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Research Article

Keywords: Bacteriocin, Antibacterial activity, Isolation and identification, Growth dynamics, Batch fermentation

Posted Date: June 16th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-605763/v1>

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Isolation and Identification of *Lactobacillus plantarum* C010 and Growth Kinetics of its Batch Fermentation

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Abstract

Chilled pork is pursuit by people due to its delicious and delicate taste, but it is still susceptible to microbial contamination even under refrigerated conditions. Consequently, to explore microbial preservatives for chilled pork, in this study, the specific spoilage organism *Pseudomonas koreensis* PS1 from spoiled chilled pork as the indicator was used to isolate the bacteriocin-producing *lactic acid bacteria* from the soils and fresh cow dungs. Among six bacteriocin-producing bacteria from 182 isolates, the strain C010 with higher-yielding, broad-spectrum, subculture stability and protease (pepsin, trypsin and proteinase K) sensitive was selected and identified as *Lactobacillus plantarum* based on morphological, biochemical and 16S rDNA gene sequence analysis. Simultaneously, the crude bacteriocin of *L. plantarum* C010 was stable under high temperature and ultraviolet conditions.

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19 The kinetics of bacterial growth and bacteriocin production of *L. plantarum* C010 were analyzed in
20 batch fermentation. Bacteriocin was produced throughout the logarithmic growth phase and the
21 Leudeking-piret model could characterize the synthesis of bacteriocin well. This present study
22 indicates that bacteriocin-producing *L. plantarum* C010 has promising potentials to control the
23 specific spoilage organism and can be used as the bio-preservative in food.

24 **Keywords** Bacteriocin• Antibacterial activity• Isolation and identification• Growth dynamics• Batch
25 fermentation

26 **Introduction**

27 Meat and meat products are increasingly popular because of their ample moisture and nutrients,
28 naturally, they are also easily contaminated by microorganisms ([Woraprayote 2016](#)). Especially the
29 foodborne spoilage bacteria such as *Pseudomonas*, *Micrococcus*, *Acinetobacter*, *Brochothrix*
30 *thermosphacta*, etc., grows rapidly even at low temperatures, posing a certain microbial biological
31 risk to the freshness of chilled pork ([Bouju-Albert 2018](#); [Geeraerts et al. 2018](#); [Li et al. 2006](#)). These
32 cryophilic microorganisms act on the protein and lipids of chilled pork by producing a variety of
33 metabolic enzymes, degrading and releasing large amounts of H₂S and amine compounds, which can
34 cause serious harm to the body if ingested ([Lee et al. 2020](#)). Simultaneously, the presence of these
35 microorganisms has an adverse effect on the appearance, texture and flavor of chilled pork, which
36 will result in significant economic losses ([Woraprayote 2016](#)). Therefore, it is a great challenge to
37 extend the shelf life of chilled pork and to reduce the variety and number of cryophilic
38 microorganisms on the surface of pork.

Nowadays, food preservation can control the growth of microorganisms mainly by physical, chemical and biological methods to extend food shelf life. Unexpectedly, physical preservation has a very short shelf life, while the long-term use of chemical synthetic preservation is potentially harmful to humans (Bharti et al. 2015; Sidooski 2018). Hence, using biological substances to replace physical and chemical synthesis is an important way to maintain the microbial quality and safety of meat and meat products (Costa et al. 2019). This method can reduce the addition of exogenous chemical synthetic preservatives which will lead to the alleviation of the toxic effects on human body. *Lactic acid bacteria* (LAB) are generally regarded as safe because of their probiotic properties, and the metabolites they produce, such as organic acids, hydrogen peroxide, diacetyl and bacteriocins, etc., have strong antibacterial properties (Karoline et al. 2018; Lv et al. 2018). Among them, the presence of organic acids can not only inhibit the growth of most microorganisms to extend the shelf life of foods, but also enhances the sensory, quality and flavor of fermented foods. However, its excessive use will produce a cumulative chronic damage to the body. As a small molecule polypeptide synthesized by the ribosome, bacteriocin is a natural antibacterial peptide produced by LAB (Todorov et al. 2019). At lower concentrations, it has strong antibacterial activity and is easy to be digested by the human body because of its protein nature; more importantly, based on its colorless and tasteless characteristics, the addition of bacteriocins has little impact on the sensory quality of food (Cotter et al. 2013; Lee et al. 2020). Besides, bacteriocins can withstand higher thermal stresses and have a wider hydrogen (pH) tolerance potential than other protein antimicrobials preservatives. For these reasons, bacteriocins in food preservation and medical industry have currently a wide range of applications (Gautam and Sharma 2009; Woraprayote 2016).

60 *Pseudomonas* is a dominant specific spoilage organism in chilled pork. The biofilm formed by
61 this bacteria easily adheres to processing equipment such as steel and glass, thus increasing the risk of
62 contamination of chilled pork during its processing and transportation ([Sterniša et al. 2019](#)).
63 Presently, Nisin is the only bacteriocin recognized as a food additive in the world and is widely used
64 in the preservation of vegetables, dairy products, meat products and others. Unfortunately, its
65 antibacterial spectrum is narrow, and its application in meat and meat products preservation is not
66 ideal. The reason is that Nisin alone is not sufficient to prevent the growth of Gram-negative spoilage
67 bacteria, such as *Pseudomonas fluorescens*. However, up to now, even though many
68 bacteriocin-producing LABs have been developed, there are still few bacteriocins that have inhibitory
69 effects on Gram-negative bacteria ([Anastasiadou et al. 2008](#); [Chung et al. 1989](#); [Ercolini et al. 2010](#);
70 [Woraprayote 2016](#)). At the same time, due to low yield, unsatisfactory purification effect and
71 uncertain toxicological properties, the majority of bacteriocins can only exist in laboratories for
72 freshness preservation, but cannot be applied to industrial production ([Leroy and De 2010](#); [Silva](#)
73 [Célia C. G. 2018](#)).

