MEDIUM OPTIMIZATION FOR ANTIMICROBIAL PRODUCTION BY NEWLY SCREENED LACTIC ACID BACTERIA

Rohmatussolihat[1]*, Puspita Lisdiyanti[1], Yopi[1], Yantyati Widyastuti[1], and Endang Sukara[1]

[1]Research Center for Biotechnology, Indonesian Institute of Sciences (LIPI), Jalan Raya Bogor Km. 46, Cibinong 16911, Indonesia

Abstract

Lactic acid bacteria (LAB) are important for prevention of spoilage and pathogenic bacterial growth in foods due to their ability to generate antimicrobial substances. The objectives of this study were to screen LAB for antimicrobial activity and to optimize culture medium for antimicrobial production using Response Surface Methodology (RSM) with Central Composite Design (CCD). Optimization of antimicrobial production of selected LAB was conducted with different combinations of glucose, NaCl, inoculum, and temperature. Our experimental results show that from 129 LAB isolates, 55 show significant inhibition against *Bacillus subtilis, Escherichia coli, Micrococcus luteus, Staphylococcus aureus, Aspergillus niger*, and *Candida albicans*. No isolates inhibited the growth of *Aspergillus flavus*. *Lactobacillus plantarum* LIPI13-2-LAB011 was selected for further study on culture medium optimization to inhibit the growth of *C. albicans*. Statistical analysis reveals that the production of antimicrobial substances was significantly influenced by temperature, NaCl, and concentration of glucose. Furthermore, the optimum concentrations of glucose, concentration of inoculum, temperature, and NaCl were 1.63 %, 3.03%, 33.74°C, and 3.4%, respectively, with a maximum predicted inhibition index of 1.916, which increased 3.56-fold compared to that obtained in medium before optimization processes. The result was confirmed as when the optimum concentration of nutritions used, the inhibition index increased 3.12-fold.

Keywords: Antimicrobial, *Lactobacillus plantarum*, Response Surface Methodology

---

*Corresponding author: Rohmatussolihat
Cibinong Science Center, Jl. Raya Bogor Km. 46, Cibinong 16911, Indonesia
Tel. +62-21-8754587, Fax. +62-21-87754588
E-mail. rohmatussolihat@gmail.com

Introduction

Proliferation of spoilage and pathogenic microorganisms cause a problem in food industry. Food borne pathogens may cause human illness and death. Food-borne diseases are mainly caused by *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, parasites, and viruses (Vincent *et al*., 2010; Kadariya *et al*., 2014; Martins *et al*., 2014). The application of antimicrobial compounds is a method commonly employed to eradicate food borne pathogens. LAB are extremely important for their capacity to produce antimicrobial and their ability to prevention of the growth of spoilage and pathogenic bacteria in foods (Galvez *et al*., 2008; Saranraj, 2012). LAB are a group of bacteria that generate most of the antimicrobial compounds, are recognized as non-toxic microorganisms, and exhibits various health properties to human (Wessels *et al*., 2004; Ghrairi *et al*., 2008; Yang *et al*., 2012).

The presence of LAB in food can extend the shelf life of foods through its ability to produce various substances with antimicrobial properties such as organic acids, short chain fatty acids, diacetyl, hydrogen peroxide, bacteriocin and bacteriocin-like compounds, enzymes and antimicrobial-bioactive peptides (Liong and Shah, 2005; Gálvez *et al*., 2007;Tan *et al*., 2014).

The production of antimicrobial compounds by LAB may be influenced by various factors e.g. nutrient and fermentation conditions. Those factors may be optimized by statistical-based approaches. Response Surface Methodology (RSM) has been extensively used in fermentation media optimization

(Khurana *et al*., 2007; Yu *et al*., 2008; Yuan *et al*., 2008; Mu *et al*., 2009). This important statistical technique was employed for multiple regression analysis by using quantitative experimental data obtained from properly designed experiments using Central Composite Design (CCD) (Bezerra *et al*., 2008). This method uses mathematical models to analyze the experimental data and to predict the relationship between the response and the variables (Bezerra *et al*., 2008; Rajendran & Thangavelu, 2007). This method offers several advantages over conventional methods in being rapid and reliable and to easily shortlist significant nutrients, helping to understand the interactions among the nutrients at various concentrations and reduce the total number of experiments while improving product yield, reduce process variability, as well as reduce development time and overall costs (Chauhan *et al*., 2007; Rohmatussolihat, 2013; Yu *et al*., 2008).

The purpose of this study was to screen LAB for antimicrobial activity and to optimize culture medium for antimicrobial production using Response Surface Methodology (RSM) with Central Composite Design (CCD).

Materials and Methods

A total of 129 LAB isolates were used for antimicrobial screening. Those isolates are obtained from the Indonesian Culture Collection (InaCC), Indonesian Institute of Sciences. Those isolates undergone a long-term storage in sterile glycerol (150 $mL.L^{-1}$) and stored at -80 °C (Badis *et al*., 2004). Those isolates are also stored in L-drying ampoules. The medium used in cultivating LAB and production of antimicrobial were (i) de Man Rogosa Sharpe (MRS) broth (Merck) at pH 10 with the addition of 100 $g.L^{-1}$ NaCl (Merck) and (ii) Trypticase Soy Yeast Extract (TSYE) broth at pH 6 with or without the addition of 100 $g.L^{-1}$ NaCl (Merck) depending on the isolates used. The composition of TSYE broth was 33 $g.L^{-1}$ Trypticase Soy Broth (TSB) (Merck) and 3 $g.L^{-1}$ of yeast extract (Merck).

