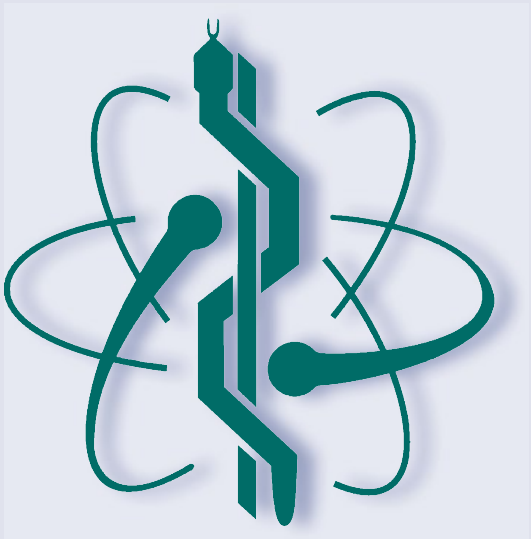
IFMBE Proceedings

Vo Van Toi · Thi-Hiep Nguyen · Vong Binh Long ·
Ha Thi Thanh Huong Editors

Volume 85

8th International Conference on the Development of Biomedical Engineering in Vietnam

Proceedings of BME 8, 2020, Vietnam: Healthcare Technology for Smart City in Low- and Middle-Income Countries



Springer

Isolation of Antimicrobial Probiotic Bacteria from Sour Shrimps in Hue City-Vietnam and Optimization for Biomass and Acid Production

Hoang Thi Kim Hong, Nguyen Thi Thu Thuy, Vo Thi Bich, and Nguyen Thi Ngoc Hanh

Abstract This study isolated antibacterial probiotic strains from sour shrimp, a Vietnamese traditional fermented food, and optimized culturing conditions for the chosen strain. Strain V101 was chosen for optimization experiments for its high antagonistic activity and production of strong antibacterial compounds against indicator harmful bacteria – *Escherichia coli*, and *Staphylococcus aureus*. Molecular identification of 16S rRNA sequencing showed that V101 strain was *Lactobacillus paracasei*. Optimization of culturing conditions found that this strain produces the highest biomass and acid accumulation on modified MRS at pH 5.5; temperature 37 °C in 60 h. Under optimum condition, biomass and acid production increased 76.61% and 10.69% respectively, and antibacterial activities against *E. coli* improved up to 20%. Pilot fermentation of L. *paracasei* V101 on production Vietnamese pickles, sour shrimp showed that the fermentation process was quicker, tastier, and fermented products could be preserved longer. These findings will be a potential prerequisite for further study on *L. paracasei* V101 as a strain not only good for food fermentation and preserving but also a helpful probiotic bacteria bring benefits to human health.

Keywords Antibacterial · *Lactobacillus paracasei* · Probiotics · LAB

1 Introduction

Lactic acid bacteria (LAB probiotics) are well-known for their benefits on health for their application as probiotics – good bacteria for the human digestive system and a popular bacteria for fermentation [1]. A wide range of antimicrobial compounds (organic acids, hydrogen peroxide, diacetyl, low molecule weight compounds, and bacteriocin) enable probiotics to inhibit both Gram (+) and Gram (–) bacteria [2].

H. T. K. Hong (✉) · N. T. T. Thuy · V. T. Bich
Hue University of Sciences, Hue University, Ho Chi Minh City, Vietnam
e-mail: hkhong@hueuni.edu.vn

N. T. N. Hanh
Hong Bang International University, Ho Chi Minh City, Vietnam

V. Van Toi et al. (eds.), *8th International Conference on the Development of Biomedical Engineering in Vietnam*, IFMBE Proceedings 85,
https://doi.org/10.1007/978-3-030-75506-5_55

Among organic acids produced by probiotics, lactic acid was produced in a noticeable amount contributing to antimicrobial activities. On the other hand, bacteriocins from LAB Probiotics were well-known for their antimicrobial activities. Bacteriocins' antimicrobial mechanisms were first studied on Nisin, a bacteriocin Gram (+). They can kill bacteria due to the capacity of changing membrane permeability by forming channels on bacteria's membrane. Moreover, bacteriocins can break down DNA and RNA and weaken the peptidoglycan layer of cell walls. These antimicrobial activities make it possible to extend the shelf life of fermented products without adding artificial preservatives [3–5].

Fermented products, such as (yogurt, sour cherry, pickled noodles, sour bamboo shoots, and sour shrimp, etc.) represented an irreplaceable part of Vietnamese cuisine. However, the quality of fermented foods fluctuated from batch to batch due to microorganisms in raw materials and the surrounding environment; skills, and workmanship of ones carrying out the fermentation process. Moreover, food preservatives added to extend the preservation period could cause bad impacts on consumer's health [1]. As a result, there was an urgent need for microorganisms to perform high antimicrobial activities against rotting microbes to improve fermented food quality and extend preservation time.

In this study, we isolated high antimicrobial probiotics strains from traditional fermented products. Optimal culture conditions for isolated strains would be screened and applied to the production of traditional fermented products.

2 Materials and Methods

2.1 Materials

A number of 25 samples of sour shrimps were collected in Hue City, Vietnam. *E. coli, S. aureus*, from the Department of Applied Biology, Faculty of Biology, University of Science, Hue University, Vietnam, were used as indicator strains for screening antimicrobial activity of isolated LAB probiotics strains.

2.2 Isolation of Potential Probiotics Strains

An amount of 100 μL of serial dilutions 10^{-1}–10^{-5} of sour shrimp samples was widely spread on MRS agar plates, followed by 48 h incubation at 30 °C. Separated colonies forming a surrounding clear zone would be transferred onto slant agar for keeping stock at 4 °C [6].