74 The search for bacteriocin-producing LABs that inhibits Gram-negative and positive bacteria has
75 a good research and application value for reducing the growth of spoilage organisms on the surface of
76 meat products at low temperatures. In this study, *Pseudomonas koreensis* PS1 isolated by our
77 research team from spoiled chilled pork ([Huang et al. 2013](#)), as a gram-negative dominant spoilage
78 bacteria, was used as the indicator for aiming to screen out the bacteriocin-producing LABs from the
79 soil, fresh cow dungs, and then the inhibitory effect and growth profile of bacteriocin production from
80 *Lactobacillus plantarum* C010 were studied.

81 **Material and methods**

82 **Sample collection and isolation of LAB**

83 Fresh soils and cow dungs were collected from the experimental field in Jiangxi Agricultural
84 University (Nanchang, China). And all samples by serial dilute were spread on De Man Rogosa
85 Sharpe (MRS) agar plates with 30 % CaCO₃ and incubated at 37 °C until colonies with the dissolved
86 ring could be clearly separated ([Amarantini et al. 2019](#); [Luo 2011](#)). Then, pick single colonies with
87 calcium-soluble circles and different morphologies and stored with 25 % glycerol at -20 °C.

88 **Microbial strains and Culture medial**

89 *P. koreensis* PS1, as a specific dominant spoilage organism isolated from chilled spoilage pork
90 by our research group, was chosen as the important indicator strain for antimicrobial assays and
91 cultured overnight in Luria broth (LB) at 37 °C ([Huang et al. 2013](#)). Meanwhile, the following strains,
92 *Staphylococcus aureus* C013, *Escherichia coli* ATC-1, *Bacillus fusiformis* J4, *Bacillus subtilis*
93 KC-08, *Bacillus putida* BP-01, *Bacillus amyloliquefaciens* JDF 002, *Serratia plymuthica* Z2, *Proteus*
94 *penneri* Z3 were used as second indicators. These strains were obtained from Jiangxi Engineering
95 Laboratory for the Development and Utilization of Agricultural Microbial Resources in Jiangxi
96 Agricultural University (Nanchang, China), and grown overnight in LB at 30 °C or 37 °C. Besides,
97 *Rhodotorula mucilaginosa* TJ1-2, *Saccharomyces cerevisiae* ATCC-5, *Aspergillus niger* HQM-1,
98 *Penicillium italicum* ACCC 30399, *Penicillium citrinum* PA-33 were all grown 3-5 d in Potato
99 Dextrose Agar (PDA) at 28 °C.

100 **Isolation of bacteriocin-producing LABs**

101 The isolated LABs were grown in MRS medium and cultured aerobically at 37 °C. After 24 h of
102 cultivation, cells were centrifuged at 4 °C, 10 000 rpm for 10 min and divided into two parts.

103 One part was adjusted to pH 5.5 with 5 M NaOH and HCl. The same pH treatment of lactic acid,
104 acetic acid and hydrochloric acid was used as the positive controls and the MRS medium had no
105 bacteria as negative control. Besides, 28 g (80 % saturation) ammonium sulfate was added to another
106 cell-free supernatant and the precipitation was dissolved by phosphate buffer solution (pH 6.0)
107 (Saelao 2017). The crude antibacterial substance was collected to against *P. koreensis* PS1 by using
108 the oxford cup diffusion method to determine the antibacterial activity (Li et al. 2019). The same
109 treatment of MRS medium without LAB was used as the control and each treatment was carried out
110 in triplicates.

111 **Screening of antibacterial LABs**

112 The antibacterial activity of crude bacteriocin was used by oxford cup diffusion method using
113 above mentioned microbiological indicators (bacteria, mold and yeast) to analyze the broad-spectrum
114 (Thirumurugan et al. 2018). Simultaneously, each LAB was subjected to passaged culture and after 5
115 generation, the crude bacteriocin was subjected to oxford cup diffusion method to determine the
116 antibacterial activity.

117 **Sensitivity of antibacterial activity of strain C010 to enzymes**

118 Aliquots of fermentation supernatant from the strain C010 were treated with 5 mg/mL catalase
119 and 1 mg/mL pepsin, trypsin and proteinase K for 2 h at 37 °C. The supernatant without proteinase
120 treatment was used as the control. The antimicrobial activity of all treatments was determined by the
121 oxford cup diffusion method in triplicate (Li et al. 2019).

122 **Identification of the bacteriocin-producing strain C010**

123 Morphological and biochemical tests were used to identify the bacteriocin-producing strain
124 C010 and selected by broad-spectrum antibacterial activity, subculture stability and enzyme
125 sensitivity. According to the criteria of Berger's Manual of Bacterial Identification (Ninth Chinese
126 Edition) (Chen and Kong 1995), the identification of strain C010 was performed, which including
127 Gram's staining, cell morphology, catalase reaction, sugar fermentation (glucose, fructose, xylose,
128 sucrose, sorbitol, mannitol), and several others (arginine ammonia produce, hydrogen sulfide
129 produce, pigment produce and gas produce). Then, strains were sent to Sangon (Shanghai, China) and
130 the partial sequence of 16S rRNA obtained was compared with the National Center for
131 Biotechnology Information (NCBI) database to evaluate their homology.

132 **The stability of antibacterial activity of *L. plantarum* C010**

133 Aliquots of 1 mL crude bacteriocin were treated with thermostatic incubated for 30 min at 60,
134 80, 100 °C and exposed to high pressure condition for 20 min at 121 °C. Similarly, crude bacteriocins
135 were placed under UV lamp for 5, 10, 20, 30, 40, 60 min. All treatments were applied to evaluate the
136 antibacterial activity against *P. koreensis* PS1 by using oxford cup diffusion method, and without any
137 treatment served as the control. Each treatment was carried out in triplicates.

