricum*, *S. thermophilus*, and *L. plantarum* between the control group and the LSP group. The results suggested that LSP had a protective effect on *B. adolescentis*. However, the LSP did not affect on the population of *L. plantarum, S. thermophilus* and *C. tyrobutyricum* in simulated intestinal fluid conditions.

4 Conclusions

In summary, the obtained results demonstrated that LSP could increase the population of *C. tyrobutyricum*, *S. thermophilus*, *B. adolescentis*, and *L. plantarum in vitro*. The developed Huang model and Reparameterized Gompertz model could be suitable for describing the growth of *C. tyrobutyricum*, *S. thermophilus*, *B. adolescentis*, and *L. plantarum* with or without LSP treatment, respectively. Additionally, LSP could improve the population of *B. adolescentis* and *C. tyrobutyricum* in simulated gastric fluid conditions. Therefore, this study can provide some theoretical bases and ideas for the investigation of the biological activity of LSP.

Future research can explore the effect of LSP on pathogens or combine with other prebiotics to evaluate the characteristics of LSP as natural prebiotic supplements in food products.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

XZ: Data curation, Formal analysis, Writing – original draft. QW: Conceptualization, Data curation, Investigation, Methodology, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. SL: Funding acquisition, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by Natural Science Foundation of Fujian Province, China [grant number: 2022J05275] and Scientific Research Foundation of Ningde Normal University [grant number: 2023T02].

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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