2.3 Primary Screening for Potential Probiotics Strain

Isolated colonies were transferred to $CaCO_3$ added MRS agar and incubated for 60 h at 30 °C. Deduction of the size of inoculum (d) from the resolution zone (D) represented the total acid production abilities of probiotics. Strain producing the highest total acid would be selected for the next experiments.

2.4 Screening for Probiotics Strains Strongly Inhibiting Indicator Bacteria

Bacteria isolates were transferred to liquid MRS medium and cultured at 30 °C for 48 h, centrifuged at 7000 rpm to obtain supernatant to apply to antibacterial assays.

2.5 Agar-Well Diffusion Assay

Indicator strains were mixed with a nutrient agar at 50 °C and shaken well before pouring into plates. An amount of 50 μL of supernatant collected from each isolate would be pumped into 10 mm wells on plates. Plates were incubated at 4 °C for 12 h in the fridge and followed by 18–24 h incubation at 35 °C. Diameters of inhibition zones were calculated as a deduction of diameters of the wells (d) from diameters of inhibition areas (D) [7].

2.6 Dual Culture Overlay Assay

LAB probiotics strains were streaked on MRS plates and incubated for 48 h. Then, the indicator strains were cultured in a nutrient agar medium at 50 °C and shaken well before overlaying MRS agar. Plates were incubated at 4 °C for 12 h in the fridge followed by a period of 18–24 h incubation at 35 °C. Diameters of inhibition zones were calculated as a deduction of diameters of colonies (d) from diameters of inhibition areas (D) [8].

2.7 Molecular Identification of Potential Strain

The probiotics strain named V101 chosen from the previous experiment was identified by 16s rRNA sequencing. After being observed for morphology, it was sent to

Nam Khoa Service and Trading Co., Ltd. to analyze 16s rRNA sequence and aligned sequences on Blast Search [9].

2.8 Optimization of Culture Conditions for V101 Growth and Total Acid Production

V101 strain was cultured in MRS broth with 10% culturing rate and shaken at 120 rpm. Optimum culturing conditions found from previous experiments would be applied to later on optimization experiments. Biomass and total acid production of the culture would be evaluated. Firstly, incubation time were optimized from 12; 24; 36; 48; 60; 72; 84; 96 h. Then initial pH were optimized: 4.0; 4.5; 5.0; 5.5; 6.0; 6.5; 7.0; 7.5; 8.0; next to NaCl concentrations 3; 4; 5; 6; 7; 8%. Optimum carbon sources were screened on mannitol, lactose, glucose, CMC, starch, molasses, and saccharose. Finally, KNO_3, NH_4NO_3, peptone, meat extract, gelatin, urea were used to screen for optimal Nitrogen sources for V101 culturing. Mc Ilvaine buffer was used for preparing medium and adjusting pH.

2.9 Growth and Antimicrobial Activities of Probiotics Strain Under Optimal Culture Conditions

V101 strain was incubated under optimal culture conditions found from previous experiments to evaluate biomass, total acid accumulation, and antimicrobial activities.

2.10 Building the Biomass Standard Curve of V101 Strain

An amount of 1 g of biomass collected from 72 h culture of V101 in MRS by centrifugation at 4000 rpm for 10 min was desiccated to constant weight. The absorbance of serial dilutions from another 1 g of biomass in sterile water was measured at 600 nm. Linear regression equation between optical density: dry biomass (g/L) was plotted by Microsoft Excel 2016 [10].

2.11 Quantitative Determination of Total Acid Concentration by Titration

After having centrifuged, 10 mL of each supernatant was transferred to a flask, then 20 mL of distilled water and 1–2 drops of phenolphthalein was added to the flask for a titration with NaOH 0.1 N. The amount of total acid in 10 mL of the supernatant was equivalent to the multiplication of the volume of NaOH 0.1 N in mL used in titration with 0.009 (1 mL of NaOH is equal to 0.009 g of total acid) [11].

2.12 Data Analysis

All experiments were triplicated and analyzed by Excel 2016 and SPSS with $p < 0.05$.

3 Results and Discussions

3.1 Isolation Potential Probiotics Strains

Bacterial densities in 25 studied samples were significantly different, from 10.71×10^6 to 43.47×10^6 CFU/mL. Around 110 LAB Probiotics strains, named from V1, V2, V3 … to V110, were isolated. Most colonies were 0.5–1.5 mm in diameter; round shape; smooth surface; round edge or lobe edge; opaque, white, cream, or milky color. Some colonies were 2–3 mm, round, smooth, opaque, or milky. There were only a few large colonies with a diameter of around 3.5–4 mm; round shape; opaque or clear white; irregular and dry edge. Among 110 isolates, 20 strains with large resolution lines were selected for the next experiments, including V9, V11, V21, V37, V38, V40, V48, V50, V51, V52, V54, V55, V62, V76, V78, V95, V96, V101, V103, and V104. Results of screening for the antimicrobial activities of selected strains by diffusion method using indicator bacteria were shown in Table 1.

Table 1 demonstrated that most strains were assumed to produce antimicrobial compounds as they could inhibit indicator bacteria. V52 and V101 performed outstanding capability of producing antimicrobial compounds and stronger inhibitory activities than other strains. Moreover, the V101 strain showed higher inhibitory ability against *Salmonella* than V52. Thus, it was selected for further experiments.

