Preliminary study for the stimulation effect of plant-based meals on pure culture *Lactobacillus plantarum* growth and acidification in milk fermentation

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ABSTRACT

Fermented dairy products have been recognized as the best carriers for the administration of probiotics. Because one of the potential probiotic strains, *Lactobacillus plantarum*, has poor proteolytic ability and weak acidifying capacity in milk fermentation, the aim of this study was to preliminarily investigate the stimulation effect of plant-based meals on *L. plantarum* CCFM8661 growth in milk, and subsequently develop a yogurt or yogurt drinks containing probiotic strain *L. plantarum* CCFM8661. Milk supplemented with different concentrations (5 to 10%, wt/wt) of oat extract and malt extract, inoculated with 2.5×10^7 cfu/mL of *L. plantarum* CCFM8661, and then incubated at 35°C. The pH value, titration acidity, and viable cell counts during 48-h fermentation at 35°C and 25-d storage at 4°C, were determined at different intervals. The results showed that the promotion effects of oat extract and malt extract on *L. plantarum* CCFM8661 growth rate in milk were much stronger than almond, walnut, sweet corn, peanut, and soybean meals. In addition, the stimulation effect of oat extract was associated with its concentration, and was much stronger than that of malt extract. Furthermore, viable counts and titration acidity of yogurt were gradually increased in the oat extract group, whereas viable counts were gradually decreased and titration acidity were slightly increased in the malt extract group during the 25-d storage at 4°C.

Key words: *Lactobacillus plantarum*, fermented milk, starter culture, growth promotion

INTRODUCTION

New yogurts with plant extracts are currently popular in the dairy market. As consumers are increasingly interested in healthy diets and wellness, with the expectation that food can help them be healthier or may even be capable of preventing illness, probiotic food products made via fermentation of milk, cereals, fruits, and vegetables are receiving attention from scientists as well as consumers (Gupta and Abu Ghannam, 2012). *Lactobacillus plantarum*, a versatile lactic acid bacterium, is encountered in a range of environmental niches, and is most frequently isolated from plant-based fermented foods as well as from meat, fish, and dairy products (Seddik et al., 2017). *Lactobacillus plantarum* is widely employed in food fermentation, with a long history and classification as “generally recognized as safe,” and has “qualified presumption of safety” status (Behera et al., 2018). *Lactobacillus plantarum* has proven ability to survive gastric transit and colonize the gut, with apparent safety to the consumer (de Vries et al., 2006). Therefore, increasing probiotic potentials of *L. plantarum* have been reported, including cholesterol-lowering (Costabile et al., 2017), immunomodulatory (Kechaou et al., 2013), and antimicrobial functions, controlling body fat gain and reducing inflammation and other adverse effects typically associated with immune and intestinal improvement (Abdelazez et al., 2018). In addition, *L. plantarum* has potential abilities to alleviate multiple species of heavy metal toxicity, such as cadmium (Zhai et al., 2014), lead (Tian et al., 2012), copper (Tian et al., 2015), and aluminum (Yu et al., 2016). For instance, *L. plantarum* CCFM8661 has excellent antioxidative activity and protective effects against lead-induced toxicity. It could decrease the lead (Pb^{2+}) levels of blood and tissue and the majority of Pb^{2+} bound on the surface of bacterial cells (Yin et al., 2016).

Received July 2, 2019.

Accepted December 19, 2019.

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Lactobacillus plantarum strains have been characterized by their enzyme systems (α -amylase, esterase, lipase, α -glucosidase, β -glucosidase, enolase, phosphoketolase, lactase dehydrogenase, and others) and bioactive compounds (bacteriocin, dipeptides, and other preservative compounds). They are easy to culture because of utilization of multiple carbon and nitrogen sources (Hedberg et al., 2008) and are widely used in fermented vegetable, cereal, meat, fish, dairy, and beverage products, and ethnic fermented foods (Behera et al., 2018). However, it has generally been considered that *L. plantarum* has low technological importance in standard milk processing (Quigley et al., 2013). Because it requires multiple amino acid and vitamin auxotrophs (Bringel and Hubert, 2003), *L. plantarum* with weak proteolytic ability was hard to grow in milk because of the lack of sufficient free amino acids and peptides to support its growth when cultured alone. In *L. plantarum*, complete whole-genome sequences have revealed that *prtB*, *prtH*, and *prtR*, the cell envelope protease genes involved in primary protein degradation, were absent (Kleerebezem et al., 2003), along with scarce proteolytic activities (Georgalaki et al., 2017). However, many genes encoding intracellular peptidases were found for peptide degradation, which was a vital reason for *L. plantarum* as an adjunct culture in cheese manufacture, for accelerating ripening (Li et al., 2017; Blaya et al., 2018).

