Research Article

Optimization of Bacterial Concentrate Media for Lactobacillus Sakei

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Abstract. Two strains of lactic acid bacteria were selected for the study (*Lactobacillus sakei Lsk-45*and*Lactobacillus sakeiDSM 20017*). Bacterial media, based on rice and rice flour, were tested as an alternative to media based on whey. A comparison of the different types of media showed that there was better growth of the selected strains on themedium based on rice flour.Statistical analyses, including factorial experiments and response surface analyses, were used to optimizethe composition of the bacterial medium for *Lactobacillus sakei* propagation. Bacteriological peptone and rice flour were found to be good growth factors for *Lactobacillus sakei*.For *Lactobacillus sakei Lsk-45*,better growth was obtained with the use of 7.75-10 g/L of peptone and 57.5-75 g/L of rice flour. For *Lactobacillus sakei DSM 20017*, better growth was obtained with the use of 7-10 g/L of peptone and 40-75 g/L of rice flour.

Keywords: starters, media, *Lactobacillus sakei*, fermentation,fish products, bacterial strains, lactic acid bacteria

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1. Introduction

Fermented fish products are consumed in many regions of the world. They include wide variety of products, including fish sauces, fish pastes, and other products [14]. Fermented fish product "omul s dushkom" have been produced in Baikal region of Russia for a long time.[12]. Enzymes and microorganisms play an important role in the process of fermentation and they are responsible for theformation of characteristic flavour and aroma of this product.

Lactic acid bacteria (LAB) are dominant bacteria in some traditionally fermented fish products (Bjerke et al., 2019; Skåra et al., 2015). In the recent years, the use bacterial starter cultures for production of fish products has great scientific interest [1].

Lactobacillus sakei is a non-pathogenic lactic acid bacterium, which was first described as a contaminant of Japanese rice wine "sake" [7]. It was also found in various fermented products, such as sourdoughs and fermented cabbage. Lactobacillus sakei is characteristic for meat products. It was found naturally on fresh meat and fish,

vacuum-packed meat and fish, naturally fermented sausages. They are used as starters for fermentation of fish and meat product [5].

For the normal growth of LAB,richbacterial media containingvariouscompounds such as amino acids,vitamins, minerals, and other compounds is required.Lactobacillussakei is known as the most fastidious of all LAB [4].

The standard recommended media for cultivation of lactic acid bacteria is MRS (De Man, Rogosa and Sharpe), which contains meat extract, yeast extract, peptone, and growth components.However, the growth of LAB in MRS medium can be inhibited due to production of organic acids and nutrients limitation [10]. Probiotic bacterial concentrates are widely produced with the use of whey-based medium [3,6].

In the present times, there is a growing demand for production of food ingredients, including bacterial starter cultures, which are produced with the use ofkosherraw materials. There is a great scientific interest in development of non-dairy/ non-bovine/non-porcine alternative culture media for bacterial concentrates [4].Therefore, the use of components of plant origin and fish-based ingredients for production of bacterial concentrates is of great interest to scientists and engineers.

Thus, the main aim of the study was to explore the potential of use of bacterial media based on rice flourfor growing Lactobacillus sakei,

2. Materials and Methods

2.1. Microbial strains

Microbial strains used in this study included*Lactobacillussakei Lsk-45, Lactobacillus sakei DSM 20017 (JCM 1157)*. These strains wereobtainedfrom the collection of State Research Institute of Genetics and Selection of Industrial Microorganisms («Genetika») (Moscow, Russia).

Table 1 shows thecharacteristicsof bacterial strains used in the present study, which are available from «Genetika»(https://vkpm.genetika.ru).

2.2. Bacterial culture media

For the optimization bacterial media with different compositions were used.MRS medium; media, based on whey; media, based on rice decoction; media, based on rice flour.

Table 1: Characteristics of *Lactobacillus sakei*strainsused in the study

	Lactobacillus sakei Lsk-45	*Lactobacillus sakei DSM 20017 (JCM 1157)*
The source of the strain	Horse meat sausage	Moto starter of sake
Optimal growth temperature	37 °C	30 °C
Recommended bacterial culture media	MRS	MRS
Inhibition of food contaminants		
Escherichia coli	+	No data
Proteus vulgaris	+	Nodata
Salmonella typhimurium	+	Nodata
The ability to produce:		
Lactic acid	+	No data
Ammonia	+	No data
+: positive -: negative		

The composition of MRS medium was as follows:meat extract – 8 g/L; peptone – 10 g/L; yeastextract – 4 g/L; tween 80 – 0.1%; glucose – 20 g/L; dipotassium hydrogen phosphate – 2 g/L; manganese sulfate tetrahydrate – 0.05 g/L; magnesium sulfate heptahydrate – 0.2 g/L; sodium acetate trihydrate – 5 g/L; triammonium citrate – 2 g/L; agar – 1.5 g; bromocresol green – 0.04 g/L, distilled water – up to 1 L.

