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Large-scale fermentation of *Lactiplantibacillus pentosus* 292 for the production of lactic acid and the storage strategy based on molasses as a preservative

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Abstract

Background *Lactiplantibacillus pentosus* 292 is a lactic acid bacterium (LAB) with significant probiotic potential, but large-scale production is often limited by high production costs and preservation challenges. This study aimed to develop a cost-effective medium to enhance lactic acid production and establish a feasible preservation strategy to support the strain's large-scale application.

Results A low-cost medium containing glucose, yeast powder, K_2HPO_4, and Tween-80 was formulated, enabling *Lactiplantibacillus pentosus* 292 to achieve a lactic acid yield of 16.24 g/L, representing an 83.48% increase compared to the traditional MRS medium. Fermentation kinetics models for bacterial growth, substrate consumption, and product generation were established in a 200-L fermenter using the Logistic, Luedeking-Piret-like, and Luedeking-Piret models, and the R^2 values from the model equation were 0.9921 (OD_{600nm}), 0.9942 (dry weight), 0.9506 (total protein), 0.8383 (lactic acid), 0.8898 (total sugar), and 0.8585 (reducing sugar), respectively, indicating that these models were suitable for accurately simulating the growth, nutrient production, and substrate consumption of *L. pentosus* 292. Additionally, a preservation strategy was developed by using 1–3% molasses as a preservative for the fermentation broth, and its efficacy was verified through temperature acceleration experiments.

Conclusion In this work, a cost-effective medium that significantly increased lactic acid yield and a preservative based on molasses as a strategy to extend the storage period of fermentation products were developed for large-scale production of *L. pentosus* 292, a member of probiotic LAB. Additionally, large-scale fermentation kinetics models were constructed, providing valuable technical insights for the large-scale production and application of this LAB, highlighting its significant potential for industrial applications.

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Keywords Lactic acid bacteria, Lactic acid, Large-scale fermentation kinetics model, Storage strategy, *Lactiplantibacillus pentosus*

Background

Lactic acid bacteria (LAB), a class of spore-free, gram-positive bacteria with the capability of fermenting carbohydrates to produce lactic acid (LA), are widely distributed in nature [1], such as *Acetilactobacillus*, *Bombilactobacillus*, *Companilactobacillus*, *Dellaglioa*, *Fructilactobacillus*, *Schleiferilactobacillus*, *Lactiplantibacillus*, etc. LAB, as a widely used microbial preparation, not only improves t he nutritional value and imparts unique flavors to products in the food industry [2], but also serves as a promising substitute for antibiotics. In the field of aquaculture, LAB can antagonize intestinal pathogens, improve body immunity, prevent/cure diseases, and promote the growth of aquatic organisms [3–7]. In the medical field, LAB has been shown to induce apoptosis in cancer cells and antagonize clinical pathogens [8, 9].

LA, as the important metabolites of LAB, is widely used in cosmetics, food, pharmaceuticals, polymers, and agriculture [1, 10]. Reportedly, biodegradable polylactic acid materials, which serve as green alternatives to petroleum-derived plastics, have garnered significant attention and demand in various countries, thereby driving the demand and development of the LA industry [11, 12]. According to statistics, the global total LA market in 2022 is about 1.5 million tons, which will grow to around 2.8 million tons by 2030 (https://www.statista.com/). However, the current cost of producing LA is still high, limiting its competitiveness in biotechnology and industrial applications [13]. Therefore, optimizing fermentation media and process parameters remains a favorable strategy to achieve high-yield production systems at a lower cost [14].

Our team has long been committed to the development of LAB for aquaculture, and some LAB strains have been confirmed with the functions of improving the growth performance, antioxidant capacity, immunity, and disease resistance of aquatic animals, such as *Lactococcus lactis* L280, *Lactococcus lactis* S1, *Lactococcus lactis* S2, *Enterococcus faecalis* F3, *Enterococcus faecalis* F7, etc [1, 15]. We consider that fermentation and storage techniques are fundamental and critical processes for the large-scale application of functional strains, ensuring the production of active products and the maintenance of their activity [16]. At the phase of storage, some researchers have adopted the method of adding ingredients such as oligosaccharide [17], mannitol [18], and maltose [19] to maintain/enhance the activity of LAB and LA [20]. Of which, molasses, which is rich in sugars and proteins, is an affordable option and can serve as a preservative for microbial storage by regulating pH [21].

Lactiplantibacillus pentosus 292, stored in our lab (the State Key Laboratory of Marine Resource Utilization in the South China Sea; isolated from shrimp intestine), has been confirmed to product high activity of LA and exhibit excellent probiotic function in aquatic animals, particularly shrimp, whereas knowledge of the fermentation and storage strategy for this strain remains limited. Herein, we aim to develop a cost-effective culture medium, identify optimal culture conditions, build large-scale fermentation kinetics models, and construct a storage strategy to facilitate the large-scale fermentation and LA production of *L. pentosus* 292, as well as extend the storage period of its fermentation broth. These efforts will support the large-scale application of LAB in aquaculture and other fields.

Materials and methods

Microorganism

Lactiplantibacillus pentosus 292 was isolated from the intestine of *Litopenaeus vannamei* at the Wenchang breeding base in Hainan, China, and is stored in the State Key Laboratory of Marine Resource Utilization in the South China Sea.

Development of a culture medium and culture conditions for the fermentation of *L. pentosus* 292

Five types of mediums, namely MRS medium (8 g/L beef extract, 4 g/L yeast powder, 10 g/L peptone, 2 g/L K_2HPO_4, 2 g/L $C_6H_{14}N_2O_7$, 5 g/L CH_3COONa, 0.25 g/L $MnSO_4$, 0.58 g/L $MgSO_4$, 20 g/L glucose, and 1% Tween – 80), freshwater medium (1 g/L yeast extract, 3 g/L beef extract, 5 g/L peptone, and 5 g/L NaCl), 2216E medium (3 g/L beef extract, 10 g/L peptone, and 5 g/L NaCl), beef extract-peptone medium (5 g/L peptone, 1 g/L yeast powder, 0.1 g/L $FePO_4$, and NaCl 30 g/L), and LB medium (10 g/L peptone, 5 g/L yeast powder, and 10 g/L NaCl), were selected to culture *L. pentosus* 292 at 30 °C for 30 h with 180 rpm of shaking speed in 7.0 ~ 7.2 of pH in the shake flasks. The biomasses of samples at 2 h-intervals were estimated by optical density (OD) at 600 nm wavelength.