Over the last decades, the health-promoting benefits of probiotics have attracted considerable attention. Hitherto, fermented dairy products (yogurt and cheese) have been recognized as the best matrix for the administration of probiotics (Georgieva et al., 2009). Recently, an increasing number of strains of *L. plantarum* with promising probiotic properties have been isolated from dairy-related sources. Therefore, some studies have made efforts to stimulate the growth and acidification of *L. plantarum* in milk using growth-promoter supplements at laboratory scale. Excellent sources of antioxidant vitamins, bioactive compounds, and minerals dramatically promoted the biomass yield of *L. plantarum* in skim and whole milk, such as 6 key amino acids and 1 purine required for *L. plantarum* ST-III (Ma et al., 2016), glycine and dipeptide (Leu-Leu and Gly-Gly) for *L. plantarum* N4 (Saguir et al., 2008), 10% blackberry juice (Yang et al., 2014), and fermented carrot juice (Demir et al., 2006). Although previous studies have showed certain promotion of the growth and acidification of *L. plantarum* in milk, at present there are no practice standards and guidelines for commercial food-grade ingredients on pure *L. plantarum* strain application in yogurt preparations.

It is known that *L. plantarum* is commonly used as a starter culture in plant-based fermentation, and plant-

based meals should stimulate the growth and acidification of *L. plantarum* in milk. In this study, the growth and acidification of *L. plantarum* in milk supplemented with commercial plant-based meals were investigated, and pH, titration acidity, and viable cell counts during 48-h fermentation at 35°C were initially determined. Malt extract and oat extract with outstanding stimulation effects on *L. plantarum* were further studied. Additionally, the growth-promoting effects among different *L. plantarum* strains were compared. Subsequently, a yogurt fermented only by *L. plantarum* was successfully developed, and the viable counts and titration acidity of the yogurt at different intervals during 25-d storage at 4°C were determined.

MATERIALS AND METHODS

Chemicals and Reagents

The skim milk powder (0.8% fat, 33.4% protein, 54.1% lactose, 7.9% minerals, and 3.8% moisture) used for milk fermentation was purchased from Fonterra Ltd. (Auckland, New Zealand). Oat extract [11.2% protein, 1.54% moisture, 1.64% sulfated ash, and dextrose equivalent (DE) value 30%] and malt extract (4.96% protein, 1.63% moisture, 1.75% sulfated ash, and DE value 47%) were purchased from Döhler Food and Beverage Ingredients (Jinshan) Co. Ltd. (Shanghai, China). Almond (21.8% protein, 19.2% fat, 1.76% ash, and 2.73% moisture), walnut (12.4% protein, 39.1% fat, 29.4% carbohydrate, and 2.46% moisture), sweet corn (2.8% protein, 1.4% fat, 83% carbohydrate, and 2.91% moisture), peanut (12.1% protein, 15.3% fat, 61.2% carbohydrate, and 2.89% moisture), and soybean (41.4% protein, 21.6% fat, 28.0% carbohydrate, and 3.54% moisture) meals were purchased from Xuzhou Nature Biotechnology Co. Ltd. (Xuzhou, China). Phenolphthalein (98%) was purchased from Canspec Inc. (Shanghai, China). All other chemicals were obtained from commercial sources and were of analytical grade.