138 **Growth curve and kinetic analysis of *L. plantarum* C010**

139 The suspension of *L. plantarum* C010 was added into fresh MRS medium at a 2 % volume and
140 cultivate at 37 °C for 24 h. Samples were taken out every 2 h and the total number of bacteria was
141 measured by plate count method, while the pH, antibacterial activity and reducing sugar were also
142 determined at the same interval. Then, the relationship among bacterial growth, product synthesis and

143 substrate consumption during the batch fermentation of *L. plantarum* C010 was explored to provide a
144 theoretical basis for the subsequent large-scale fermentation and condition optimization.

145 **Determination of the total number of bacteria**

146 Plate colony counting method was used to determine the total number of bacteria, and the
147 specific method was as follows: Samples of fermentation broth were taken at different time points
148 and diluted at appropriate multiples, then 0.1 mL was spread on MRS medium and evenly coated, and
149 incubated at 37 °C until single colony clearly appeared. Subsequently, the number of colonies for
150 50-300 plate were selected for counting and record.

151 **Determination of reducing sugar content**

152 The 3,5-Dinitrosalicylic acid (DNS) method was used to determine the reducing sugar content of
153 the sample solution, and the glucose standard curve was shown in [Fig. 1](#). Then the reducing sugar
154 content in the fermentation broth was determined and calculated with reference to the standard curve
155 determination method.

156 [\(Fig. 1 about here\)](#)

157 **Statistical analysis**

158 All results were expressed as mean value of the inhibitory diameter $\pm$ standard deviation.
159 Significant differences were determined by independent sample T test and full single factor statistical
160 analysis by DPS (version 7.0, Canada). The Origin (version 9.0, Origin Lab, USA) would be adopted
161 for statistical and graphical analysis.

162 **Results**163 **Isolation of bacteriocin-producing LABs**

In this study, 182 strains with calcium dissolution circles were screened from soils and fresh cow
dungs using MRS agar containing 30 % CaCO₃ as the initial screening medium for LABs. All these
isolates were shown to be gram positive and catalase negative. After eliminating the differences
between the morphology and the samples, 6 strains were found to produce antibacterial substances
that removed the acidic interference by pH-adjusted and still had an inhibitory on Gram-negative *P.*
koreensis PS1 (Table 1). Interestingly, further re-screened by ammonium sulfate precipitation and the
antibacterial activity of antibacterial substance from these strains all had obvious effect on *P.*
koreensis PS1 than the treatment of removing the acid interference. Hence, the crude extract
bacteriocin from the precipitation of ammonium sulfate would be used for subsequent experiments.

173 (Table 1 about here)

174 **Screening of antibacterial LABs**

In order to obtain crude bacteriocin with good inhibitory effect, the antibacterial activities of 6 strains were determined as shown in Table 2. The crude bacteriocins of 6 LABs exhibited broad antibacterial effect against bacterium both Gram-positive and Gram-negative and molds, especially Gram-positive bacteria such as *S. aureus* C013, *B. amylolyticus* JDF 002 and *B. subtilis* KC-08, etc. but were slightly less effective against Gram-negative bacteria (*P. koreensis* PS1, *E. coli* ATC-1, *B. putida* BP-01, etc.). In comparison, the crude bacteriocin of strains C010 and C001 showed more significant antibacterial effect against indicators than the other 4 strains. Interestingly, the crude

182 bacteriocins of six isolates did not produce antibacterial effects against yeasts, suggesting that the
183 crude bacteriocin of LABs had no antibacterial effects against some fungus.

184 (Table 2 about here)

185 Strain degradation can occur during the subculture, and it is necessary that determine the number
186 of subcultural to ensure the stability of metabolically active substances of LABs. Accordingly, the
187 isolates were analyzed for the genetic stability during subculture, and 6 strains were conducted 5
188 passages to evaluate the stability of antibacterial activity. Table 3 showed that the bacteriocin
189 produced by 5 strains (R1, N109, C010, J001, C001) maintained great antimicrobial stability after 5
190 generations, especially the strain C010, which had the highest antibacterial activity against *P.*
191 *koreensis* PS1 among all 6 strains, and the diameter of inhibition zone more than 14 mm. Whereas
192 strain H011 had poor inhibition stability, and its inhibition effect decreased after three generations.

193 The above conclusions collectively suggested that strain C010 had the properties of
194 higher-yielding, broad-antibacterial and stable-subculture, which could be chosen for subsequent
195 testing.

196 (Table 3 about here)

197 **Sensitivity of antibacterial substance from the strain C010 to enzymes**

198 The antibacterial substance degraded by proteases means that they can be digested by human.
199 Table 4 showed that the CFS after the removal of organic acids still had antibacterial effect. A number
200 of studies have reported that LABs can produce a variety of antibacterial substances during
201 metabolism, of which including organic acids, hydrogen peroxide and bacteriocins dominate. Then
202 compared with the unprocessed control, the antibacterial activity of strain C010 treated with catalase

203 did not change significantly, while produced a significant decrease when treated with pepsin, trypsin,
204 proteinase K and the deactivation rate reached 56.59 %, 28.06 %, 77.73 %, respectively. These
205 findings indicated that there was almost no hydrogen peroxide in the antibacterial substances
206 produced by strain C010, but was sensitive to protease. And this feature exhibits the protein property
207 of the antimicrobial substance, and it is speculated that it may be a bacteriocin.