MRS medium compared with others displayed the higher growth capability of *L. pentosus* 292. Therefore, based on the MRS medium, the effects of carbon sources (molasses, brown sugar, maltose, lactose, glucose, and maltodextrin), nitrogen sources (yeast powder, yeast extract, malt extract powder, urea, and soybean peptone), inorganic salts ($MgSO_4$, CH_3COONa, $(NH_4)_2SO_4$,

$MnSO_4$, NaCl, and K_2HPO_4), pH (4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, and 8), temperature (20, 25, 30, 35, and 40 °C), and inoculation amount (1%, 3%, 5%, 7%, and 10%) on the growth of *L. pentosus* 292 and its capability of LA production were estimated, respectively. Subsequently, according to the above results, glucose, yeast powder, inorganic salt, and pH were selected to perform the orthogonal test (orthogonal design is shown in Tables S1 and S2) to obtain the optimal condition for the fermentation and LA production of *L. pentosus* 292.

Large-scale fermentation and kinetics models of *L. pentosus* 292

The developed culture medium (30 g/L glucose, 15 g/L yeast powder, 8 g/L K_2HPO_4, and 1% Tween − 80) from the above experiments was used for large-scale fermentation of *L. pentosus* 292 in a 200-L stirred-tank fermenter (Zhenjiang Dongfang Biological Engineering Equipment Technology Co., Zhenjiang, China). The fermentation conditions were as follows: aeration rate 1.5 m^3/h, tank pressure 0.03–0.07 MPa, agitation speed 80 rpm, pH 6, temperature 35 °C, and inoculation amount 3%. The fermentation kinetics curves and models of bacterial growth, substrate consumption, and product formation were constructed by the following equations.

Kinetics model of bacterial growth

The Logistic equation (bacterial count equation) was used to describe the growth law (including bacterial biomass and dry weight) of *L. pentosus* 292 in a 200-L fermenter [22].

$$d_x/d_t = \mu_m X\left(1-\frac{X}{X_m}\right) \tag{1}$$

When $t=0,\ X=X_0$, integrate in 0-t:

$$X=\frac{X_0 e^{\mu_m t}}{1-\frac{X_0}{X_m}(1-e^{\mu_m t})} \tag{2}$$

d_x/d_t: bacterial growth rate, g/(L·h); X: bacterial mass concentration, g/L; t: fermentation time, h; μ_m: maximum bacterial growth rate, g/(L·h); X_m: maximum bacterial mass concentration, g/L; X_0: initial bacterial mass concentration, g/L.

Kinetics model of product synthesis

Luedeking-Piret equation was used to describe the product generation (LA and total protein) of *L. pentosus* 292 in a 200-L fermenter [23].

$$d_p/d_t = \alpha\frac{dX}{dt}+\beta X \tag{3}$$

When $t=0,\ X=X_0$, integrate in 0-t:

$$\begin{aligned} P=\alpha X_0[&\frac{e^{\mu_m t}}{-\frac{X_0}{X_m}(1-e^{\mu_m t})}-1]\\ &+\beta\frac{X_m}{\mu_m}\ln[1-\frac{X_0(1-e^{\mu_m t})}{X_m}] \end{aligned} \tag{4}$$

d_p/d_t: the product synthesis rate, g/(L·h); P: the mass concentration of product, g/L; α: growth-related constant of the product; β: non-growth-related constant of the product; t: fermentation time, h; μ_m: maximum specific product generation rate, g/(L·h); X_m: maximum specific product concentration, g/L; X_0: initial mass concentration of specific product, g/L.

Kinetics model of substrate consumption

Luedeking-Pire-like equation was used to describe the substrate consumption (total sugar and reducing sugar) of *L. pentosus* 292 in a 200-L fermenter [24].

$$-d_s/d_t=\frac{1}{Y_X}\frac{dX}{dt}+\frac{1}{Y_P}\frac{dX}{dt}+\lambda X \tag{5}$$

When $t=0$, S=S_0, integrate in 0-t, $b=\frac{1}{Y_X}+\frac{\alpha}{Y_P}$:

$$\begin{aligned} S=S_0-bX_0[&\frac{e^{\mu_m t}}{-\frac{X_0}{X_m}(1-e^{\mu_m t})}-1]\\ &+c\frac{X_m}{\mu_m}\ln[1-\frac{X_0(1-e^{\mu_m t})}{X_m}] \end{aligned} \tag{6}$$

d_s/d_t: the substrate consumption rate, g/ (L·h); S: the substrate concentration; Y_X: the yield of bacteria to substrate, g/g; Y_P: the yield of product to substrate, g/g; λ: the bacterial maintenance constant. S_0: the initial substrate concentration.

Fermentation monitoring of *L. pentosus* 292

The biomasses (OD_{600nm}), dry weight, total sugar, reducing sugar, total protein, and LA of the samples at 2 h-intervals were estimated by the following methods during the fermentation.

OD_{600nm} was measured by a microplate reader (BioTek Epoch 2 Microplate Spectrophotometer; Agilent Technologies, Winooski, VT, USA).

Dry weight was determined by the drying method. Briefly, a certain amount of fermentation liquid was centrifuged at 8,000 rpm in 28°C for 5 min to obtain the supernatant and bacterial cells. The bacterial cells were washed twice with normal saline, and dried by freeze-drying machine (Kejin Vacuum Freeze-drying Technics Co., China) until the weight was stable for weighing.

Reducing sugar was measured by the 3, 5-dinitrosalicylic acid (DNS) method with some modifications, as has been previously described [25–28]. A reaction mixture of 2.5 mL of the relevant reducing sugar solution and 2.5 mL of DNS reagent was boiled for 5 min and cooled to room temperature in a water-ice bath, then 20 mL of distilled water was added to dilute the mixture. The absorbance of this mixture was detected at 540 nm by a microplate reader (BioTek Epoch 2 Microplate Spectrophotometer; Agilent Technologies, Winooski, VT, USA).

Total sugar was measured by the phenol-sulfuric acid method [15]. A reaction mixture of 0.2 mL of the relevant total sugar solution, 0.2 mL of 6% phenol solution, and 1.5 mL of concentrated sulfuric acid was boiled for 15 min and cooled to room temperature in a water-ice bath. The absorbance of this mixture was detected at 490 nm by a microplate reader (BioTek Epoch 2 Microplate Spectrophotometer; Agilent Technologies, Winooski, VT, USA).

LA was measured by the spectrophotometer method [29]. A reaction mixture of 50 mL of the relevant LA solution and 2 mL of FeCl3 solution (0.2% w/v)was stirred vigorously. The absorbance of this mixture was detected at 390 nm by a microplate reader (BioTek Epoch 2 Microplate Spectrophotometer; Agilent Technologies, Winooski, VT, USA).

Total protein was determined by a BCA Protein Assay Kit (Sangon Biotech, China) according to the instructions.