Bacterial Strains and Culture Conditions

Lactobacillus plantarum CCFM8661, 8610, and 8742 were obtained from the Culture Collections of Food Microbiology, Jiangnan University (Wuxi, China). *Lactobacillus plantarum* ST-III was purchased from State Key Laboratory of Dairy Biotechnology (Shanghai, China). Before the experiments, these frozen stock cultures were inoculated in de Man, Rogosa, and Sharpe medium and transferred 3 times at 37°C for 16 h, respectively. After culture, cells were harvested and centrifuged at $5,000 \times g$ for 5 min and washed twice with PBS (0.01 M, pH 7.4). The harvested cells were resuspended and

concentrated with 10% (wt/vol) skim milk, which was sterilized in a YXQ-LS-70A autoclave (Shanghai Boxun Industry and Commerce Co. Ltd., Shanghai, China) at 108°C for 15 min. The milk was subsequently dried in a Labconco 12L lyophilizer (Labconco Corp., Fort Scott, KS). The number of bacteria in the lyophilized preparations was determined via plate colony counting before later experiments.

Preparation of Fermented Milk

Fermented milk was prepared using the following procedure. Whole milk powder was reconstituted at a concentration of 200 g/L and then mixed and stirred moderately with a mechanical stirrer (Eurostar 40, IKA Werke GmbH and Co., Staufen, Germany) at room temperature for 30 min to dissolve fully. The dispersion was then stored overnight at 4°C for complete hydration of casein micelles before use. Ingredients for improving the yogurt texture and flavor were blended initially and then gradually added to 60°C water, heated using a thermostated hotplate magnetic stirrer (C-MAG HS 7, IKA Werke). The volume of the water was equal to that of the reconstituted milk. The addition rates of sugar, plant-based meals, Novation 2300 modified starch (Ingredion China Ltd., Shanghai, China), low-methoxyl pectin, and natural whey were 70 g/L, 75 g/L, 5 g/L, 1.5 g/L, and 2 g/L, respectively. After fully dissolving, the prepared solution and reconstituted milk were mixed at 65°C and then homogenized by a 2-stage high-pressure GJJ-0.05/40 homogenizer (Shanghai Nuoni Light Industry Machinery Co. Ltd., Shanghai, China), using pressures around 15 MPa for the first stage and 5 MPa for the second stage, to break large fat globules down to smaller ones and prevent the separation of cream from milk (Geciova et al., 2002). The milk obtained (60 mL) was subpacked in 100-mL glass jars and then sterilized at 108°C for 15 min in a YXQ-LS-70A autoclave (Shanghai Boxun Industry and Commerce Co. Ltd.). We chose these sterilization conditions (108°C/15 min) because *L. plantarum*, with weak growth and acid production capacity in milk, could not inhibit the growth of the other microorganisms, such as spores of *Bacillus*, at low-temperature pasteurization (80 to 85°C/15 min) in the laboratory (Halvorson and Church, 1957; Sadek et al., 2018), which would result in the deterioration of milk quality.

Proper cell concentrations of solution containing *L. plantarum* were prepared and inoculated, with the inoculum dose calculated as 2.0×10^7 cfu/mL. The jars were shaken gently to distribute the starter and then incubated statically at 35°C in a BSP-150 biochemical incubator (Boxun Ltd., Shanghai, China) for 12 h. Aliquots of samples were removed and collected at 2- to

4-h intervals for determination of titration acidity, pH, and viable cell counts.

Measurement of TA and pH

The pH of fermented milk at different intervals was measured with an OHAUS ST3100 digital pH-meter (Changzhou, China). The titration acidity was determined according to China National Food Safety Standard GB 5413.34-2010 (IDF, 1978, 1981). In brief, 10 g of sample was mixed with 20 mL of freshly boiled and cooled distilled water and then titrated with 0.1 mol/L NaOH using 0.5% phenolphthalein as indicator until the color changed to pink and disappeared for 30 s. The volume consumed by titration of NaOH standard solution was recorded, and then the titration acidity (degrees Thorner, °T) of sample was calculated according to the following equation (Jia et al., 2016):

$$^{\circ}\text{T} = c \times v \times 100 / (m \times 0.1), \quad [1]$$

where c is the molarity of NaOH standard solution; v is the volume consumed by titration of NaOH standard solution; and m is the mass of the fermented milk sample.