Table 1 Sizes of inhibition zones

Strain	Agar-well diffusion assay		Dual culture overlayer assay	
	E. coli	*E. coli*	*S. aureus*	*S. aureus*
V9	54.5 ± 0.3	31.5 ± 0.5	–	9.1 ± 0.8
V11	43.5 ± 0.3	–	27.5 ± 0.5	–
V21	–	–	–	10.5 ± 0.1
V37	65.5 ± 0.2	22.8 ± 0.1	32.5 ± 0.3	–
V38	37.4 ± 0.6	–	–	–
V40	34.5 ± 0.9	–	–	19.5 ± 0.4
V48	61.3 ± 0.2	–	28.8 ± 0.8	23.8 ± 0.1
V50	72.0 ± 0.5	33.5 ± 0.5	12.0 ± 0.1	25.3 ± 0.9
V51	31.0 ± 0.8	–	–	11.6 ± 0.2
V52	72.8 ± 0.6	36.7 ±.0.8	31.5 ±.0.5	32.5 ± 0.5
V54	–	–	–	11.8 ± 0.3
V55	37.0 ± 0.1	12.5 ± 0.6	–	–
V62	–	–	–	–
V76	29.5 ± 0.1	–	–	–
V78	50.5 ± 0.7	–	16.2 ± 0.7	–
V95	–	28.0 ± 0.5	–	30.5 ± 0.1
V96	21.2 ± 0.8	–	–	–
V101	63.3 ± 0.7	33.5 ±.0.3	29.6 ±.0.4	29.2 ± 0.1
V103	14.3 ± 0.9	–	–	–
V104	35.5 ± 0.7	–	22.7 ± 0.5	–

3.2 Colony Morphological Analysis and Sequencing 16S rRNA of V101 Strain

Colony morphological analysis. V101 strain was cultured on MRS plates in 48 h. Morphology of colonies was observed. V101 colonies were convex, 1.0–1.5 mm in diameter, ivory-white. Gram staining showed that V101 was a Gram-positive strain. Under the microscope, V101 cells were scattered, long rod-shaped (Fig. 1).

Sequencing analysis. Sequencing analysis of 16 s rRNA, shown in Fig. 2 was carried out in Nam Khoa Service and Trading Co., Ltd. Molecular identification resulted was analyzed similarities with sequences in Genbank by BLAST.

It was found that the V101 16S rRNA sequence having 99% similarity with *Lactobacillus paracasei* Strain IIA, ID CP014985.1. Thus it was identified as *L. paracasei* (Table 2).

In Thai fermented shrimp or Kung-Som, isolated LAB strains were *L. futsaii* CS3 and *L. futsaii* CS5 [12]. LAB Probiotics strain found on meat traditional product

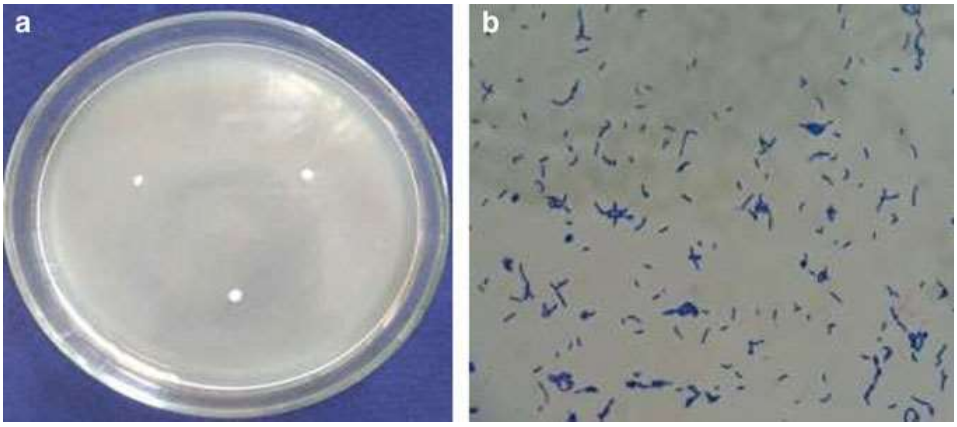


Fig. 1 **a** V101 colonies on MRS plates. **b** V101 Gram staining under the microscope

of Portugal was *Lactobacillus sakei* [13]. *L. plantarum* was used to ferment and improve the nutritional values of fermented silage from Korea [14]. *Lactobacillus futsaii* CS3 was found as the main fermentation bacteria for Kung-Som (a Thailand fermented shrimp) [12]. Potential LAB probiotics isolated from Nem Chua, another Vietnamese fermentation food were found as *Lactobacillus plantarum* and *Pediococcus pentosaceus* [1]. It was found that *L. plantarum* could prevent the occurrence of yeast, molds, and other harmful microorganisms in silage. It was also proven as a good probiotic strain for its characteristics including bile salt tolerance, non-virulent and non-hemolysis characteristics, high survival rate, or ability to grow in low pH from 2.5 to 4.0 [15].

3.3 Biomass Standard Curve

The standard curve of biomass was built against biomass and OD at 600 nm. The graph was plotted in Fig. 3. From the standard curve, R^2 is 0.9988, which is near 1. Thus this standard curve was reliable to be used for interpreting data from OD to biomass content.

3.4 Optimization of Appropriate Culturing Conditions for Bacterial Growth and Total Acid Accumulation

Factors affecting the fermentation process including incubation time, pH, NaCl concentrations, carbon sources, and nitrogen sources were optimized. Overall, biomass accumulation and acid production correlated with each other in which they peaked up at the same culturing conditions in 4 out of 5 optimization experiments.