Storage strategy of the fermentation broth

Molasses (sugarcane molasses, molasses content is 45%, and pH = 4.84) was selected to investigate the effects of sugar resources on the growth and preservation of *L. pentosus* 292 fermentation broth. The different proportions (1%, 2%, 3%, 5%, and 10%) of molasses were added to the developed culture medium and the fermentation broth of *L. pentosus* 292 to detect the changes of bacterial activity (CFU/mL) and pH. Additionally, the storage period of *L. pentosus* 292 was estimated by the method of temperature-accelerated test. Briefly, the fermentation broth was incubated at 4, 10, 15, 20, 25, and 30°C, respectively, and the bacterial survival rates were calculated by the bacterial cell counting method. Then, the storage period of *L. pentosus* 292 fermentation broth was predicted by linear equation.

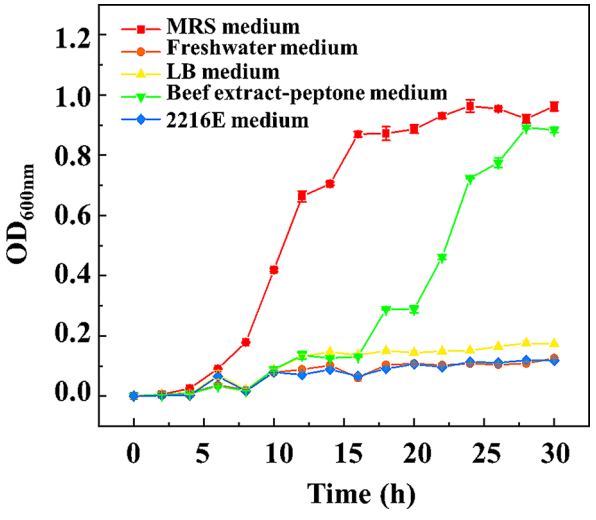

Fig. 1 The effect of MRS medium, freshwater medium, 2216E medium, beef extract-peptone medium, and LB medium on the growth of *L. pentosus* 292

Statistical analysis

The statistical differences in data were analyzed by the least-significant difference test ($P < 0.05$) using SPSS 22.0 for Windows (SPSS Inc., Chicago, USA). All of the models (including Logistic, Luedeking-Pire, and Luedeking-Piret-like), R^2, and diagrams were fitted, calculated, and generated in Origin 2022 software (OriginLab, Northhampton, MA, USA).

Results

Development of a culture medium and culture conditions for the fermentation of *L. pentosus* 292

Five types of culture media were tested to select an appropriate medium for *L. pentosus* 292. The result showed that MRS medium, compared to freshwater medium, 2216E medium, beef extract-peptone medium, and LB medium, was more suitable for the growth of *L. pentosus* 292 (Fig. 1), and the biomass reached the highest value (OD_{600nm} of 0.96) at 24 h. Consequently, MRS medium was selected as the base medium for subsequent fermentation investigation.

Six carbon sources as the sole carbon source (each at a concentration of approximately 12 g/L,), including molasses, lactose, maltose, brown sugar, maltodextrin, and glucose, were selected to investigate their effects on the growth of *L. pentosus* 292 and its capability of producing LA. As shown in Fig. 2A, lactose, brown sugar, and glucose not only supported the growth of *L. pentosus* 292, but also enhanced the production of LA.

Five nitrogen sources as the only nitrogen source (each at a concentration of approximately 8.5 g/L), including urea, malt extract, yeast extract, yeast powder, and soybean peptone, were selected to investigate their effects on the growth of *L. pentosus* 292 and its capability of producing LA. As shown in Fig. 2B, yeast powder and yeast extract, compared with other nitrogen sources, were beneficial for both the growth of *L. pentosus* 292 and itsLA production.

Five inorganic salts as the only inorganic salt (each at a concentration of approximately 1%), including $MgSO_4$, CH_3COONa, $(NH_4)_2SO_4$, $MnSO_4$, NaCl, and K_2HPO_4,