Plate Count

The viable counts of cells in the milk during fermentation were determined using the plate count method. In detail, 10 g of each sample at 0-, 2-, 4-, 6-, 8-, 10-, and 12-h intervals after inoculation were aseptically collected and diluted with 90 mL of 0.85% (wt/vol) sterile saline solution and then serially diluted 10-fold to appropriate concentrations. One milliliter of diluted solution was pipetted into aseptic Petri dishes, and then the de Man, Rogosa, and Sharpe agar culture medium, melted and cooled to proper temperature, was poured into dishes and shaken. After the medium cooled and solidified, these plates were inverted and incubated in a BSP-150 biochemical incubator (Boxun Ltd., Shanghai, China) at 35°C for 48 h. The numbers of *L. plantarum* were expressed as colony forming units per milliliter. The stimulation effects of malt extract and oat extract were compared by growth curve parameters. To investigate the shelf life of the fermented milk, the titration acidity and viable counts of the yogurt at different intervals were determined during 25 d of storage at 4°C.

Kinetics of Growth Curves

In general, when the bacteria growth curve is defined as the logarithm of the viable cell number plotted against time, the growth rate changes and results in a

sigmoidal curve, with a lag phase before the beginning of exponential growth. After inoculation (N_0), growth rate often starts at a value of 0 and then accelerates to a maximal value (μ_m) in a certain period of time. At the end of the exponential phase, the growth rate gradually decreases and finally reaches 0, so that an asymptote (N_{max}) is reached. The maximum specific growth rate (μ_m) is a valuable parameter of the growth curve, which can illustrate the stimulation effects on *L. plantarum* by supplemented plant-based meals with different concentrations. The logistics equation (Chowdhury et al., 2007) was usually selected and fitted to growth data by nonlinear regression according to equation [2] with a Levenberg-Marquardt algorithm:

$$\text{Log}_{10}N_t = A + B / \{1 + \exp[-\mu_m \times (t - t_m)]\}, \quad [2]$$

where $\text{Log}_{10}N_t$ is the number of viable cells at the observed time (t) during the different growth phases after the initial inoculation; A is the asymptotic log count as t decreases indefinitely (i.e., $\text{Log}_{10}N_0$); B is the asymptotic amount of growth as t increases indefinitely; μ_m is the maximum specific growth rate at t_m during the exponential phase; and t_m is the time at which the maximum specific growth rate was achieved (Fujikawa et al., 2004). For each curve of this experiment, A , B , μ_m , and t_m can be calculated after data fitting under different experiment conditions. After data fitting, the parameters $\text{Log}_{10}N_t$, μ_m , t_m , A , and B were derived from each curve. The obtained parameters of growth curve were further analyzed, to study the stimulation effects of malt extract and oat extract at different concentrations.

Statistical Analysis

All data were expressed as means and standard deviation (SD) from 3 replicates. All statistical analyses were performed using the Statistical Analysis Software (SAS 9.2, SAS Institute Inc., Cary, NC). The confidence level was set at $P < 0.05$ for statistical significance. Origin 8.0 software (OriginLab Corp., Northampton, MA) was selected for data plotting.

We selected 1stOpt1.5 PRO Software (7D-Soft High Technology Inc., Beijing, China) for data fitting, using Levenberg-Marquardt and universal global optimization (UGO) algorithms (Zhang et al., 2013). The reliability of the logistics equations describing the growth of *L. plantarum* in milk was determined via root mean square error (RMSE), sum of squares (SSE), and coefficient of determination (R^2) analysis. R^2 is defined as the ratio of explained variation to total variation and is a measure of the degree of fit.

RESULTS AND DISCUSSION

Stimulation Effects of Different Plant-Based Meals

The acidification and growth of *L. plantarum* CCFM8661 in milk supplemented with plant-based meals at different intervals are shown in Figure 1. The pH value of fermented milk is shown in Supplemental Figure S1 (<https://doi.org/10.3168/jds.2019-17200>). *Lactobacillus plantarum* CCFM8661 grew significantly in the milk supplemented with plant-based meals compared with the control group without supplementation ($P < 0.05$). Additionally, in group 6, the titration acidity reached $104 \pm 2^\circ\text{T}$ (pH 3.82) and $117 \pm 2^\circ\text{T}$ (pH 3.70), and the logarithms of viable counts reached 9.04 and 8.92 cfu/g at 24 and 48 h, respectively. In group 7, the titration acidity was $90 \pm 1^\circ\text{T}$ (pH 3.95) and $112 \pm 2^\circ\text{T}$ (3.73), and the logarithms of viable counts were 9.08 and 9.04 cfu/g at 24 and 48 h, respectively. The titration acidity and viable counts in groups 6 and 7 were much higher than other groups at the observed intervals ($P < 0.05$).