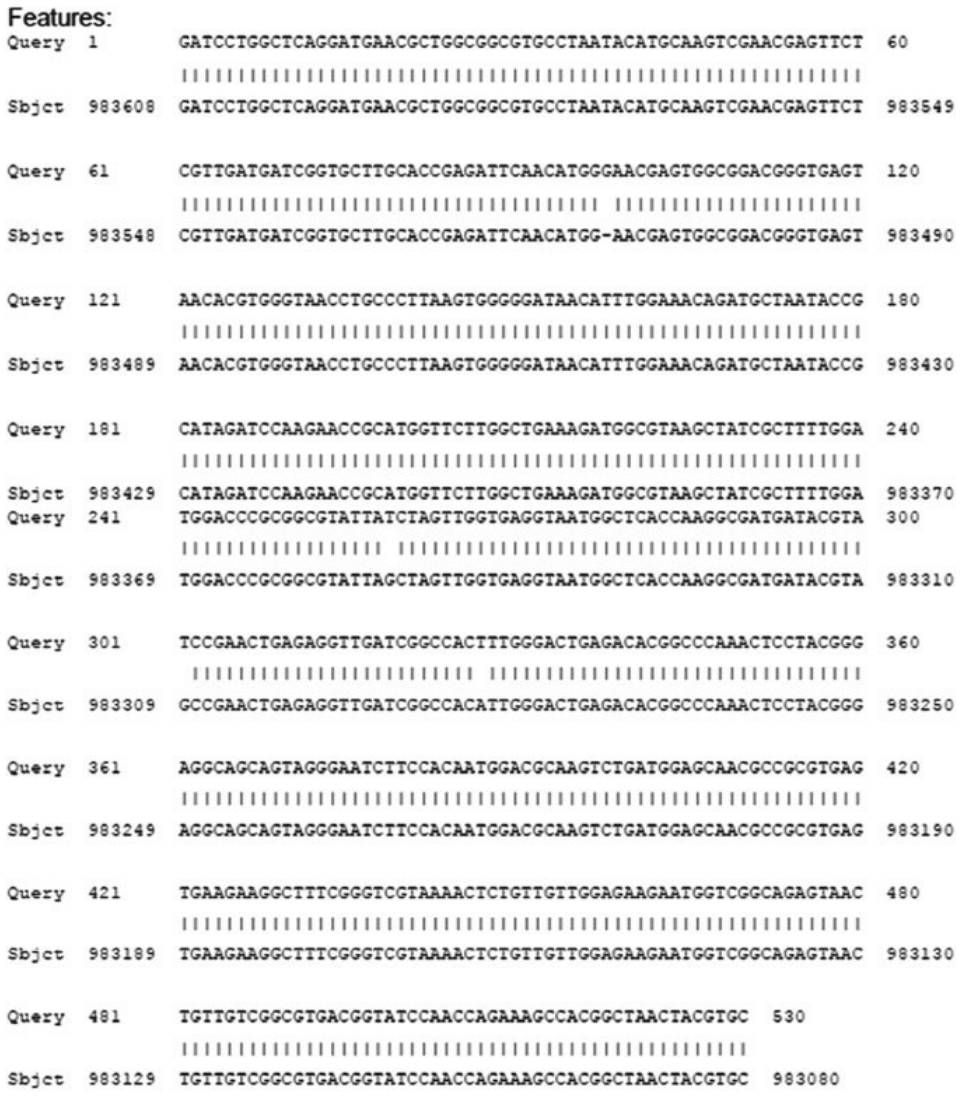


Fig. 2 16S sequence of V101 strain

Table 2 Identification of V101 strain

Species name	Strain	ID	Similarity
Lactobacillus paracasei	IIA	CP014985.1	99%

Effects of fermentation time. Firstly, the effect of incubation time was screened from 12 to 96 h. Both biomass and acid production rose from 0.65 and 10.17 g/L to their peaks at 60 h – biomass 2.01 g/L and acid content 17.25 g/L then gradually declined. As a result, 60 h was chosen as the optimum incubation time for V101 for further experiments (Fig. 4).

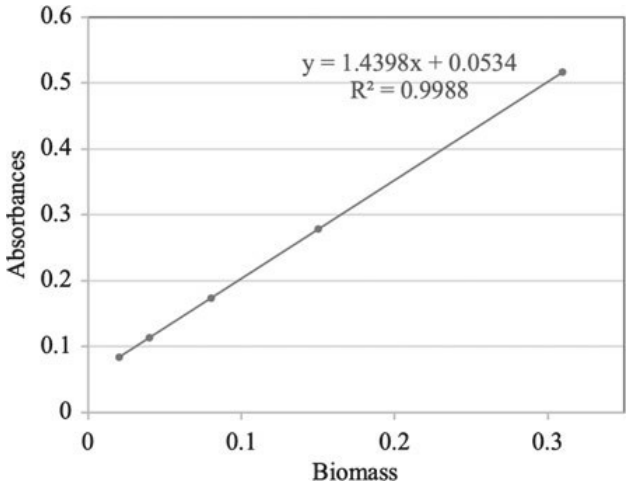


Fig. 3 Biomass standard curve of V101

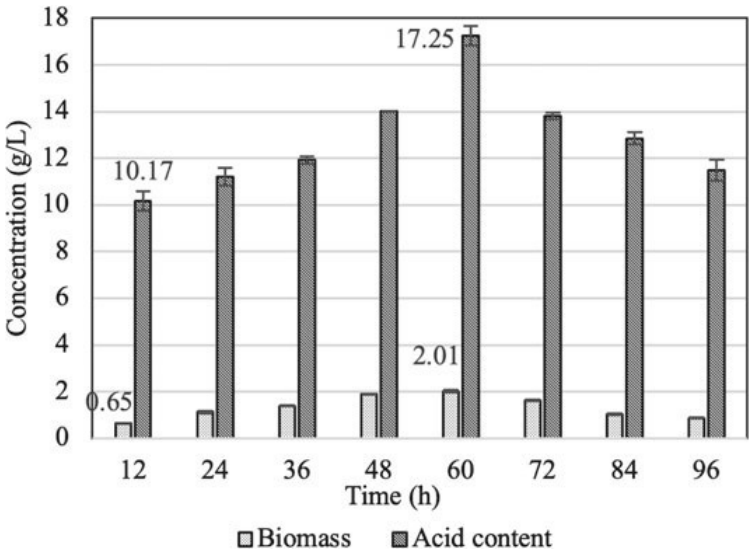


Fig. 4 Effects of fermentation time

In a previous study, it took only 19 h for LAB probiotics *Lactobacillus sakei* to produce the highest bacteriocin ST22Ch at 1600AU/mL and remained unchanged for the next 5 h before dropping down [13].