208 (Table 4 about here)

209 Identification of Strain

210 Through the test of morphology (plate morphology, Gram staining and scanning electron
211 microscopy), strain C010 was found to present Gram-positive and rod-shaped LAB (Fig.2). At the
212 same time, the results of physiological and biochemical measurements were compared with the
213 identification standards in Bergey's System Bacteriology Manual (The Ninth Edition)(Chen and
214 Kong 1995), and the strain C010 could be preliminarily identified as *Lactobacillus plantarum* (table
215 5). Since phenotypic features are sometimes misidentified, for further confirmation, the partial of 16S
216 rDNA (approximately 1431 bp) sequence was obtained after comparing with the sequences from
217 NCBI 's Gene Bank to confirm the strain C010, and it was found to have 100 % homology with *L.*
218 *plantarum* DSM 10667 (Fig.3). Therefore, the strain C010 was identified as *L. plantarum* C010, and
219 the GenBank access number is MW925129.

220 (Fig. 2, Fig. 3 and table 5 about here)

221 The stability of antibacterial substance from *L. plantarum* C010

222 The stability of crude bacteriocin at different temperatures and UV irradiation were shown in
223 Table 6. The crude bacteriocin of *L. plantarum* C010 maintained their original activity under high

temperature and UV irradiation conditions, which had no significant difference between any treatment and the unprocessed control ($P>0.05$), and even exposed at 121°C for 20 min. The result demonstrated that crude bacteriocin was performed excellent heat resistance and UV irradiation tolerance, which also laid the theoretical foundation for the subsequent cooperative action of bacteriocin that could be associated with other preservation methods to prolong the freshness of foods.

([Table 6 about here](#))

Growth curve and kinetic analysis of *L. plantarum* C010

Growth curve and bacteriocin-production of L. plantarum C010

As shown in Fig.4, the bacterial growth was accompanied by bacteriocin synthesis and pH reduction during the batch fermentation process. *L. plantarum* C010 entered logarithmic growth phase at the second hour, and reached stable when it fermented to 12th h. During the logarithmic growth period, the rapid growth of bacteria was accompanied with rapid bacteriocin production and glucose consumption. After 12 h fermentation, changes of biomass and glucose consumption became slowly. During the entitle fermentation, the pH dropped rapidly at initial 20 h, and eventually stabilized around 3.72. This pH is in agreement with the finding of *L. pentosus* DZ35, which bacteriocin-producing LAB fermentation pH reduced to below 4.0 ([Allende et al. 2007](#); [Lee et al. 2020](#)).

The antibacterial activity of bacteriocin appeared at 4th hour and reached maximum (15.74 mm) at 20th h. Meanly, it was produced both at logarithmic growth and stabilization phase, suggesting that the bacteriocin might include two components both primary and secondary metabolites. With the

245 growth of the bacterium, the antibacterial activity of bacteriocin decreased, which may be due to the
246 depletion of nutrients in the culture medium. At this moment, the bacterium needs to degrade proteins
247 to obtain the energy needed for its own growth.

248 (Fig. 4 about here)

249 *Kinetic model construction*

250 The kinetic model of microorganism can express the most fundamental observations related to
251 microbial growth progress, like growth of bacteria, product synthesis and substance consumption.
252 Bacterial growth requires the consumption of more substrates, during which time metabolites with
253 inhibitory effects can be synthesized. Thus, it is useful to characterize the changes in the basic
254 indicators of the bacterium by growth kinetic models. In the illustrated model, the equation for the
255 formation rates of total number of bacteria (X), synthesis of bacteriocins (P) and consumption of
256 glucose (S) are used. The meanings and units of letters and symbols are listed in the table 7 (Vázquez
257 and Murado 2008).

258 (Table 7 about here)

259 *Bacterial growth kinetics*

260 There are two models (Monod model and logistic model) to describe the growth process of
261 microorganisms. However, the Monod model expresses the growth of the bacterium in a single way.
262 Compared with this model, the logistic model can better describe the inhibitory effect along with the
263 growth of bacteria during the batch fermentation process, so it was used to determine the growth
264 kinetics in this experiment. And by integrating equation (1), the equation (2) can be obtained and
265 reflects the law of bacterial growth versus time:

$$\frac{dy}{dx} = \mu_m \left(1 - \frac{X}{X_m}\right) X \quad (1)$$

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

The maximum specific growth rate μ_m and the initial and maximum total number of bacteria X_0 and X_m can be obtained by fitting the data nonlinearly with Origin 9.0 through equation (2), and the resulting correlation R^2 was used to assess the degree of fit of the model.

Bacteriocin-producing kinetics

The products are synthesized by microorganisms in different ways during the batch fermentation process, based on the difference between primary and secondary metabolism. And the relationship between the bacteriocin production and the growth of bacteria can be divided into three types during the batch fermentation process: growth-coupled ($\alpha \neq 0, \beta = 0$), non-growth-coupled ($\alpha = 0, \beta \neq 0$) and partial growth-coupled ($\alpha \neq 0, \beta \neq 0$). As mentioned above, the bacteriocin synthesis increased during the logarithmic growth period and reached optimal level during the growth stabilization, which demonstrated the bacteriocin produced by *L. plantarum* C010 is linked to the growth of cell. Therefore, this experiment explored the Leudeking-piret equation to describe the bacteriocin synthesis.

$$\frac{dP}{dt} = \alpha X + \beta \frac{dX}{dt} \quad (3)$$

And by integrating equation (3), the equation (4) can be obtained and reflects the bacteriocin synthesis versus time:

$$P = P_0 + \alpha \left(\frac{X_m X_0 e^{\mu_m t}}{X_m - X_0 + X_0 e^{\mu_m t}} - X_0 \right) + \frac{\beta X_m}{\mu_m} \ln \frac{X_m - X_0 + X_0 e^{\mu_m t}}{X_m} \quad (4)$$

285 A nonlinear fit to the data was performed by Origin 9.0 through equation (4), which allowed the
 286 derivation of the constants α , β to determine the model for growth and product synthesis of the
 287 bacteriophage, and the resulting correlation R^2 was used to assess the degree of model fit.