Similar promotive effects on growth were observed in experiments supplemented with adenine, amino acids, and blackberry juice for other *L. plantarum* strains (ST-III, WCFS1, and LPHS; Ma et al., 2016; Yang et al., 2014). The logarithms of viable counts of *L. plantarum* in fermented milk supplemented with 0.1 g/L adenine and 0.2 g/L amino acids (Ile, Leu, Val, Tyr, Met, and Phe) were 8.34 and 9.18 cfu/g at 24 and 48 h, respectively. In contrast, the logarithms of viable counts in control group were only 7.65 and 7.59 cfu/g at 24 and 48 h, respectively. The pH values of cultures with the *L. plantarum* ST-III strain in the supplemented group were 5.11 and 4.08 at 24 and 48 h, respectively. However, the pH values of the control group were only 6.06 and 5.77 at 24 and 48 h, respectively. The pH values of fortified fermented milk with the *L. plantarum* WCFS1 strain and the control group were 4.89 and 5.87 at 24 h, respectively. As for the *L. plantarum* LPHS strain, the pH value of fortified fermented milk was 5.00, and that of control group was only 5.97 at 24 h. Moreover, we found that the growth of *L. plantarum* was significantly stimulated by blackberry juice in skim milk, and the logarithms of viable counts in fermented milk with blackberry juice were 10.02 and 11.3 cfu/g at 24 and 48 h, respectively; whereas the logarithms of viable cell counts in the control group were 6.93 and 7.72 cfu/g at 24 and 48 h, respectively.

According to manufacturer's instructions, malt extract is prepared from barley malt and barley, and the barley malt is treated with hot water extraction, saccharification by amyloglucosidase and glucose isomerase, filtration, concentration, and vacuum drying.

Figure 1. The acidification (A) and growth (B) of *Lactobacillus plantarum* CCFM8661 in milk supplemented with different plant-based meals. The addition rates were 5% (wt/wt) for all meals. Control group was unsupplemented milk; groups 1–7 were milk supplemented with almond meal, walnut meal, sweet corn meal, peanut meal, soybean meal, malt extract, and oat extract, respectively. Columns are shown as mean $\pm$ SD. Within each group, means with different lowercase letters were significantly different ($P < 0.05$) among different groups. Within each time period, means with different capital letters were significantly different among different time points ($P < 0.05$).

Malting is basically the germination process carried out under controlled conditions (Østergaard et al., 2002), during which several seed biopolymers, including the storage proteins, must be hydrolyzed. The barley proteins are initially solubilized by endoproteases and then further degraded by exopeptidases (Jones, 2005). Oat extract is prepared from the enzymatic pretreatments of oat flour, protease, and β -glucanase, derived from bacteria or fungi, followed by filtration, concentration, and vacuum drying. Whether endogenous or exogenous proteases, proteins in barley and oat meal are partially hydrolyzed and thus provide peptides needed for growth of *L. plantarum*.

Although the stimulation effects of malt extract and oat extract on the growth of *L. plantarum* in milk were not as great as those of blackberry juice, growth rates from malt and oat extracts were much faster than those of supplementation with 0.1 g/L adenine and 0.2 g/L amino acids. In addition, as food-grade ingredients, malt extract and oat extract have many functional aspects. For instance, oat extract contains relatively hydrophilic phenolic compounds, including avenanthramides and tocopherol, which have very obvious antioxidant abilities, known to protect the body from damage by free radicals. The antioxidants in oat extract can inhibit the oxidation of low-density lipoproteins (LDL), prevent development of atherosclerosis, and decrease levels of LDL cholesterol, reducing risks of cardiovascular diseases such as coronary heart disease (Gray et al., 2002). Malt extract also has obvious antioxidant activity and anti-aging effects; it can prevent the decrease of antioxidant enzyme activity and reduce the contents

of malondialdehyde and carbonyl groups in liver and brain tissues, to prevent cancer, inflammation, Parkinson's disease, and other health problems (Yang et al., 2010). Therefore, the stimulation effects of malt extract and oat extract on *L. plantarum* in milk fermentation need to be further investigated in greater detail.