Media based on whey and hydrolyzed wheycontainedwhey and hydrolyzed (with β-Galactosidase) whey with added growth components including trisodium citrate, monopotassiumphosphate, ascorbic acid, anhydrous magnesium chloride, peptone, and agar.

Medium based on rice decoctionwas prepared from decoction of white rice (75 g/L) with glucose (15 g/L), trisodium citrate, dipotassium hydrogen phosphate; magnesium sulfate heptahydrate, peptone.

Medium based on rice flour contained white rice flour instead of white rice and the same components as medium based on rice decoction.

The chemical composition of rice and rice flour is given in Table 2.

2.3. Microbiological analyses

Cultures were inoculated in the media (3% v/v), then media were kept at 37 °C for 24 hours. Inoculum was grown on standard MRS.

Table 2: Chemical composition of rice and rice flourin 100 g of product (USDA, 2019)

	Rice, white, medium-grain, raw	Rice flour, white
Protein	6.61 g	5.95 g
Total lipid	0.58 g	1.42 g
Carbohydrate	79.34 g	80.13 g
Ash	0.58 g	0.61 g
Fiber	N.D.	2.4 g
Total sugars	N.D.	0.12 g
Calcium	9 mg	10 mg
Iron	0.8 mg	0.35 mg
Sodium	1 mg	0 mg
Magnesium	35 mg	35 mg
Phosphorus	108 mg	98 mg
Potassium	86 mg	76 mg
Zinc	1.16 mg	0.8 mg
Copper	0.11 mg	0.13 mg
Manganese	1.1 mg	1.2 mg
Selenium	N.D.	15.1 µg
Thiamine	0.07 mg	0.138 mg
Riboflavin	0.048 mg	0.021 mg
Niacin	1.6 mg	2.59 mg
Pantothenic acid	1.342 mg	0.819 mg
Vitamin B-6	0.145 mg	0.436 mg
Folate, total	9 µg	4 µg
Choline, total	N.D.	5.8 mg
N.D.: no data		

Serial dilution technique was used for estimation of LAB. Bacteria were incubated for 24 - 48 h at 37°C on MRS agar.

2.4. Chemical analyses

The pH of the media was analyzed with the use of pH-meter Anion-4100 (InfraspakAnalit, Novosibirsk, Russia).

2.5. Statistical analyses

Each experiment was repeated twice. Lactic acid bacteria viable counts were converted into log CFU/ml, then the mean values and standard deviations were calculated.

Full factorial design withthree levels was used for the optimization of bacterial media. Two factors (peptone content, rice flour content) were used for the study. Microsoft Excel 2010 with XLSTAT add-on was used for the study.

3. Results and Discussion

3.1. Microbial strains

Two bacterial strains were selected for analysis from the collection of State Research Institute of Genetics and Selection of Industrial Microorganisms. One of them was isolated from meat product, and the other one from rice drink, sake.

3.2. The selection of Bacterial Culture Media

On the first step of the study the growth of bacteria on different media was studied. For the analysis four different media were chosen: MRS broth; medium based on whey; medium based on rice decoction; medium based on rice flour.

For better growth and development of *Lactobacillus sakei* Mg^{2+} and Mn^{2+} are required (Lechiancole et al., 2002).For the growth of Lactobacillus sakeiamino acids, minerals and vitamins are required. The peptone was added to the bacterial media as a source of amino acids. Rice and rice flour contain vitamins and minerals, they are reach in magnesium (Table 2), therefore, no magnesium-containing salts were added to the media based on rice and rice flour.

Lactobacillus sakeipreferably ferment glucose, N-acetyl-D-glucosamine,sucrose, fructose, and mannose [17]. Some of the strains can ferment lactose[2]. Probably, because the strains used for the study couldn't ferment lactose, the growth on whey medium was very limited. The growth on other media, containing glucose, was relatively good. The better growth was observed on the media, based on the rice flour. Therefore, this medium was selected for the further optimization.

3.3. Optimization of Bacterial Culture Medium

For the further studies the experimental plan was designed (Table 4). Two factors (peptone content and rice flour content) were selected for the study.