288 *Substrate consumption kinetics*

289 In general, carbon source is an important component in medium for cell growth, metabolite
 290 accumulation and some cell function maintained. Its consumption can characterize the growth of
 291 bacteria. Thus, the experiment can determine the growth of *L. plantarum* C010 by measuring the
 292 content of reducing sugars. And the consumption of glucose throughout the process can be modelled
 293 using a Leudeking and piret-like equation (5).

$$294 \quad \frac{dS}{dt} = -\frac{1}{Y_x} \frac{dX}{dt} - \frac{1}{Y_p} \frac{dP}{dt} - K_e X \quad (5)$$

295 And by integrating equation (5), the equation (6) can be obtained to reflect substrate
 296 concentration versus time:

$$297 \quad S = S_0 - m \left(\frac{X_m X_0 e^{\mu_m t}}{X_m - X_0 + X_0 e^{\mu_m t}} - X_0 \right) - \frac{n X_m}{\mu_m} \ln \frac{X_m - X_0 + X_0 e^{\mu_m t}}{X_m} \quad (6)$$

298 A nonlinear fit to the data was performed by Origin 9.0 through equation (6), which obtained the
 299 substrate yields Y_x and Y_p of the bacteria and products, and the resulting correlation R^2 was used to
 300 assess the degree of model fit.

301 **Batch fermentation kinetics fitting solution**

302 *Kinetic parameters evaluation*

303 The equation (2), (4) and (6) can be evaluated to solve three major models of fermentation
 304 kinetics, respectively. The values of each parameter and the correlation coefficient R^2 can be obtained

305 by nonlinear fitting. Besides, the specific parameter values obtained from the above kinetic model are
 306 shown in [Table 8](#). After batch fermentation of *L. plantarum* C010, the growth rate of the strain could
 307 be calculated as 0.8239 (h⁻¹) by nonlinear fitting, while the predicted initial (X_0) and maximum (X_m)
 308 total number of bacteria were 2.0736 (10⁸ CFU/mL) and 214.55 (10⁸ CFU/mL), which were not
 309 significantly different from the actual fermentation. Then α and β were obtained as 0.021 8 and 0.000
 310 7 after fitting the Leudeking-piret equation, which indicated the bacteriocin showed a partial
 311 growth-coupled relationship with the growth of the bacteria. In addition, in the substrate consumption
 312 model, m and n were 0.0005 and 0.0591, respectively. Due to m less than n, it could be seen that the
 313 effect of bacteriophage growth rate on glucose consumption was much less than the effect of
 314 bacteriophage concentration on glucose consumption, and the predicted initial substrate content (S_0)
 315 was only 15.4826 g/L, which was a decrease compared to the initial added glucose (20 g/L). This
 316 finding may be the reaction of glucose occur during the sterilization process and lead to loss.

317 [\(Table 8 about here\)](#)

318 *Kinetic nonlinear fitting model equation solving*

319 The models for *L. plantarum* C010 growth kinetics (7), bacteriocin synthesis kinetics (8), and
 320 reducing sugar consumption kinetics (9) can be solved by bringing the evaluated values of the
 321 parameters in table 8 into equations (1), (3), and (5), and are shown in detail of kinetic model equation
 322 as follows.

$$323 \quad X = \frac{444.87e^{0.82389t}}{212.47+2.0735e^{0.82389t}} \quad (7)$$

$$324 \quad P = 8.0290 + 4.6382 \left(\frac{e^{0.82389t}-1}{103.4674+e^{0.82389t}} \right) + 1.8229 \ln(0.9903 + 0.0097e^{0.82389t}) \quad (8)$$

$$S = 15.4826 - 0.0005 \left(\frac{e^{0.82389t} - 1}{103.4674 + e^{0.82389t}} \right) - 15.390 \ln(0.9903 + 0.0097e^{0.82389t}) \quad (9)$$

Comparison between experimental and simulated values of nonlinear fitted curves

The non-linear fitting of the kinetic equations can obtain correlation fitting curves of the three kinetic models, which can better describe the growth and metabolic consumption of *L. plantarum* C010 (Fig. 5). Among them, the correlation coefficients R^2 of the kinetic models of bacteriophage growth (a), bacteriocin synthesis (b) and reducing sugar consumption (c) are 0.9994, 0.9104, 0.9029, respectively, which indicated that the experimental and predicted values of equations were well fitted. These results provide a theoretical basis for the subsequent study on the optimization of the liquid fermentation process of *L. plantarum* C010 and the enhancement of bacteriocin production.

(Fig. 5 about here)

Discussion

Bio-preservation is a significant approach to maintain the quality and safety of meat and meat products, which can be applied to extend the shelf-life of food (Da Costa et al. 2019). Currently, bacteriocin, has high-efficiency against microorganism at low concentration and less likely to induce resistance, which can meet the demand of human for natural preservatives. According to reports, the bacteriocin produced by Gram-positive bacteria has strong antibacterial activity, such as LABs (Mendoza et al. 1999; Sanni et al. 1999). Unfortunately, up to now, although a large number of bacteriocin-producing LABs have been developed worldwide, most of them only have strong bacteriostatic effect on Gram-positive bacteria, whereas they have less even no influence on Gram-negative bacteria (Mendoza et al. 1999; Suhartono et al. 2021).