Effects of pH. Biomass and acid content were optimized against initial pH, from acidic conditions to slightly alkaline of pH range – from 4 to 8. Biomass accumulation, acid content produced by V101 strain climbed up to a high at pH 5.5, with biomass at 17.46 g/L and acid content at 2.31 g/L. pH higher than 5.5 then reduced biomass and acid accumulation of V101, to a low at pH with biomass at 9.66/L and acid content at 0.69 g/L. Hence, pH 5.5 was chosen as optimum pH and would be applied for the next experiments (Fig. 5).

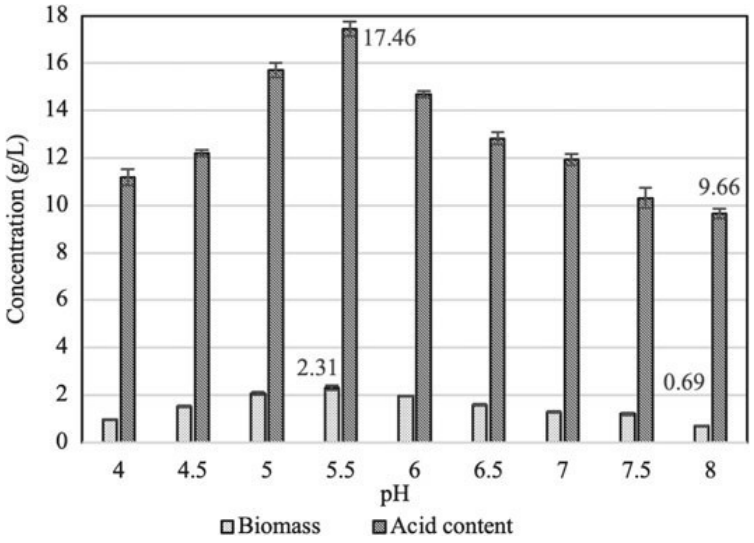


Fig. 5 Effects of pH

It was reported that LAB probiotics could develop, grow, and accumulate acid in a wide range of pH. pH 5.5 was found as the optimum pH for LAB probiotics. *L. sakei* from meat traditional product produced the highest bacteriocin at pH 4 [13], lower than results found in this study. On the other hand, the optimum pH for bacteriocin LAB probiotics was 6.2–8.5 [10].

Effects of NaCl. NaCl concentration did affect growth and acid production of V101 strain and the effect of its on V101 was studied from 3 to 8%. While biomass fluctuated, acid content performed an old pattern of rising to a peak then declined. However, they still exhibited correlation when both peaked at 6% of NaCl – 17.64 g biomass/L and 2.36 g acid/L. NaCl concentration of 8% yielded minimal growth and acid content, 9.66 g/L, and 1.22 g/L, respectively. As a result, 6% of NaCl was chosen as an optimum NaCl concentration.

It was reported that *Lactobacillus plantarum* from the shrimp gut performed great antibacterial activities against many harmful bacteria. It produced the highest bacteriocin at 0.9% NaCl [16], less than 1/6 of optimum NaCl concentration found in this study. Strain V101 here was isolated from quite salty fermented food such as Vietnamese pickles, sour shrimps. Thus this strain might prefer a higher NaCl concentration for its growth (Fig. 6).

Effects of carbon sources. Effects of various carbon sources on growth and acid production of V101 were screen on a medium containing mannitol, lactose, glucose, CMC, starch, molasses, and saccharose (Fig. 7).

Glucose beat other carbon sources to induce the best growth and acid production of the strain, with 3.08 g biomass/L and 18.21 g acid/L. CMC was the worst studied carbon source, yielded 4.13 g/L for acid content, only less than ¼ of that with medium prepared by glucose, and biomass only 1.68 g/L – nearly a half of that in medium

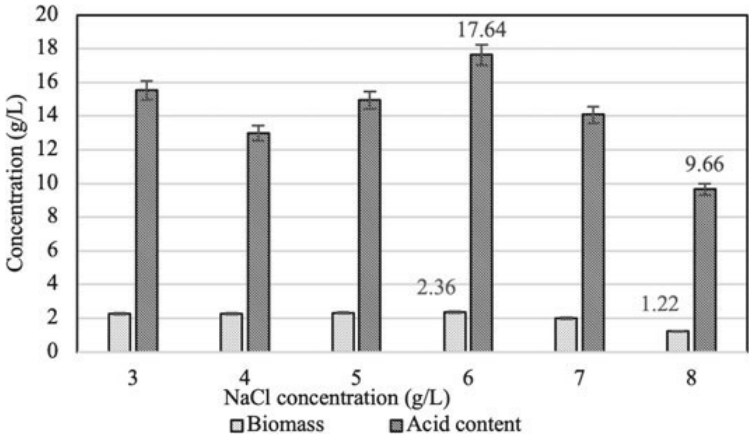


Fig. 6 Effects of NaCl concentrations

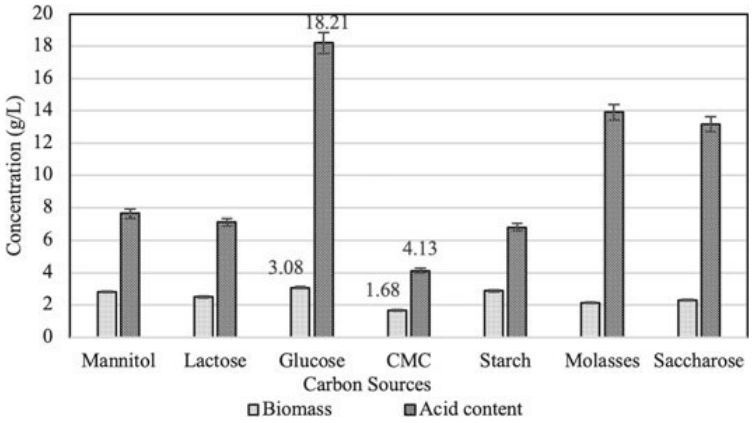


Fig. 7 Effects of carbon sources

with glucose. It could be explained that glucose was simple and ready to use carbon source as it was a monosaccharide and the main material for many main biochemical reactions in the cells.