Milk Fermentation Supplemented with Malt Extract and Oat Extract

The acidification and growth of *L. plantarum* CCFM8661 in milk supplemented with different concentrations (3.5%, 5%, 7.5%, and 10%) of malt extract and oat extract during 12-h fermentation, which were measured at 2-h intervals, are shown in Figure 2. The pH values of fermented milk are presented in Supplemental Figure S2 (<https://doi.org/10.3168/jds.2019-17200>). Compared with the control group, both malt extract and oat extract significantly stimulated the acidification and growth of *L. plantarum* CCFM8661 in milk ($P < 0.05$). The stimulation effects on *L. plantarum* CCFM8661 were associated with addition rates. The titration acidities of fermented milk with malt and oat extracts at 10 h were between 45 and 85°T. The rates of acidification and growth of *L. plantarum* CCFM8661 in oat extract were much stronger than those of malt extract, which might be due to the higher protein content and greater levels of peptides in oat extract, compared with malt extract. In the case of oat extract, the stimulation effects on *L. plantarum* CCFM8661 were dependent on the addition rates. However, in the case of malt extract, the stimulation effects as func-

tions of addition rates were not very linear, with the optimal concentration at 7.5%. The titration acidities of fermented milk were highly associated with viable cell counts; the higher the titration acidity achieved, the greater viable counts were obtained in the sample.

As the curves grew exponentially, the experimental data were fitted with the formula of logistic equation [2], using Levenberg-Marquardt and UGO algorithms, to describe the sigmoidal growth of viable cells. 1stOpt1.5 PRO software is an optimization analysis software package for mathematics with independent intellectual property rights. Compared with Matlab, Origin Pro, SAS, SPSS, and other software packages, 1stOpt can enable calculations to achieve the optimal convergence without providing initial values of parameters. Due to

its strong optimization and fault tolerance abilities in most situations, this software is widely used in various fields of scientific research, in problems of nonlinear regression, curve fitting, calculation of nonlinear model parameters, and linear and nonlinear programming (He and Yu, 2013).

These simplified assumptions were indicated with fixed values, to account for the slight deviations of the model from the data. The model of logistic curve was proposed to simulate experimental data with variable parameter $\log_{10} N_t$ and t_m , and the parameter μ_m represented the maximum specific growth rate. It has been previously observed in experiment that growth rate varies with the addition of different concentrations of malt extract and oat extract. As shown in Table 1, the

Figure 2. Milk fermentation supplemented with different concentrations of malt extract (A, B) and oat extract (C, D). Error bars represent SD. °T = degrees Thorner. Columns are shown as mean $\pm$ SD. Within each time period, means with different lowercase letters are significantly different ($P < 0.05$) among different time points. Within each group, means with different capital letters are significantly different among different groups ($P < 0.05$).

Table 1. Results of parameters of *Lactobacillus plantarum* 8661 growth curves in milk supplemented with different concentrations of malt and oat extracts fitting by logistic model¹

Addition rate	<i>L. plantarum</i> CCFM 8661 with malt							<i>L. plantarum</i> CCFM 8661 with oat						
	<i>A</i>	<i>B</i>	μ_m	t_m	RMSE	SSE	R ²	<i>A</i>	<i>B</i>	μ_m	t_m	RMSE	SSE	R ²
Control	6.929	0.633	0.732	3.083	0.0368	0.0095	0.971	6.896	0.674	0.628	2.894	0.0347	0.0084	0.974
3.50%	6.853	1.936	0.741	3.925	0.0739	0.0382	0.989	6.684	2.227	0.668	3.668	0.0299	0.0063	0.999
5.00%	6.885	1.946	0.800	4.058	0.0801	0.0449	0.988	6.807	2.279	0.699	3.998	0.0397	0.0110	0.998
7.50%	6.860	2.086	0.869	3.974	0.0552	0.0214	0.995	6.859	2.341	0.728	4.289	0.0552	0.0213	0.996
10.00%	6.842	2.173	0.733	4.092	0.0711	0.0353	0.992	6.788	2.518	0.650	4.441	0.0732	0.0375	0.994

¹*A* = asymptotic log count as time (*t*) decreases indefinitely; *B* = asymptotic amount of growth as *t* increases indefinitely; μ_m = maximum specific growth rate at t_m during the exponential phase; t_m = time at which maximum specific growth rate was achieved; RMSE = root mean square error; SSE = sum of squares.