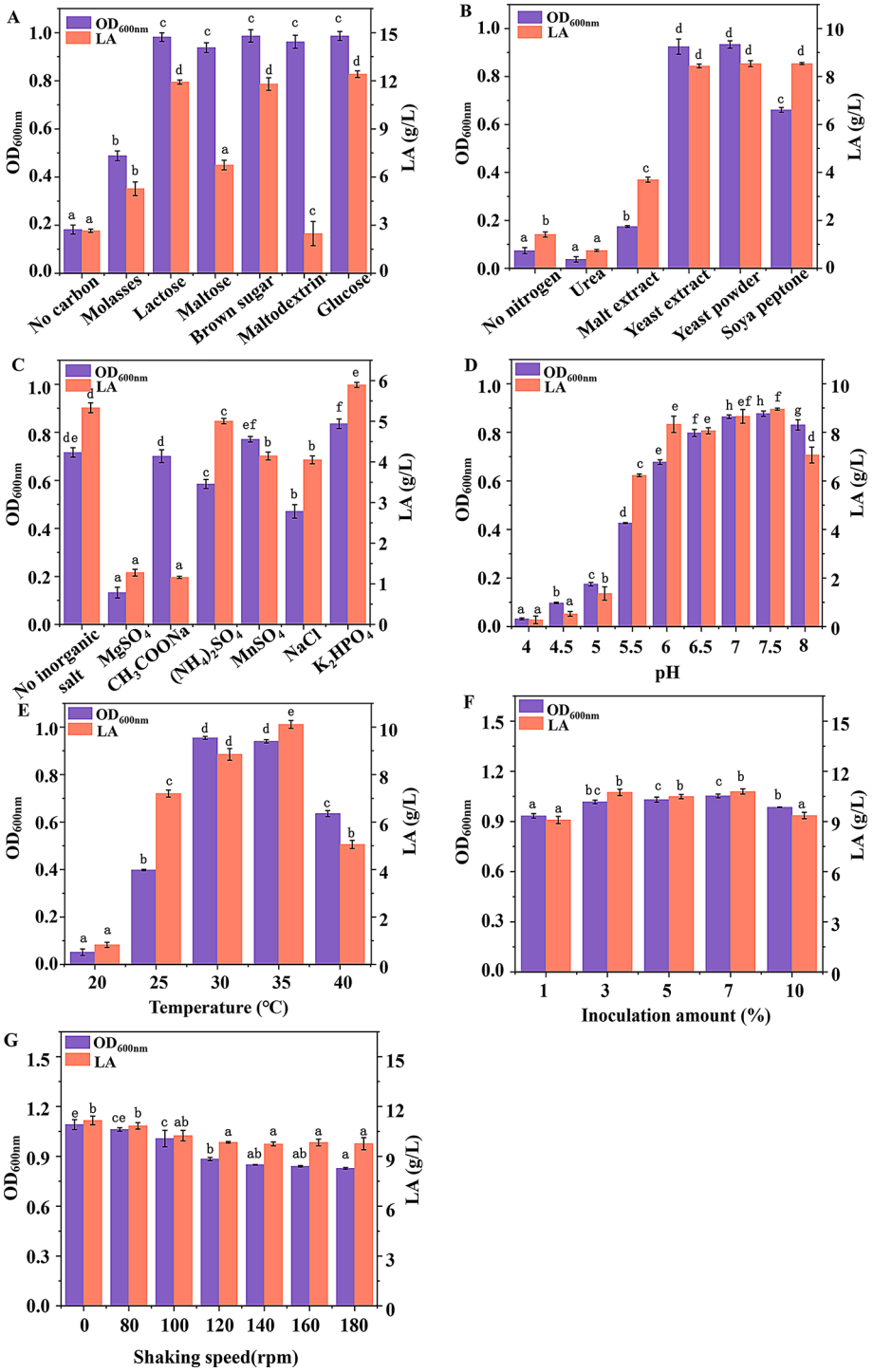

Fig. 2 The effects of carbon sources (**A**), nitrogen source (**B**), inorganic salt (**C**), pH (**D**), temperature (**E**), inoculation amount (**F**), and shaking speed (**G**) on the growth of *L. pentosus* 292 and its LA production after culturing for 24 h. Data were reported as the mean ± standard deviation. Mean values with different letters above the bars differ according to Tukey's test at $P < 0.05$

were selected to investigate their effects on the growth of *L. pentosus* 292 and its capability of producing LA. As shown in Fig. 2C, K_2HPO_4 displayed the highest capability for promoting *L. pentosus* 292 growth and increasing LA yield (5.89 g/L).

Additionally, *L. pentosus* 292 exhibited a relatively high growth capability and LA yield (approximately 9–10 g/L) at temperatures of 30–35 °C and a pH range of 6.5–7.5 (Fig. 2D and E). However, neither the inoculation amount nor the shaking speed had a significant effect on the

growth or LA yield of *L. pentosus* 292 within the monitoring range (Fig. 2F and G).

Based on the above results and considering the importance of carbon source, nitrogen source, inorganic salt, and pH during the strain fermentation and product generation, an orthogonal test with four factors (glucose as the carbon source, yeast powder as the nitrogen source, K_2HPO_4 as the inorganic salt, and pH) was performed to determine the relatively suitable conditions for the growth and LA production of *L. pentosus* 292. The main results are shown in Tables S3 and S4. After preliminary optimization, the relatively optimal fermentation medium of *L. pentosus* 292 consisted of 30 g/L glucose, 15 g/L yeast powder, 8 g/L K_2HPO_4, and 1% Tween-80. T he relatively optimal fermentation conditions for *L. pentosus* 292 fermentation were a temperature of 35 °C, pH of 6, shaking speed of 80 rpm, and inoculation amount of 3%. Under the optimal conditions, the biomass of the 292-strain increased by 40.33% (OD_{600} reached up to 1.35) and the LA yield increased by 83.48% (up to 16.24 g/L) after 24 h of fermentation (Fig. 3).

Large-scale fermentation and kinetics models of *L. pentosus* 292 in fermenter

The pilot fermentation test was carried out under the optimal culture conditions, and the changes of biomass, dry weight, reducing sugar, total sugar, total protein, and LA of *L. pentosus* 292 were monitored at 2 h intervals. As shown in Fig. 4, the growth of 292 strain was in a lag phase at the early fermentation stage of 0 to 8 h. The *L. pentosus* 292 strain entered the logarithmic growth phase during 8–24 h of fermentation, and all of the substrate consumption and product generation of *L. pentosus* 292 peaked at 24 h, such as the reducing sugar content decreased to 1.05 g/L, the total sugar content decreased to 2.46 g/L, the OD_{600nm} reached 1.63, and the bacterial dry weight reached 3.76 g/L. The maximum bacterial growth rate was 0.234 g/(L·h) during the logarithmic stage. It's worth noting that the LA content and residual sugar content tended to stabilize during 24–48 h, with the highest LA content (16.24 g/L) being generated at 28 h.

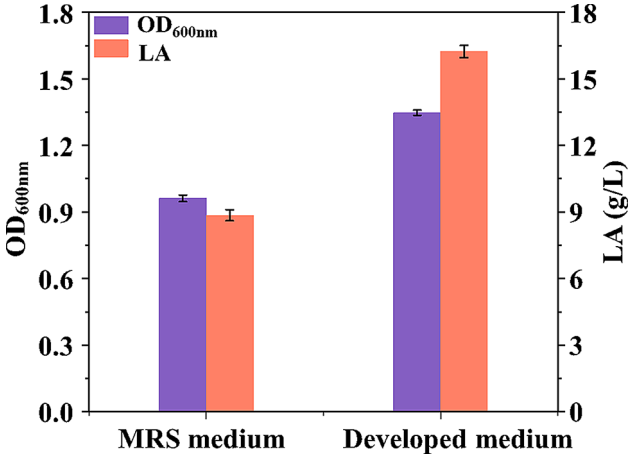

Fig. 3 The capability of growth and LA production of *L. pentosus* 292 in the MRS medium under normal conditions (temperature of 35 °C, pH of 7, shaking speed of 180 rpm, and inoculation amount of 3%) and the developed medium under the suitable condition (temperature of 35 °C, pH of 6, shaking speed of 80 rpm, and inoculation amount of 3%) after 24 h of fermentation

The fermentation kinetics models of bacterial growth, substrate consumption, and product generation of *L. pentosus* 292, including biomass (OD_{600nm}), dry weight, LA, total protein, total sugar, and reducing sugar of *L. pentosus* 292 were established (Fig. 5), and the main kinetic model fitting parameter values are shown in Table S5.

The kinetics curves of biomass (OD_{600nm}) and dry weight of the 292-strain were better fitted to the model of Logistic equation. The kinetic models are represented by Eq. (7) for biomass (OD_{600nm}) with an R^2 of 0.9921 and Eq. (8) for dry weight with an R^2 of 0.9942.

$$X_g = \frac{0.000195e^{0.658t}}{1 - 0.000118(1 - e^{0.658t})} \tag{7}$$

$$X_w = \frac{0.013e^{3.767t}}{1 - 0.034(1 - e^{3.767t})} \tag{8}$$

The kinetics curves of total protein and LA of the 292-strain were better fitted to the model of the Luedeking-Piret equation. The kinetic models are represented by Eq. (9) for total protein with R^2 of 0.9506 and Eq. (10) for LA with R^2 of 0.8383.