CMC, a kind of cellulose, on the other hand, was not only a polysaccharide but also has a low conversion rate or slow breaking down reaction speed. *L. sakei* from meat traditional product produced the highest bacteriocin – 1600AU/mL on medium containing glucose, fructose, lactose, and saccharose [13]. This finding consistent with our results, as LAB PROBIOTICS, tended to use simple carbon sources as monosaccharides and disaccharides for its growth. All in all, glucose was chosen as an optimum carbon source for growing V101 for outstanding biomass and acid yield.

Effect of nitrogen sources. The effects of various nitrogen sources on growth and acid production of V101 were screened on a medium containing KNO_3, NH_4NO_3, peptone, meat extract, gelatin, and urea (Fig. 8).

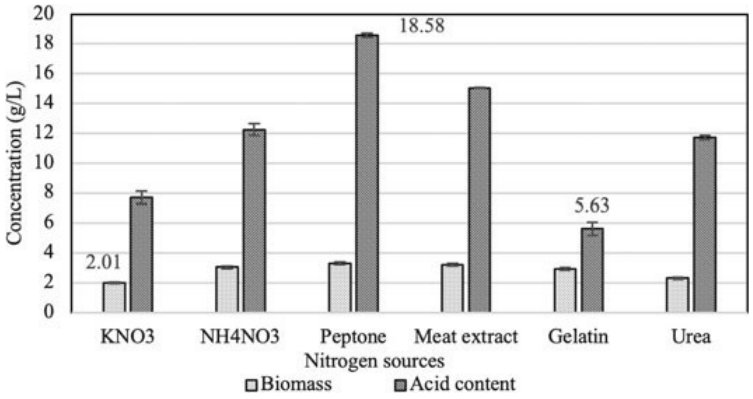


Fig. 8 Effects of nitrogen sources

Total acid content was highest on medium containing peptone, 5.63 g/L, and lowest on medium with gelatin, 18.58 g/L. Biomass was not significantly different in medium containing NH_4NO_3, peptone, meat extract.

Organic nitrogen sources were found to induce significantly higher inorganic sources as there would be metabolism precursors or trait concentration of growth factor, vitamins, hormones, etc. which enhanced the growth of microorganisms in dried organic nitrogen sources. Thus, peptone was selected as the optimum nitrogen source.

3.5 Culturing V101 Under Optimum Conditions

All in all, optimum conditions for culturing V101 strain were found as pH 5.5, 6% NaCl on medium using glucose and peptone as carbon and nitrogen sources in 60 h. Under optimum conditions, the V101 strain produced 3.55 g/L biomass and 19.44 g/L acid content, 76.61%, and 10.69% higher than previous non-optimal conditions, respectively (Table 3).

V101 acid production doubled that of L. *helveticus* and L. bulgaricus, which were 10.1 g/L and 9.6 g/L, respectively [17]. However, total acid content produced by the V101 strain was a little lower than that ability of *L. pentosus* ATCC 8041 – 21.8 g/L [18], or nearly a half of lactic acid produced by *L. bulgaricus* NRRL B-548-38.7 g/L [19]. Differences in acid production could be caused by different LAB strains, fermentation conditions, and substrates.

Table 3 Dry biomass and acid content in optimum culture condition

	Dry biomass (g/L)	Acid content (g/L)
Optimum conditions	3.55 ± 0.01	19.14 ± 0.16
Traditional MRS	2.01 ± 0.12	17.51 ± 0.13

The culture supernatant of V101 under optimum conditions was collected by centrifugation and applied to antibacterial assays. Large and clear inhibition zones were observed by both dual culture overlay assay and agar-well diffusion assay on agar plates against *E. coli* and *S. aureus* (Figs. 9 and 10).

Table 4 showed that most inhibition zones against both *E. coli* and *S. aureus* were moderately improved. Sizes were statistically significantly different.

Among 2 indicating bacteria, optimization of culturing conditions improved antibacterial of V101 against E. *coli* better and more impressive than *S. aureus.*

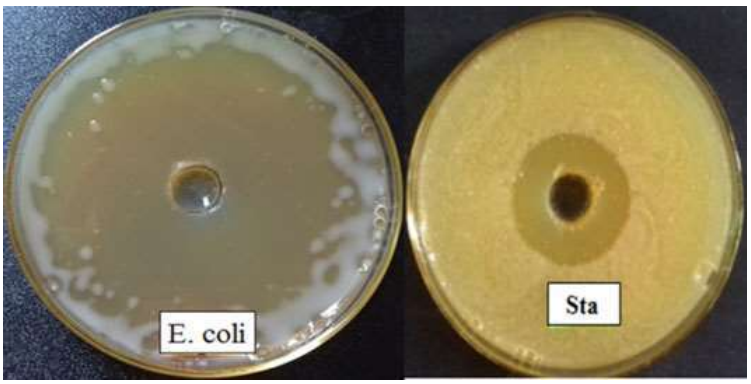


Fig. 9 Inhibition zones by agar-well diffusion assay

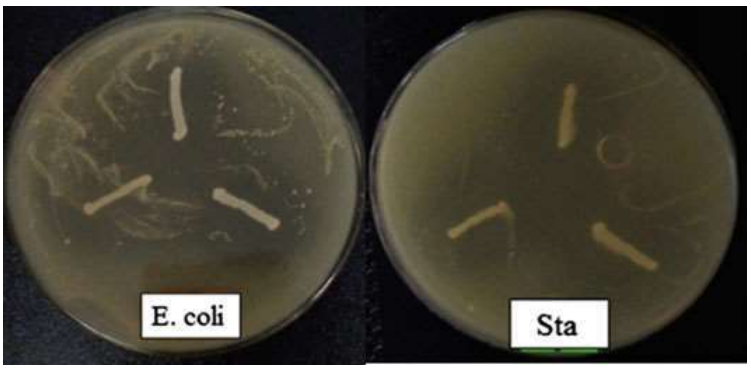


Fig. 10 Inhibition zones by dual culture overlayer assay

Table 4 Inhibition zones of indicator bacteria inhibited by V101 on optimum medium