values of μ_m of different addition rates (0%, 3.5%, 5%, 7.5%) were 0.732, 0.741, 0.800, and 0.869 and 0.628, 0.668, 0.699, and 0.728 with malt and oat, respectively, showing positive growth trends with addition of malt and oat extracts. In Figure 2B and 2D, the tendency of the experimental data coincided with the hypothesized growth and rose slowly, which could demonstrate that kinetic growth of *L. plantarum* CCFM8661 was influenced by the addition rates of malt and oat extracts. However, when the addition rate increased to 10%, the value of μ_m decreased slightly along with counts of viable cells, which was suspected to be caused by the growth limit of *L. plantarum* CCFM8661. Similarly, with the same inoculum size, the calculated parameter *A* was very close, and the parameter *B*, which referred to the asymptotic amount of growth with indefinite time, increased according to the same rule of different addition rates of malt and oat extracts (Table 1). In conclusion, the parameter of the equation showed the growth specificity of probiotics, and the stimulation effects (μ_m) on *L. plantarum* CCFM8661 were found to be positively associated with addition rates of malt and oat extracts.

The reliability and precision of the equations were determined through RMSE, SSE, and R² analysis (Zhang et al., 2018). A measure of the degree of fit, R² is defined as the ratio of explained variation to total variation. We used RMSE and SSE to measure the deviation between observed values and predicted values. Low dispersion between the experimental and predicted values, which indicates the higher precision and greater fit of the logistic model, can be illustrated by the low values of RMSE and SSE and high value of R². In all experiments with addition of malt and oat extracts, we found the maximum values of RMSE and SSE to be 0.0801 and 0.0449, respectively. The logistic equation exhibited high R² values, ranging from 0.971 to 0.999. These low RMSE and SSE values and high R² values indicate the goodness of fit of the logistic model (Taheri Garavand et al., 2011). Therefore, the logistic model

could be used to describe the growth of *L. plantarum* strains in fermented milk.

Stimulation Effects Among Different *L. plantarum* Strains

The growth of *L. plantarum* strains in fermented milk supplemented with 7.5% malt and oat extracts during 12-h incubation, at 4-h intervals, are shown in Figure 3. The study was performed in multiple groups, and the stimulation effects on growth were analyzed at time points of 0, 4, 8, and 12 h among 4 kinds of *L. plantarum* strains, including ST-III, CCFM8661, CCFM8610, and CCFM8742.

In this experiment, the logarithms of viable counts of *L. plantarum* CCFM8661 in fermented milk were calculated and analyzed to significantly define the stimulation effects among different *L. plantarum* strains. As shown in Figure 3, after inoculation of *L. plantarum* 8661 with malt and oat extracts, the logarithms of viable counts at intervals of 0, 4, 8, 12 were 6.97, 7.97, 8.84, and 9.02 cfu/g in the malt extract group and 6.99, 7.94, 8.99, and 9.15 cfu/g in the oat extract group, respectively. We see that the number of viable cells increased with the fermentation time, and the growth rate was first increased to μ_m , then gradually decreased to maintain stability after inoculation.

In Figure 3, the growth rates of 4 *L. plantarum* strains in milk supplemented with malt and oat extracts increased obviously compared with the control group ($P < 0.05$), which illustrates that the growth in milk of these *L. plantarum* strains could be definitely stimulated by malt and oat extracts. Interestingly, we discovered a difference among the 4 *L. plantarum* strains in the growth in the milk supplemented with malt extract and oat extract. In addition, ST-III had weaker growth with oat extract than with malt extract ($P < 0.05$), which was not observed in the other 3 strains. Among the 4 *L. plantarum* strains, the highest logarithms of viable counts were 9.02 cfu/g of *L. plantarum* 8661

Figure 3. The growth of control and *Lactobacillus plantarum* ST-III, 8661, 8610, and 8742 with 7.5% malt extract (A) and oat extract (B) at time points of 0, 4, 8, and 12 h. The control group was defined as logarithm of viable counts of strain 8661, which is the main target strain of this article, at different time intervals (0, 4, 8, and 12 h) without the addition of malt and oat extracts. Columns are shown as mean $\pm$ SD. Within each time period, means with different lowercase letters are significantly different ($P < 0.05$) among different time points. Within each group, means with different capital letters are significantly different among different groups ($P < 0.05$).

and 9.12 cfu/g of *L. plantarum* 8742 with malt extract, and 9.15 cfu/g of *L. plantarum* 8661 and 9.26 cfu/g of *L. plantarum* 8742 with oat extract. In contrast, the logarithm of viable counts was only 7.64 cfu/g in the control group when the incubation time reached 12 h. Therefore, it can be determined that *L. plantarum* 8742 and 8661 both have better growth abilities than those of *L. plantarum* ST-III and 8610.