$$P_{prot} = 0.00012\left[\frac{e^{0.658t}}{1 - 0.000118\,(1 - e^{0.658t})}\right] - 0.00718\ln\left[1 - 0.000118(1 - e^{0.658t})\right] \tag{9}$$

$$P_{LA} = 0.002\left[\frac{e^{0.658t}}{1 - 0.000118\,(1 - e^{0.658t})}\right] - 0.0242\ln\left[1 - 0.000118(1 - e^{0.658t})\right] \tag{10}$$

The kinetics curves of total sugar and reducing sugar of 292-strain were better fitted for the model of the Luedeking-Piret-like equation. The kinetic models are represented by Eq. (11) for total sugar with R^2 of 0.8898 and Eq. (12) for reducing sugar with R^2 of 0.8585.

$$S_T = 17.082 - 0.0015\left[\frac{e^{0.658t}}{1 - 0.000118\,(1 - e^{0.658t})}\right] + 1.091\ln\left[1 - 0.000118(1 - e^{0.658t})\right] \tag{11}$$

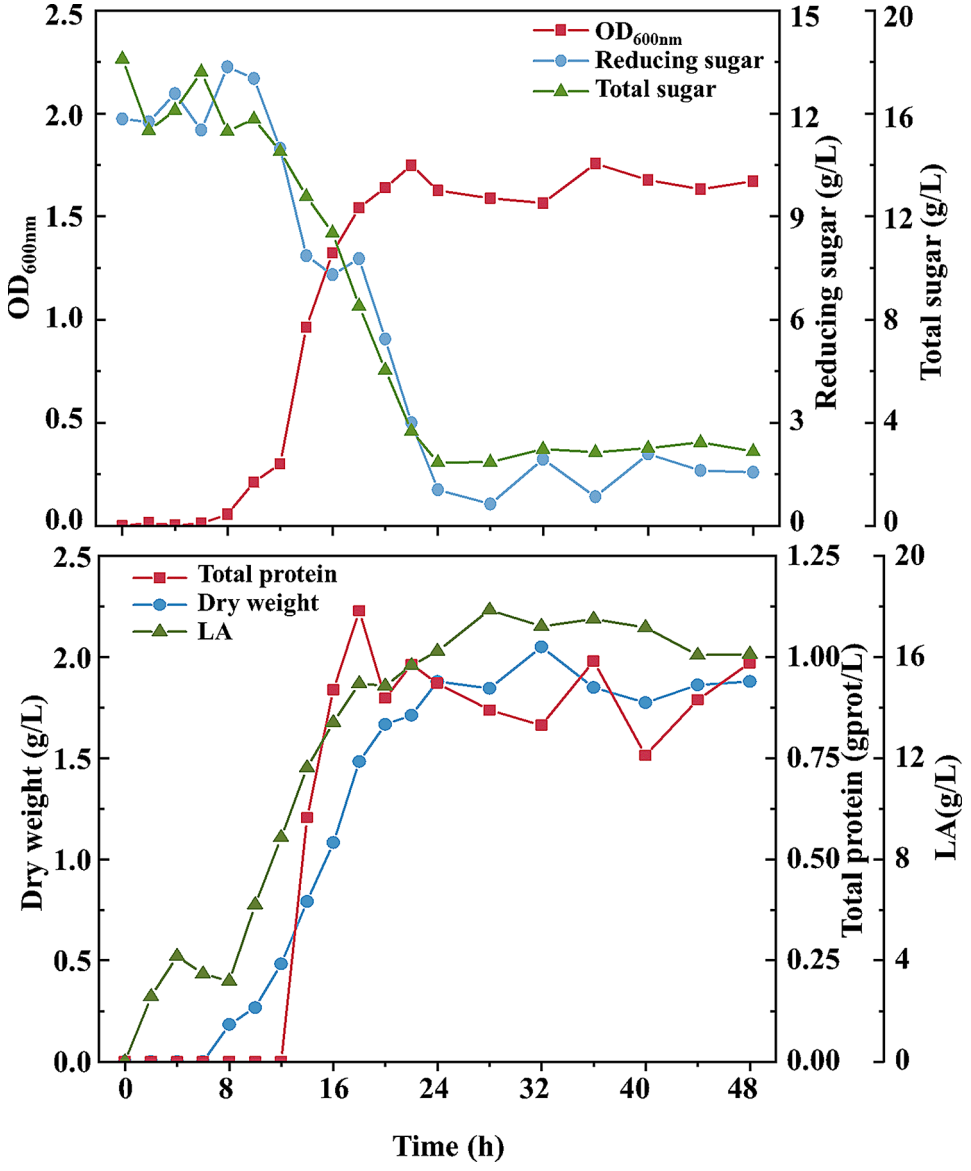

Fig. 4 Fermentation kinetics curve of bacterial growth, substrate consumption, and product generation of *L. pentosus* 292 in 200-L fermenter, including OD_{600nm}, total sugar, reducing sugar. dry weight, total protein, and LA

$$S_R = 12.762 - 0.00119\left[\frac{e^{0.658t}}{1-0.000118\,(1-e^{0.658t})}\right] + 1.091\ln\left[1-0.000118(1-e^{0.658t})\right] \quad (12)$$

Storage strategy and storage period prediction of *L. pentosus* 292

As shown in Fig. 6 and Table S6, the strategy of adding 1–3% molasses into *L. pentosus* 292 fermentation broth could maintain relatively high bacterial activity and a relatively low pH value (less than 6.5) within 72 h of preservation, whereas adding more than 3% molasses into the fermentation broth inhibited the bacterial activity. However, the strategy of adding less than 3% of molasses into the *L. pentosus* 292 strain medium didn't improve the growth or bacterial activity of *L. pentosus* 292, while adding more than 3% molasses inhibited the growth and bacterial activity of this strain. It is worth noting that the addition of molasses, either before inoculation of the medium or 24 h after fermentation, can reduce the pH value of the fermentation solution.

Additionally, in this work, the storage period of *L. pentosus* 292 fermentation broth was predicted by a temperature-accelerated test (Fig. 7). Briefly, the survival rates of viable bacteria of *L. pentosus* 292 in the fermentation broth were investigated at different test temperatures at different storage stages, and the linear regression was used to determine the thermal degradation rate and storage period at specific test temperature (Fig. 7A).