	Dual culture overlay assay		Agar-well diffusion assay	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*
Optimum conditions	65.5 ± 0.9	31.2 ± 0.5	35.5 ± 0.6	31.5 ± 0.8
Traditional MRS	63.3 ± 0.7	33.5 ± 0.3	29.6 ± 0.4	29.2 ± 0.1

Inhibition zones by agar-well diffusion assay against *E. coli* increased 19.93%, from 29.6 mm to 35.5 mm.

It could be explained that a 76.61% increase in biomass accumulation might lead to the secretion of more bacteriocins and other antibacterial bioactive compounds. Together 10.69% higher acid produced and secreted, supernatant from culture under optimum conditions performed significant improvement on antibacterial activities.

3.6 Pilot Fermentation

L. paracasei V101 was applied to ferment sour shrimp under pilot productions. Shrimps were prepared as the same protocol for traditional sour shrimp fermentation. Samples inoculated with 3% *L. paracasei* V101 was fermented and compared with control without adding *L. paracasei* V101. Results of changes in sensory assessments were summarized in Table 5.

It took less time for *L. paracasei* V101to ferment Vietnamese pickles and sour shrimp. When both cultures were not different on Day 1, fermentation was quicker in the sample with *L. paracasei* V101 with the appearance of broth and slightly good smell.

On the other hand, sour shrimp fermented by *L. paracasei* V101 could be well preserved almost double the preservation period of control samples. More impressively, during preservation time, the texture of sour shrimps fermented by *L. paracasei* V101 was firmer. It was until Day 35 that shrimps *L. paracasei* V101 start their degrading phase while control samples appeared degrading signals from Day 20. Moreover, the shrimp body was intact and remain their shape for a longer time (up to 35 days) while control shrimp lost their heads after 20 days and shrank their bodies at 35 days.

Acid accumulation in the culture of *L. paracasei* V101 fermented and control sour shrimps was recorded in Fig. 11.

The acid content in culture was recorded from Day 1 to Day 35. In general, the acid content in both cultures climbed up to a peak then declined. Acid contents in culture fermented with *L. paracasei* V101 were significantly higher than that of control culture in all studied periods. In *L. paracasei* V101 fermented culture, acid content rose to a high on Day 25, at 39.90 g/L, and decreased to around 32 g/L on Day 35. The acid content in the control batch was much lower. It peaked earlier on Day 20, only reached 29.16 g/L then declined. Lactic acid bacteria added to culture would increase the LAB probiotics population and thus, increase the capacity of total acid-producing of the culture. Higher acid content could explain why fermentation by *L. paracasei* V101 was quicker with longer preservation time than non-L. *paracasei* V101 adding samples.

As a result, products were more delicious and more impressive, could be preserved longer. Fermentation processes induced by potential *L. paracasei* V101 should be studied more carefully in the future to be applied as a helpful probiotics strain to

Table 5 Quality of sour shrimp fermented by *L. paracasei* through time

Time (day)	Sample	Sensory assessments				Shrimp status
		Color		Flavor		
		Shrimp	Broth	Shrimp	Broth	
1	Control	Transparent grey	Transparent grey	Fishy	No broth	Intact, firm texture
	L. paracasei	Transparent grey	Transparent grey	Fishy	No broth	Intact, firm texture
2	Control	Transparent grey	Coral	Fishy	Slight flavor	Intact, firm texture
	L. paracasei	Coral	Coral	Slight flavor	Slight flavor	Intact, firm texture
3	Control	Orange	Coral red	Sour, good flavor	Sour, good flavor	Intact, firm texture
	L. paracasei	Coral red	Coral red	Sour, good flavor	Sour, good flavor	Intact, firm texture
5	Control	Coral red	Coral red	Sour, good flavor	Sour, good flavor	Intact, firm texture
	L. paracasei	Coral red	Coral red	Sour, good flavor	Sour, good flavor	Intact, firm texture
20	Control	Coral red	Coral red	Too sour, good flavor	Too sour, good flavor	Head detached, loose texture
	L. paracasei	Coral red	Coral red	Too sour, good flavor	Too sour, good flavor	Intact, firm texture
35	Control	Coral red	Coral red	Too sour, good flavor	Too sour, good flavor	Head detached, loose texture
	L. paracasei	Coral red	Coral red	Too sour, good flavor	Too sour, good flavor	Head detached, firm texture

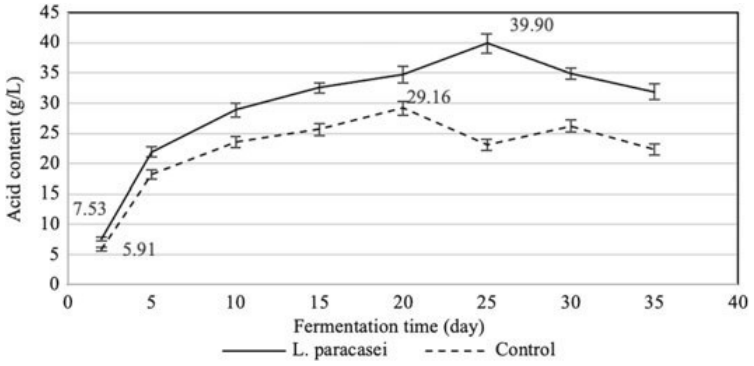


Fig. 11 Acid content in sour shrimp culture through time

bring good benefits for human health, traditional fermentation food improvement, and industrialization.