As a result of statistical analysis contour plots (Fig.1) and the following models were obtained:

Table 3: Growth data of two strains of Lactobacillus sakei

Strains	Media	LAB viable counts, log CFU/ml	pH
Lactobacillus sakei Lsk-45	MRS	9	4.59
	Medium based on whey	3.3	6.0
	Medium based on hydrolyzed whey	7.47	4.85
	Medium based on rice decoction	6.77	5.42
	Medium based on rice flour	7.3	4.9
Lactobacillus sakei DSM 20017 (JCM 1157)	MRS	8.3	4.62
	Medium based on whey	3	6.2
	Medium based on hydrolyzed whey	7.6	4.86
	Medium based on rice decoction	7	5.0
	Medium based on rice flour	8	4.72

Table 4: Experimental plan.

Observation	Sortorde	Runorder	Repetition	Peptone, g/L	Riceflour, g/L	Number of LAB counts, log CFU/ml
Obs1	1	1	1	0	25	
Obs2	2	2	1	5	25	
Obs3	3	3	1	10	25	
Obs4	4	4	1	0	50	
Obs5	5	5	1	5	50	
Obs6	6	6	1	10	50	
Obs7	7	7	1	0	75	
Obs8	8	8	1	5	75	
Obs9	9	9	1	10	75	

1. For *Lactobacillus sakei Lsk-45*:

Number of LAB counts = 6,93+1,78*peptone+1,15*rice flour-0,51*peptone^2-0,31*rice flour^2+0,0025*peptone*rice flour

1. For *Lactobacillus sakei DSM 20017*:

Number of LAB counts = 7,13+1,7*peptone+0,58*rice flour-0,68*peptone^2-0,33*rice flour^2-0,075*peptone*rice flour

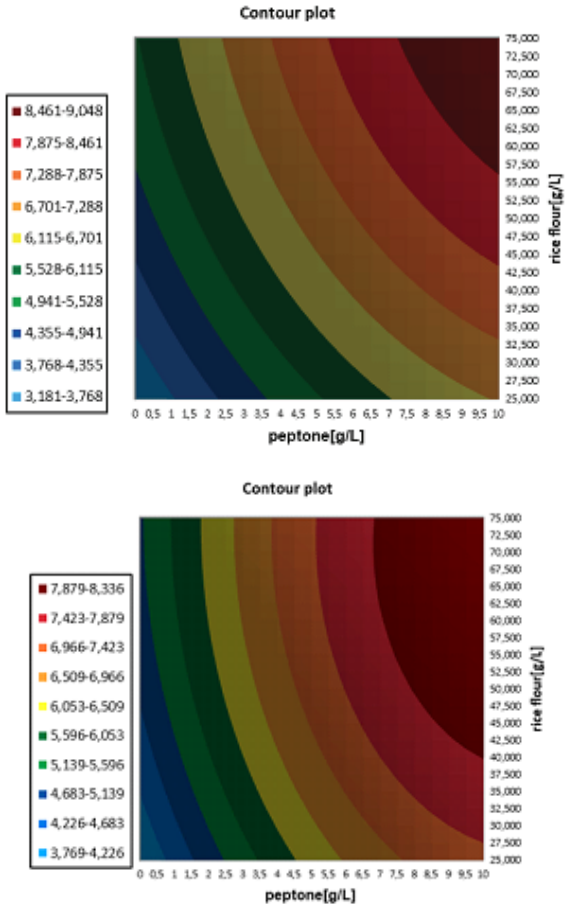

Figure 1: Response surfaces showing the predicted growth of Lactobacillus sakei: a) Lactobacillus sakei Lsk-45; b) Lactobacillus sakei DSM 20017 (graphs show the influence of two factors (peptone content and rice flour content) on number of LAB counts).

For *Lactobacillus sakei Lsk-45* better growth can be obtained with the use of 7.75-10 g/L of peptone and 57.5-75 g/L of rice flour. For *Lactobacillus sakei DSM 20017*better growth can be obtained with the use of 7-10 g/L of peptone and 40-75 g/L of rice flour.

Growth of both bacterial strains was relatively good. Both peptone and rice flour stimulated growth of Lactobacillus sakei. However, the content of peptone had more significant effect on growth of *Lactobacillus sakei Lsk-45*, while, the rice flour content had more significant effect on growth of*Lactobacillussakei DSM 20017*, probably, due to different origins of the strains.

4. Conclusions

In this work, the effect of use of different media on the growth of Lactobacillus sakei was studied. Two parameters (peptone content and rice flour content) of media composition for the cultivation of two strains of Lactobacillus sakeiwereoptimized.

The study showed that the addition of peptone and rice flour stimulate the activity of Lactobacillus sakei.

Further experiments to optimize bacterial medium for Lactobacillus sakeiare required. The influence of medium components and factors such as pH and temperature of medium should be studied.

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