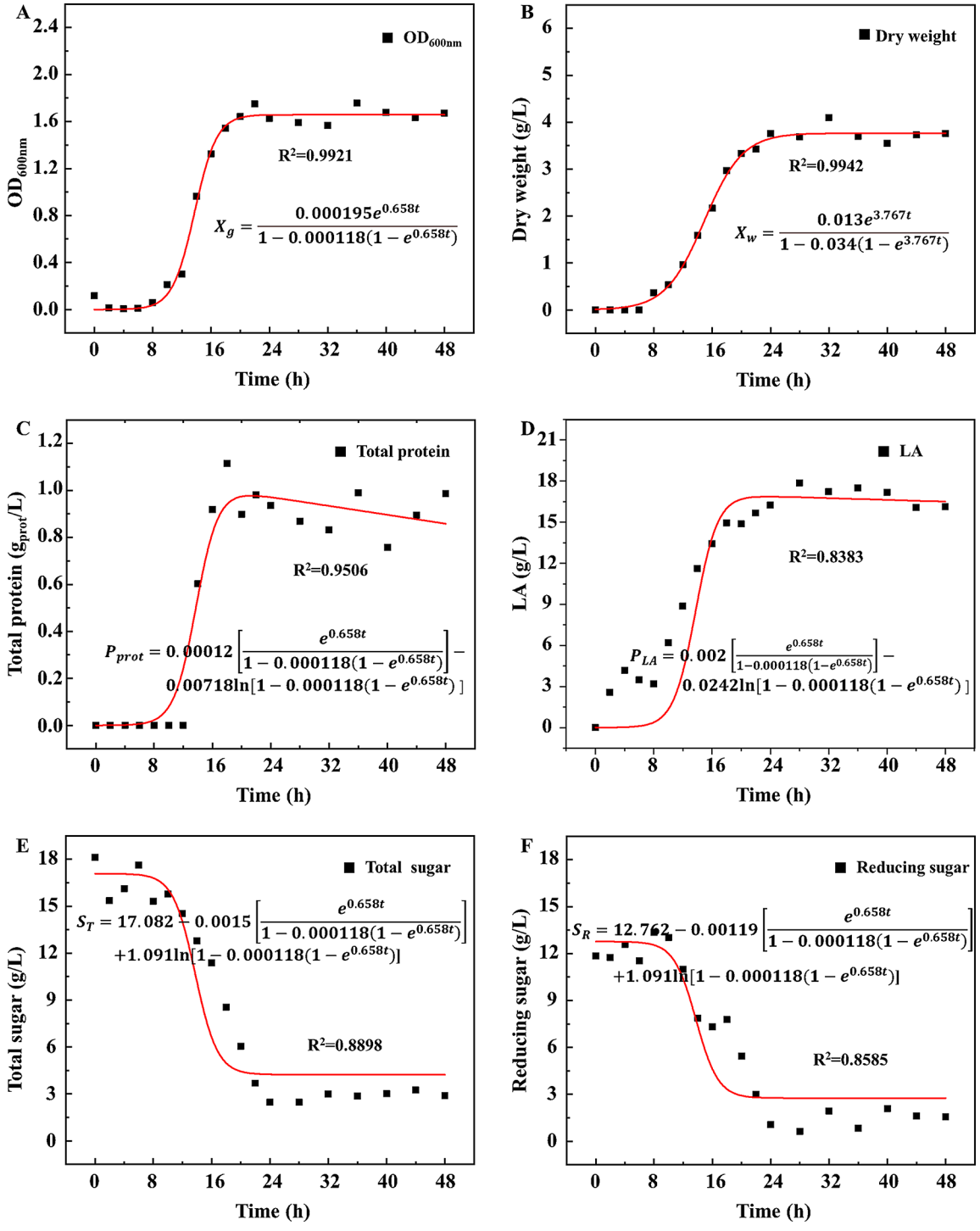

Fig. 5 Fermentation kinetics models of bacterial growth (**A**), dry weight (**B**), LA (**C**), total protein (**D**), total sugar (**E**), and reducing sugar (**F**) of *L. pentosus* 292

Subsequently, the correlation of thermal degradation rate and temperature was established by exponential function to adjust the thermal degradation rate (Fig. 7B), and then the adjusted thermal degradation rate was correlated with the storage period at the test temperature through linear regression (Fig. 7C). Finally, a storage period model of *L. pentosus* 292 fermentation broth was established (Fig. 7D). As shown in Fig. 7D, the fermentation broth could be preserved for about 36 days at 4 °C, 16 days at 25 °C, and 9 days at 30 °C. However, when the temperature exceeded 45 °C, the fermentation broth would

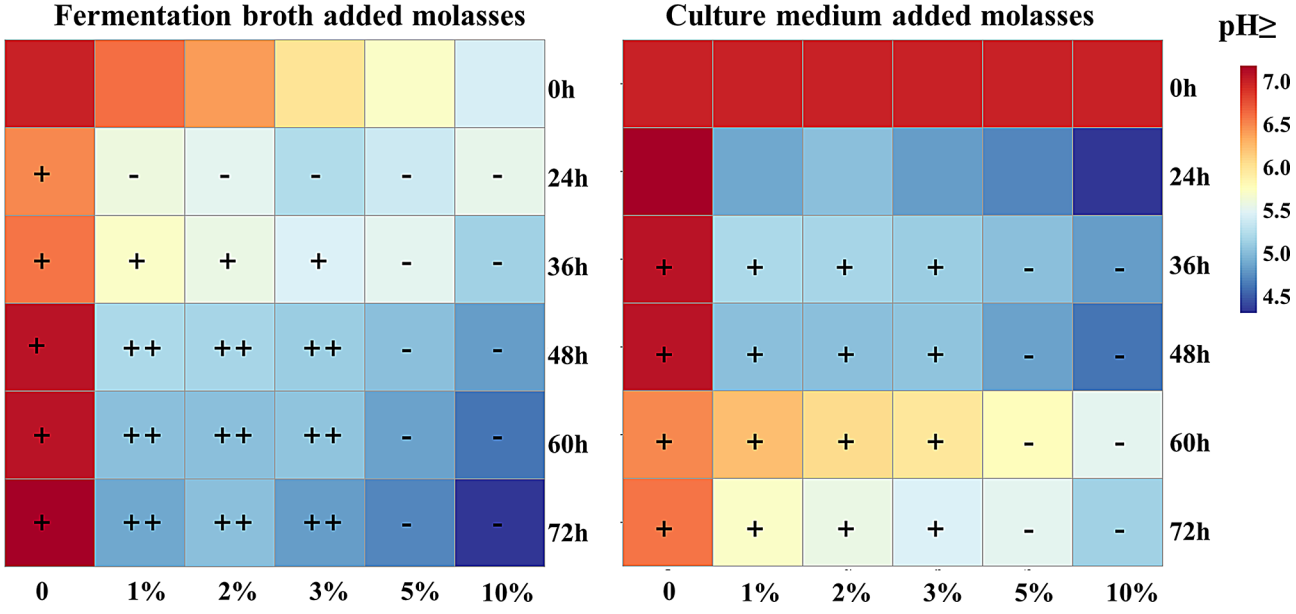

Fig. 6 The influence of molasses added into the fermentation broth and culture medium on changes in bacterial biomass and pH of *L. pentosus* 292 for the construction of a storage strategy for the 292-strain. Bacterial biomass is marked with "++" (improving), "+" (normal), and "-" (inhibiting), while the pH value is shown by the heatmaps

quickly lose its activity, according to the storage period preservation model.