4 Conclusions

V101 strain isolated from traditional fermented food was identified as *L. paracasei.* It exhibited optimum cell growth and biomass, total acid production on modified MRS with 10 g/L meat extract, 5 g/L yeast extract, 10 g/L peptone, 20 g/L glucose, 5 g/L CH_3COONa, 2 g/L K_2HPO_4, 0.2 g/L $MgSO_4$, 0.05 g/L $MnSO_4$, 1 mL/L Tween 80, pH 5.5 and 60 h. Under optimal culture conditions, biomass and total acid accumulation increased by 76.61% and 10.69% respectively, and antimicrobial activities increased by 20%. *L. paracasei* V101 was a potential LAB probiotic for the improvement of fermented food and benefits for human health.

Conflicts of Interest The authors have no conflict of interest to declare.

References

1. Phong HX, Van TH, Thanh NN, Long BHD, Dung NTP (2017) Antifungal activity of lactic acid bacteria isolated from nem chua. Chem Biomol Eng 1(2):49
2. Kaškonienė V, Stankevičius M, Bimbiraitė-Survilienė K, Naujokaitytė G, Šernienė L, Mulkytė K, Malakauskas M, Maruška A (2017) Current state of purification, isolation, and analysis of bacteriocins produced by lactic acid bacteria. Appl Microbiol Biotechnol 101(4):1323–1335
3. Thomas LV, Ingram RE, Bevis HE, Davies EA, Milne CF, Delves-Broughton J (2002) Effective use of nisin to control *Bacillus* and *Clostridium* spoilage of a pasteurized mashed potato product. J Food Prot 65(10):1580–1585
4. Reis J, Paula A, Casarotti S, Penna A (2012) Lactic acid bacteria antimicrobial compounds: characteristics and applications. Food Eng Rev 4(2):124–140

5. Gautam N, Sharma N (2009) Bacteriocin: safest approach to preserve food products. Indian J Microbiol 49(3):204–211
6. Zhang B, Wang Y, Tan Z, Li Z, Jiao Z, Huang Q (2016) Screening of probiotic activities of lactobacilli strains isolated from traditional Tibetan Qula, a raw yak milk cheese. Asian Aust J Anim Sci 29(10):1490
7. Gharib SA (2020) Antimicrobial activity and probiotic properties of lactic acid bacteria isolated from traditional fermented dairy products. J Mod Res
8. De Las Cardoso M, Manzo RM, Tonarelli GG, Simonetta AC (2012) Characterisation of a cell-free supernatant obtained from cultures of *Enterococcus faecalis* DBFIQ E24 with antagonistic activity against bacteria, yeasts and moulds. Int J Dairy Technol 65(4):568–577
9. Magnusson J, Ström K, Roos S, Sjögren J, Schnürer J (2003) Broad and complex antifungal activity among environmental isolates of lactic acid bacteria. FEMS Microbiol Lett 219(1):129–135
10. Yang E, Fan L, Yan J, Jiang Y, Doucette C, Fillmore S, Walker B (2018) Influence of culture media, pH and temperature on growth and bacteriocin production of bacteriocinogenic lactic acid bacteria. AMB Exp 8(1):10
11. Khusniati T, Choliq A, Rahayu RD (2020) The organoleptic and physic characteristics and lactic acid contents of yoghurt with commercial starter added *Bifidobacteria bifidum*. J Bio Res 17:87–90
12. Sanchart C, Rattanaporn O, Haltrich D, Phukpattaranont P, Maneerat S (2017) Lactobacillus futsaii CS3, a new GABA-producing strain isolated from Thai fermented shrimp (Kung-Som). Indian J Microbiol 57(2):211–217
13. Todorov SD, Oliveira R, Vaz-Velho M (2012) Media optimization of bacteriocin ST22CH production by *Lactobacillus sakei* ST22CH isolated from salpicao, a traditional meat-product from Portugal. Chem Eng Trans 27:283–288
14. Arasu MV, Jung MW, Kim DH, Ilavenil S, Jane M, Park HS, Al-Dhabi NA, Jeon BT, Choi KC (2014) Enhancing nutritional quality of silage by fermentation with *Lactobacillus plantarum*. Indian J Microbiol 54(4):396–402
15. Arasu MV, Jung M-W, Kim DH, Ilavenil S, Jane M, Park HS, Al-Dhabi NA, Jeon BT, Choi KC (2014) Enhancing nutritional quality of silage by fermentation with Lactobacillus plantarum. Indian J Microbiol 54(4):396–402
16. Karthikeyan V, Santosh S (2009) Isolation and partial characterization of bacteriocin produced from *Lactobacillus plantarum*. Afr J Microbio Res 3(5):233–239
17. Plessas S, Bosnea L, Psarianos C, Koutinas A, Marchant R, Banat I (2008) Lactic acid production by mixed cultures of *Kluyveromyces marxianus*, *Lactobacillus delbrueckii* ssp. bulgaricus and *Lactobacillus helveticus*. Bioresour Technol 99(13):5951–5955
18. Bustos G, Moldes AB, Cruz JM, Domínguez JM (2004) Production of fermentable media from vine-trimming wastes and bioconversion into lactic acid by *Lactobacillus pentosus*. J Sci Food Agric 84(15):2105–2112
19. Burgos-Rubio CN, Okos MR, Wankat PC (2000) Kinetic study of the conversion of different substrates to lactic acid using *Lactobacillus bulgaricus*. Biotechnol Prog 16(3):305–314