345 In this study, 6 strains, named as J001, N109, R1, H001, C010 and H013, were isolated from
346 soils and cow dungs by using the specific dominant bacteria *P. koreensis* PS1 as the indicator, and
347 these strains were found to produce antibacterial substances other than organic acids and hydrogen
348 peroxide. This is similar to the vast majority findings reported. Chen et al. showed inhibitory
349 activity of *Lactobacillus plantarum* CKXP13 and CWXP24 against the indicator bacteria even after
350 neutralization pH and degradation of hydrogen peroxide, and these antibacterial substances were
351 confirmed as bacteriocins (Chen et al. 2021). In addition, the LABs isolated and screened from the
352 mangrove sediments of Logending Beach, Kebumen by Dyah Fitri Kusharyat, also exhibited
353 antibacterial activity after crude extraction with ammonium sulfate (Kusharyati et al. 2021).
354 Besides, the antibacterial substance produced by 6 strains all showed a wide and strong inhibitory
355 effect on bacteria and molds, especially strain C010, which is similar to the report of Abo-Amer
356 A.E., who found that bacteriocins produced by 4 *L. plantarum* screened from yogurt were strongly
357 inhibitory effects to both Gram-negative and positive bacteria (Abo-Amer 2007). Moreover, the
358 antibacterial substance produced by strain C010 remained stable antibacterial activity after 5
359 generations, demonstrating a good reference value for subsequent fermentation cultures and
360 industrial applications. Furthermore, the antibacterial substance possessed antibacterial activity after
361 elimination of organic acids and partial loss of activity after proteinases degradation such as pepsin
362 and proteinase K. These finding was also further observed in *Oenococcus oeni* CECT 217T
363 (Lasik-Kurdyś et al. 2018), which reflects its safety and also indicates its protein properties, similar
364 to bacteriocin produced by bacteria. However, different bacteriocin has different sensitivity to
365 proteases and varying degradation effects. Yasmeen YA (Elyas et al. 2015) found that the
366 antimicrobial activity of 18 bacteriocin-producing strains was completely disappeared only after

367 pepsin degradation. Instead, the bacteriocin-produced strain (T1 and T2), according to the report of
368 Luo F, were insensitive to pepsin, but could lost antibacterial activity after treatment with proteinase
369 K and trypsin (Luo et al. 2011). Yet, Todorov S D (Todorov and Dicks 2006) concluded that three
370 major proteases showed significant degradation of the metabolites of LABs screened from boza. To
371 date, most LABs are more easily degraded by pepsin, which can inhibit 90 % or more of the
372 bacteriocin produced by LABs isolated from meat and meat products (Bromberg et al. 2004).

373 The bacteriocin produced by strain C010, maintained its original antimicrobial activity under
374 heat treatment and UV irradiation conditions, even exposure to 121°C for 20 min. The capability of
375 heat resistance was similar to that of bacteriocins synthesized by lactic acid bacteria such as
376 *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus brevis*, et al. (Ohenhen et al.
377 2015), *L. pentosus* DZ35 (Yi et al. 2020) and *L. plantarum* AMT11 (Thirumurugan et al. 2018).
378 Based on the heat resistance, the bacteriocin of *L. plantarum* C010 can be classified as heated stable
379 class (class I or class II), and this thermal stability has typical excellent properties for food
380 preservation during high temperature processing (Sifour et al. 2010). Instead, the stability of UV
381 irradiation allows bacteriocin to work synergistically with UV irradiation for food freshness
382 preservation and can reduce the requirements and costs of preservation, which promotes the
383 development of the food preservation in the industry.

384 Fermentation kinetics has wide application and important guiding significance during the
385 fermentation process. Ziadi *et al.* provided a reliable regulatory strategy and means for efficient
386 lactic acid synthesis by studying the kinetic characteristics of wholesale fermentation lactic acid
387 production by *Enterococcus faecalis* SLT13 in M17 and cheese whey medium, respectively (Ziadi
388 et al. 2019). In this research, bacteriocin synthesis of *L. plantarum* C010 was studied by fractional

389 fermentation kinetics, and a relevant fermentation kinetic model, and the basic rules of growth and
390 metabolism of the bacterium were established and figured out by fractional fermentation
391 experiments. The correlation coefficients R^2 of the three major simulation curves obtained by
392 kinetic curve fitting were all greater than 0.9, indicating that the experimental and predicted values
393 were well fitted and could better evaluate the bacterial growth, product synthesis and substrate
394 consumption. Simultaneously, the parameters of Luedeking-Piret model can be calculated that $\alpha \neq 0$
395 and $\beta \neq 0$, indicating that bacteriocin synthesis and strain growth were partially growth-coupled,
396 which also coincided with the fermentation process curve, which can reflect the bacteriocin
397 fermentation and be used to analyze the bacteriocin production process of *L. plantarum* C010
398 wholesale fermentation. This can accumulate experience for the subsequent fine control of
399 fermentation process, process improvement, target product accumulation and subsequent batch
400 replenishment model establishment.

401 In conclusion, *L. plantarum* C010 has broad-spectrum bacterial inhibition and stable
402 subculture, while its production of inhibitory substances is sensitive to proteases and tolerant to heat
403 and UV irradiation. Meanwhile, the growth kinetics of the strain provided insights into the
404 properties of bacteriocin-producing *L. plantarum* C010. All findings provide insights into
405 understanding the properties of bacteriocin-producing *L. plantarum* C010. And the antibacterial
406 mechanism of *L. plantarum* C010 against foodborne spoilage organisms will be investigated in the
407 further study. This also provides a theoretical basis for the application of preserving chilled pork
408 and its products.

409 **Acknowledgments** The author thanks the teacher (Dr. Lin Huang) and colleagues who provided many
410 stimulating discussions and help on this experiment. And also gratefully acknowledge the financial support

411 provided by the Natural Science Foundation of China (Grant No. 31560482), the Graduate student research
412 Innovative Project of Jiangxi Higher Education Institutions (Grant No. YC2020-S250).

413 **Author contribution statement** Jinyue Dai and Limin Fang participated in experimental design, experiment
414 performed, data analysis and wrote the manuscript. Manmin Zhang and Huaili Deng performed the experience
415 and collect the data. Xin Cheng and Minyin Yao participated in the grammar revision of this manuscript, and
416 Lin Huang adjusted the overall framework of the manuscript, full text content and grammar modification.