Discussion

While LAB are recognized for their probiotic functions through secretion of bioactive compounds (e.g., organic acids, antimicrobial peptides, exopolysaccharides) [12], our study specifically elucidates how *L. pentosus* 292's secretory profile, particularly its LA, which serves as a raw material for green degradable plastic products. Among prebiotics, LA has been designated as Generally Recognized as Safe (GRAS) by the U.S. Food and Drug Administration and has garnered significant attention due to its versatile applications. LA is extensively utilized in food, chemical, cosmetic, and pharmaceutical industries as an antioxidant, pH regulator, green solvent, metal chelator, moisturizer, and more. Specific functionalities include prebiotic activity, skin rejuvenation, anti-acne effects, controlled drug delivery, and applications in medical devices (e.g., dialysis solutions, prostheses, surgical sutures). The global lactic acid market will increase to 1,960.1 kilotons in 2025 [11] and 2.8 million tons by 2030 (https://www.statista.com/). Despite the prominence of microbial fermentation, particularly via LAB, as a primary LA production method, key challenges persist. These include the scarcity of high-yield LAB strains, cost-effective fermentation medium, optimized fermentation techniques, and efficient storage strategies for fermentation broth to facilitate downstream processing.

L. pentosus 292 stored in the State Key Laboratory of Marine Resource Utilization in the South China Sea; isolated from shrimp intestine as a kind of LAB with a high capability of producing LA displayed excellent probiotic function to aquatic animals, particularly shrimp aquaculture, which was similar to the function of *Lactococcus lactis* L280, *L. lactis* S1, *L. lactis* S2, *E. faecalis* F3, and *E. faecalis* F7 previously reported by our lab [1, 15]. Reportedly, *L. pentosus* strains as probiotics had many advantages since their excellent features of high acid tolerance, high yield, and productivity [30], such as antagonistic against pathogens [31], broad-spectrum gram-positive bacteriocin preparation [25], improving the resistance of prawn [32], reducing carcinogenic substances by secreting N-nitrosodimethylamine [33], particularly the LA generation by the fermentation of this kind of strains [34–37].

MRS medium is usually the reference medium of LAB, which can provide a large amount of nutrients for the growth of LAB. However, due to the high cost of this medium, it is not suitable for large-scale fermentation. In this work, we developed a culture medium (30 g/L glucose, 15 g/L yeast powder, 8 g/L K_2HPO_4, and 1% Tween – 80) based on MRS medium for *L. pentosus* 292 to generate a high amount of LA under a suitable condition (temperature of 35 °C, pH of 6, shaking speed of 80 rpm, and inoculation amount of 3%), and the LA yield was up to 16.24 g/L increased by 83.48% compared with the 292-strain cultured in MRS medium after fermentation for 24 h. As shown in Table S7, Compared to MRS medium (8 g/L beef extract, 4 g/L yeast powder, 10 g/L peptone, 2 g/L K_2HPO_4, 2 g/L $C_6H_{14}N_2O_7$, 5 g/L CH_3COONa, 0.25 g/L $MnSO_4$, 0.58 g/L $MgSO_4$, 20 g/L

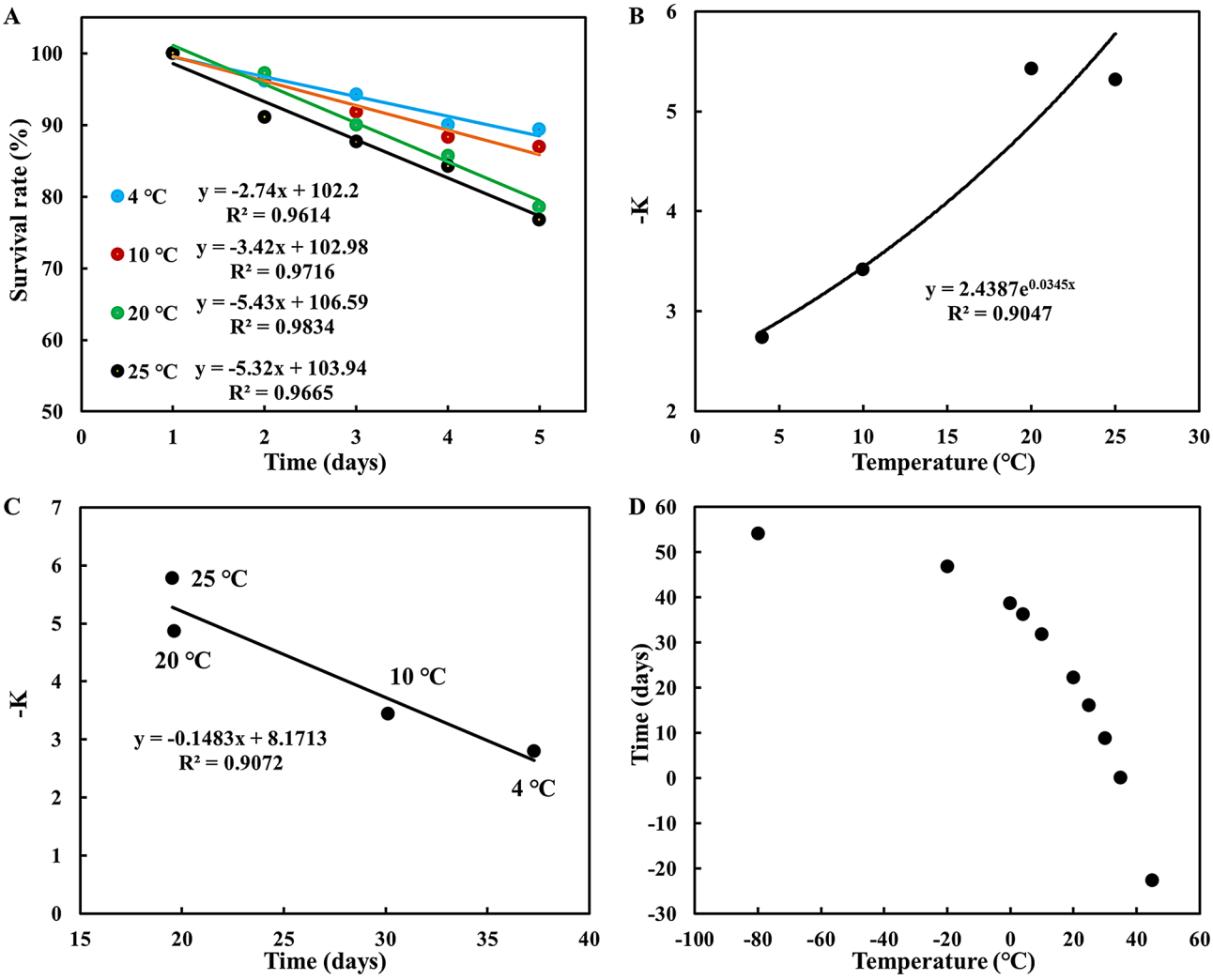

Fig. 7 Storage period prediction of *L. pentosus* 292 fermentation broth by the temperature-accelerated test. (**A**) The thermal degradation rate of *L. pentosus* 292 (the coefficient of the linear regression equation is defined as *K*, representing the thermal degradation rate). (**B**) The relationship between the thermal degradation rate and temperature. (**C**) The simulation of the thermal degradation rate and storage days. (**D**) Estimation of the storage period at different temperatures