On the basis of the experiment data analyzed above, the fermented milk with the addition of malt and oat extracts can be verified to effectively stimulate the growth of *L. plantarum* strains. In this sense, to achieve higher cell viability of *L. plantarum* strains, this method can be transferred to the industrial production of fermented milk with varieties of *L. plantarum* strains, to produce more effectiveness of *L. plantarum* strains in the functional food industry. However, because the severe thermal treatment applied in our experiment (108°C/15 min) possibly causes greater nutritional loss in the milk than do those used in industrial thermal processes (that is, pasteurization and UHT treatment), the critical nutrients in malt and oat extracts supporting the substantial growth of *L. plantarum* in milk still need to be further studied.

Shelf Life of Yogurt Fermented by *L. plantarum* CCFM8661

Milk supplemented with 7.5% malt extract and oat extract was fermented for 8 h after inoculation and then rapidly cooled and stored in a refrigerator at 4°C. The titration acidity and viable counts of fermented

milk supplemented with 7.5% malt extract and oat extract during 25-d storage are shown in Figure 4. At the end of fermentation, the titration acidities of milk containing malt extract and oat extract were $72 \pm 2^\circ\text{T}$ and $67 \pm 1^\circ\text{T}$, respectively. The slightly higher titration acidity for the malt extract group might be due to the higher initial titration acidity and faster growth of

Figure 4. The titration acidity ($^\circ\text{T}$ = degrees Thorner) and viable cell counts of fermented milk supplemented with 7.5% malt extract and oat extract during 25-d refrigerated storage. Error bars represent SD. The solid lines with solid squares (■) and solid circles (●) represent the titration acidity of fermented milk containing malt extract and oat extract, respectively; the gray line and red line depict the viable counts in fermented milk containing malt extract and oat extract, respectively.

L. plantarum CCFM8661 (Figure 2). Interestingly, the post-acidification of the fermented milk containing oat extract was much stronger than that of the malt extract group, which coincided with the continuous growth of this strain during the first 12-d storage and then slowly declined. However, the viable counts in fermented milk containing malt extract gradually decreased, which might be due to the depletion of available nitrogen sources, so that they could not sustain the growth. At the end of 25-d storage, the logarithms of viable counts in fermented milk supplemented with malt extract and oat extract were 7.45 cfu/g and 8.61 cfu/g, respectively.

For application of malt extract and oat extract alone as growth promoters of *L. plantarum* in yogurt preparation, the weakness of post-acidification and reduction of viable cells are now being investigated. Therefore, it is very valuable to study the combination of these 2 components and to reduce the adverse effects of post-acidification and viable count reduction by themselves.

CONCLUSIONS

In conclusion, the investigated plant-based meals have different promotion effects on the growth of *L. plantarum* CCFM8661 in milk compared with control. The promotion effects of malt extract and oat extract on CCFM8661 growth rates in milk were much stronger than those of the other meals. The stimulation effects of malt extract and oat extract were associated with their addition rates. Furthermore, the stimulation effect of oat extract was much stronger than that of malt extract. We also found certain differences in the growth among different *L. plantarum* strains in milk supplemented with malt extract and oat extract. As widely used food-grade ingredients, malt and oat extracts can be applied in the development of novel probiotic yogurt and yogurt drink using only *L. plantarum* strains or coupled with other lactic acid bacterial strains. Extensive research is needed to study the critical nutrients in malt and oat extracts that support the substantial growth of *L. plantarum* in milk.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (No. 31771953; Beijing), the National First-Class Discipline Program of Food Science and Technology (JUFSTR20180102, Wuxi City, China), and the Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province (Wuxi, China). The authors have not stated any conflicts of interest.

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