417 **Availability of data and material** Data will be made available on reasonable request.

418 **Declarations**

419 **Conflict of interest** The authors declare that there is no conflict of interest.

420 **Ethical approval** This article does not contain any studies with human participants or animals performed by
421 any of the authors.

422 **Reference**

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 543

544 **Figure and table captions**

545 **Fig. 1** The standard curve of glucose

546 **Fig. 2** The colony morphology of the strain C010 on the MRS plate (a), the cell
547 morphology under the optical microscope (1000×) (b) and scanning electron
548 microscope (12000×) (c)

549 **Fig. 3** The phylogenetic tree of the strain C010 based on 16S rDNA sequence

550 **Fig. 4** Growth curve and the bacteriocin production of *L. plantarum* C010 in batch
551 fermentation. Note the differences in the time scale on the x-axis and also the
552 variation in the y-axis scale showing the data. Among them, ● represent pH, ■
553 represent total bacteria count ($\times 10^8$ CFU/mL), ▲ represent reducing sugar content
554 (mg/mL) and the bar chart represent diameter of inhibition zone (mm).

555 **Fig. 5** The fitting curve model of growth kinetics (a), bacteriocin synthesis (b) and
556 substrate consumption (c) of *L. plantarum* C010 during batch fermentation process.

557 **Table 1** Isolation and screening of bacteriocin-producing LABs

strains	Diameter of inhibition zone (mm)	
	Exclusion of organic acid	Crude extract of ammonium sulfate
	interference	precipitate
R1	12.00±0.36	13.27±0.07
C010	12.12±0.89	14.64±0.30
N109	12.25±0.88	12.01±0.53
J001	11.92±0.83	12.24±0.33
C001	8.18±0.21	11.54±0.54
H011	8.32±0.21	11.89±1.16
MRS	-	-
Lactic acid at pH 5.5	-	-
acetic acid at pH 5.5	-	-
hydrochloric acid at pH 5.5	-	-

558 (-) present no antibacterial activity

559 **Table 2** Broad-spectrum determination from 6 LABs

Indicator strains	Diameter of inhibition zone (mm)					
	R1	C010	N109	J001	C001	H011
<i>Serratia plymuthica</i> Z2	11.36±0.82 ^{AB}	12.86±1.07 ^A	11.38±1.67 ^{AB}	11.11±1.33 ^B	12.47±0.60 ^{AB}	12.81±0.19 ^A
<i>Proteus penneri</i> Z3	11.25±1.16 ^{AB}	10.67±1.43 ^{AB}	10.94±0.63 ^{AB}	10.08±1.36 ^B	11.62±0.30 ^{AB}	13.27±0.00 ^A
<i>Bacillus fusiformis</i> J4	16.14±1.40 ^{AB}	17.26±1.23 ^A	16.33±0.63 ^{AB}	15.12±0.96 ^{BC}	15.82±0.38 ^{AB}	16.46±0.55 ^{AB}
<i>Escherichia coli</i> ATC-1	13.03±1.44 ^{ABC}	14.09±1.20 ^A	11.97±0.67 ^{CD}	12.31±1.13 ^{BCD}	11.62±0.26 ^D	13.34±0.38 ^{AB}
<i>Bacillus subtilis</i> KC-08	18.78±0.74 ^C	23.22±1.20 ^A	19.19±0.68 ^C	19.04±1.10 ^C	18.66±1.13 ^C	20.95±1.44 ^B
<i>Staphylococcus aureus</i> C013	17.49±0.87 ^{AB}	18.48±0.42 ^A	17.08±2.39 ^{AB}	17.16±1.90 ^{AB}	14.68±0.34 ^C	15.53±0.48 ^{BC}
<i>Bacillus putida</i> BP-01	13.70±0.61 ^{BC}	15.22±0.66 ^A	13.15±0.42 ^{BC}	12.54±0.34 ^C	15.50±0.22 ^A	13.70±0.28 ^{BC}
<i>Bacillus amyloliquefaciens</i> JDF 002	20.39±1.45 ^{ABC}	21.46±1.61 ^{AB}	22.01±1.83 ^A	21.41±1.71 ^{AB}	19.51±0.66 ^{BC}	20.00±0.93 ^{ABC}
<i>Aspergillus niger</i> HQM-1	15.73±1.17 ^{BC}	16.85±1.08 ^B	14.54±0.52 ^C	14.53±0.48 ^C	18.22±0.14 ^A	16.56±0.89 ^B
<i>Penicillium italicum</i> ACCC 30399	18.11±0.15 ^{AB}	18.98±0.02 ^{AB}	18.05±1.18 ^{AB}	18.51±0.16 ^{AB}	18.16±1.54 ^{AB}	15.95±1.01 ^B
<i>Penicillium citrinum</i> PA-33	16.17±0.46 ^A	17.17±0.53 ^A	16.51±0.59 ^A	17.09±0.29 ^A	17.80±1.22 ^A	18.11±1.03 ^A
<i>Rhodotorula mucilaginosa</i> TJ1-2	-	-	-	-	-	-
<i>Saccharomyces cerevisiae</i> ATCC-5	-	-	-	-	-	-

560 ^{A-D} are compared horizontally and indicates analysis of significant differences in the inhibition of the same
561 indicator bacteria by different isolates (P<0.05).

562 (-) represent no inhibit activity.