glucose, and 1% Tween – 80), the medium we developed uses a single carbon source (glucose) and inorganic salt (K_2HPO_4), which caused a reduction in the cost of the medium. Based on existing statistical data, it is preliminarily inferred that the optimized medium formulation demonstrates a cost reduction of ¥ 1.0404 per gram of LA produced, highlighting its economic viability for industrial-scale fermentation processes (Table S7). In the developed culture medium for 292-strain fermentation, the glucose, yeast powder, and K_2HPO_4 were selected as carbon source, nitrogen source, and inorganic salts, which is similar to the medium developed by Zhang et al. that was suitable for the production of bacteriocin by *Lactobacillus plantarum* [38]. Reportedly, LA yields typically range from 0.01 to 1.2 g/g (LA/Carbohydrate) due to variations in carbon source types, quality, and metabolic characteristics of LAB [11], *Lactobacillus coryniformis* subsp. *Torquens* achieved a yield of 0.46 g/g representing a comparable acid production to the strain 292 [39], *Lactobacillus rhamnosus* ATCC 7469 achieved a higher yield of 0.73 g/g representing a 35% increase over the strain 292 [40], *Lactobacillus pentosus* CECT 4023T achieved yield of 0.45 g/g representing a 1.3-flod lower than the strain 292 [36], and *Lactobacillus acidophilus* achieved a higher yield of 0.87 g/g representing a 61% increase over the strain 292 [41]. These comparisons suggest that the LA production capacity of strain 292 falls within a moderate level among reported studies.

Glucose exists as a carbon source is one of the main factors stimulating the production of biomass, whereas excessive glucose content will inhibit the production of biomass [42]. Reportedly, LA production yield by LAB is generally related to pH ranging from 3.5 to 9.6 and temperature ranging from 5 to 45 °C [11]. *L. pentosus* 292

could survive better in pH 5–8 at 25–35 °C with a relatively high LA production and productivity.

Based on the developed medium, we conducted a pilot experiment to expand the fermentation of *L. pentosus* 292. The kinetic model can effectively describe the fermentation performance of bacteria [43]. The fermentation kinetics models of bacterial growth (OD_{600nm} and dry weight), substrate consumption (total sugar and reducing sugar), and product generation (LA and total protein) of this strain were successfully built, which were better fitted by the model of Logistic equation, Luedeking-Piret-like equation, and Luedeking-Piret equation, respectively, thereby facilitating the construction of the production strategy of fermentation products and exploring what fermentation stage the bacteria were in. According to the large-scale fermentation kinetics models of *L. pentosus* 292, we reason that both substrate consumption and product generation are closely related to bacterial growth. Reportedly, the parameter $v\,(v = \beta/\mu\,\alpha)$ in the product generation kinetics model can be expressed as the relationship between the specific growth rate (μ) and the constants associated and unrelated to bacterial growth, and when the v value is closer to 0, indicating that the product generation is more related to the growth of biomass [44]. Additionally, G.A. Dominguez-Gutierrez et al. reported that v <0 indicates that the products are suffering from decomposition in the fermentation process [42]. In this work, the v values of protein and LA were -1.373×10^{-6} and -2.83×10^{-7} according to the pretion kinetics generation model (Eq. 9) and LA generation kinetics model (Eq. 10), respectively, indicating that the LA and protein production of *L. pentosus* 292 were significantly related to bacterial growth. Although the v values of protein and LA were negative, they were very close to 0, which may indicate that the products began to suffer decomposition, but the degradations of these two products were very weak.

Molasses, as a readily fermentable sugar, enhances fermentation quality by serving as a substrate for LA accumulation and pH reduction [45]. Its organic acids (e.g., benzoic and sorbic acids) exhibit antifungal properties, enabling dual functionality as both a fermentation substrate and preservative to inhibit mould/yeast growth via pH reduction [21, 46]. Notably, molasses addition (1–3%) in *L. pentosus* 292 fermentation broth effectively maintained bacterial activity and prolonged storage by sustaining a low-pH environment without significantly altering LAB counts [47]. Given the important role of preservation strategy in the preservation process of fermentation broth and the preparation process of functional product from fermentation broth, this work performed a storage strategy by adding 1–3% molasses as a preservative to the fermentation broth of *L. pentosus* 292, which besides effectively maintaining the bacterial activity, also could keep the fermentation broth at a low-pH environment, thereby increasing the storage period of LA and LAB. A temperature-accelerated storage model revealed preservation durations of 36 days at 4 °C and 16 days at 25 °C, providing sufficient time for post-processing to harvest active cell preparations. Extended preservation occurred at lower temperatures (-20 °C/-80 °C), consistent with prior studies demonstrating improved microbial viability at 4 °C versus 37 °C [48], and preservation at -80 °C could lead to higher survival and activity of *Lactiplantibacillus plantarum* LIP-1 than preservation at -20 °C [49].

Conclusion

In conclusion, we reported a suitable medium for the large-scale *L. pentosus* 292, a member of LAB, which exhibited a relatively high capability of producing LA under optimal conditions. We performed a pilot fermentation test for the strain 292, and the large-scale fermentation kinetics models of bacterial growth, substrate consumption, and product generation were successfully built. Adding 1–3% molasses as a preservative could effectively maintain the bacterial activity of the fermentation broth of *L. pentosus* 292 in a low-pH environment. According to the model, after the treatment of this preservative, the *L. pentosus* 292 fermentation broth can be preserved for 36 days at 4 °C and 16 days at 25 °C. This work lays a foundation for the industrial application of *L. pentosus* 292 for the large-scale production of LAB preparation and its products.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03837-4 .

Supplementary Material 1

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Author contributions

WR and ZX provide the conceptualization and designed the experiments. XC, ZW and ZF performed the experiments and analyzed the data. YC and XW analyzed the data. HL and XC supervised the study. WR and XC wrote the manuscript and revised the manuscript. XC and ZW contributed equally to this work. All authors have read and approved the final manuscript.

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Data availability

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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