563 **Table 3** The antibacterial effect on *P. koreensis* PS1 by subculture of 6 LABs

Strains	Diameter of inhibition zone (mm)					
	F0	F1	F2	F3	F4	F5
R1	12.35±0.22 ^a	12.22±0.56 ^a	11.95±0.87 ^a	12.11±0.66 ^a	11.32±1.37 ^a	11.52±0.77 ^a
C010	14.52±0.15 ^a	14.29±1.09 ^a	14.46±0.97 ^a	14.25±0.58 ^a	14.83±0.33 ^a	14.05±0.68 ^a
N109	12.48±0.68 ^{ab}	12.47±0.56 ^{ab}	10.94±0.82 ^c	11.93±0.58 ^b	12.93±0.42 ^a	10.84±0.56 ^c
J001	11.89±0.23 ^a	11.44±0.98 ^a	10.72±0.76 ^{ab}	11.18±0.89 ^{ab}	10.41±0.76 ^{ab}	10.14±0.33 ^b
C001	12.12±0.81 ^a	12.20±1.68 ^a	10.86±0.46 ^b	11.59±1.86 ^{ab}	11.89±0.87 ^{ab}	ND
H011	11.34±0.36 ^a	11.13±1.13 ^a	11.83±1.13 ^a	10.99±0.94 ^{ab}	9.99±0.36 ^b	ND

564 (ND) represents no experiment.

565 ^{a-c} Represent differences in bacteriostatic activity between generations of fermentation medium compared by
566 levels.

Table 4 Preliminary determination of antibacterial substances in the supernatant of the strain C010

Protease treatment	Diameter of inhibition zone (mm)	Deactivation rate (%)
unprocessed	18.55±0.59 ^a	100
CFS (pH 5.5)	12.12±0.89	60.95
MRS Medium (pH 5.5)	-	-
Lactic acid (pH 5.5)	-	-
Acetic acid (pH 5.5)	-	-
HCl (pH 5.5)	-	-
Treatment with catalase	17.99±0.24 ^a	0.05
Treatment with pepsin	12.58±0.68 ^c	56.59
Treatment with trypsin	15.59±0.60 ^b	28.06
Treatment with proteinase K	10.35±0.78 ^d	77.73

^{a-d} represent the significant difference analysis between the fermentation supernatant after each enzyme treatment and the fermentation supernatant without any treatment (p<0.05).

571 **Table 5** Physiological and biochemical test of the strain C010

Experiment project		The strain of C010
Gram strain		+
Catalase		-
Shape	On the tablet	Wet, precipitated, opaque, white colonies, smooth and neat edges
	Microscope Observation	rod
Carbohydrate Fermentation	Mannitol	+
	Xylose	+
	Fructose	+
	Sucrose	+
	Glucose	+
	Sorbitol	+
	V-P	-
	Indole	-
Safety measurement	Arginine ammonia production	-
	Hydrogen sulfide	-
	Pigment	-
	Gas production	-
Identification based on 16s rRNA sequencing (100 % identity)		<i>Lactobacillus plantarum</i> DSM 10667

572 (-) present positive and (+) present negative

573 **Table 6** The antibacterial effect on *P. koreensis* PS1 of the crude substance of *L.*

574 *plantarum* C010 by UV irradiation and thermal treatment

Experimental treatment		Diameter of inhibition zone (mm)	P value
unprocessed	Unprocessed	14.20±0.47	
	60 °C 30 min	14.15±0.54	0.8929
Heat treatment	80 °C 30 min	13.82±0.40	0.2648
	100 °C 30 min	13.71±0.44	0.1829
	121 °C 20 min	13.75±0.58	0.2730
	5 min	14.67±0.55	0.2426
UV irradiation treatment	10 min	14.74±0.35	0.1116
	20 min	14.00±0.26	0.4875
	30 min	14.32±0.52	0.7430
	60 min	14.08±0.60	0.7674

575 **Table7** The symbolic notation of each unit

The units	The symbolic notation of unit
X_0	The initial total number of bacteria (10^8 CFU/mL)
$X_{\max}$	The maximum total number of bacteria (10^8 CFU/mL)
μ_m	The rate of growth (h^{-1})
t	Time (h)
α	The parameter based on Luedeking (Product synthesis constants associated with bacteriophage growth)
β	The parameter based on Luedeking (Product synthesis constants related to the growth rate of the bacteriophage)
P	The synthesis of antibacterial activity of bacteriocin (mm)
S	The consumption of glucose (g/L)

576 **Table 8** Estimated value of batch fermentation kinetic parameters

Parameters	Assessed Value	Parameters	Assessed Value	Parameters	Assessed Value
μ_m (h ⁻¹)	0.8239±0.0417	P_0	8.0290±0.4684	S_0	15.4826±0.9150
X_0 (10 ⁸ CFU/mL)	2.0736±0.5107	α	0.0218±0.0042	m	0.0005±0.0081
X_m (10 ⁸ CFU/mL)	214.55±0.8075	β	0.0007±0.0003	n	0.0591±0.0005

577 **Fig.1**

578

579 **Fig. 2**

580

581

(a)

(b)

(c)

582 **Fig. 3**

583

584 **Fig. 4**

585

587

588

589

Figures

Figure 1

The standard curve of glucose

Figure 2

The colony morphology of the strain C010 on the MRS plate (a), the cell morphology under the optical microscope (1000×) (b) and scanning electron microscope (12000×) (c)

Figure 3

The phylogenetic tree of the strain C010 based on 16S rDNA sequence

Figure 4

Growth curve and the bacteriocin production of *L. plantarum* C010 in batch fermentation. Note the differences in the time scale on the x-axis and also the variation in the y-axis scale showing the data. Among them $\square$ represent pH, $\bullet$ represent total bacteria count ($\times 10^8$ CFU/mL), $\triangle$ represent reducing sugar content (mg/mL) and the bar chart represent diameter of inhibition zone (mm).

Figure 5

The fitting curve model of growth kinetics (a), bacteriocin synthesis (b) and substrate consumption (c) of *L. plantarum* C010 during batch fermentation process.

Supplementary Files

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- [GraphicalabstractofMS.pdf](#)
- [Highlights.doc](#)