Two loops of LAB isolates taken from glycerol stock were inoculated into 10 mL of sterile MRS media and incubated at 30 °C. After 48 hours, the cell culture was centrifuged at 3,220 x g at 4 °C for 10 minutes to obtain cell-free supernatant (CFS). The CFS was used for antimicrobial testing by using disc diffusion method (Balouiri *et al*., 2016).

Test Organisms

Bacillus subtilis BTCC B-612, *Escherichia coli* BTCC B-548, *Micrococcus luteus* BTCC B-552, *Staphylococcus aureus* BTCC B-611, *Candida albicans* BTCC Y-33, *Aspergillus niger* BTCC F-62, and *Aspergillus flavus* BTCC F-69 were chosen as test organisms for antimicrobial assays. Those testing microbes were obtained from Biotechnology Culture Collection (BTCC), Research Center for Biotechnology, Indonesian Institute of Sciences. The reference of the test bacteria is the basis of initial screening in antimicrobial testing. If one isolate has activity, then continued for further test.

One loop of testing bacteria were cultured in 5 mL Nutrient Broth (NB) (Merck) at 30 °C, while testing fungi were cultured in 5 mL Potato Dextrose Broth (PDB) (Merck) at 30 °C and incubated for 24 h in a shaker incubator.

Disc Diffusion Assay for Antimicrobial Activity

Petri dish containing 15 mL of solidified Muller Hinton Agar (Merck) for bacteria or Sabaraud Agar (Merck) for fungi overlaid with 5 mL of soft agar was inoculated with each of test organism. The concentration of testing microbes was 0.1 % (v/v) for *S. aureus* and *B. subtilis*, 0.2% (v/v) for *E. coli*, and 0.5 % (v/v) for *M. luteus*, *C. albicans*, *A. niger*, and *A. flavus* (Miyadoh & Otoguro, 2004). A total of 30 µL of CFS was injected on a paper disc and left to dry. The disc was then placed on the agar containing test organisms, and were incubated at 30 °C for 24 hours (Bromberg *et al*., 2004; Saranya and Hemashenpagam, 2011).

Antimicrobial activity from CFS was evaluated by measuring the zone of inhibition/clearing zone against the test organisms. Microbial inhibition was visually appraised as the diameter of the inhibition zones surrounding the discs (disc diameter included) and recorded in millimeters. Inhibition zone formed around the paper disc was observed and measured using a caliper. The agar disc diffusion bioassay were performed in duplicates. The inhibition index was measured according to the following formulation: inhibition index= (inhibition zone

diameter (mm) - discs diameter (mm))/(discs diameter (mm)).

Optimization for Antimicrobial Production

Optimization of antimicrobial production was performed by using RSM with the CCD. The design and data analysis was carried out using Design-Expert Software Statistics 7. The used design is based upon Leal-Sánchez *et al*. (2002) that consists of four variables namely glucose (X_1), inoculum (X_2), temperature (X_3), and NaCl (X_4) with 5 level combinations (Table 1). According to this design, 30 runs were conducted containing six replications at the central point for the estimating the purely experimental uncertainly variance (Mu *et al*., 2009). The response surface analysis was based on the multiple linear regressions taking into account the main, quadratic and interaction effects, according to the following equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j \quad \text{(Eq.1)}$$

β_0: scaling constant (intersept), β_i–βij: regression estimates

Table 1. Coded and uncoded values of the experimental variables

Variables		Code Values				
		–2	–1	0	+1	+2
Glucose (%)	X_1	0.5	1.0	1.5	2.0	2.5
Inoculum (%)	X_2	1.0	2.0	3.0	4.0	5.0
Temperature (°C)	X_3	25.0	30.0	35.0	40.0	45.0
NaCl (%)	X_4	2.0	3.0	4.0	5.0	6.0

Optimization of antimicrobial production was conducted by inoculating the selected isolates into 25 mL of optimized MRS Broth medium and incubated at temperature that varies based on the design of experiments using RSM for 48 hours (Al-Allaf *et al*., 2009; Leal-Sánchez *et al.*, 2002). The cultures were centrifuged at 3,220 g at 4 °C for 10 minutes. The CFS was then analyzed for its antimicrobial.

Results

Screening for Antimicrobial Production

The CFS from 129 LAB isolates were screened for its antimicrobial properties against test organisms. The results are depicted in Table 2. Out of 129 LAB isolates, 55 isolates (42.6%) show significant inhibition against test organisms with varying degrees and 74 isolates (57.4%) did not show inhibition. Upon further screening, from 55 isolates, significant inhibition was observed against *B. subtilis*, *E. coli* (8), *M. luteus* (2), *S. aureus* (1), *C. albicans* (36), *A. niger* (32). None of the 129 isolates could inhibit *A. flavus*. The number of LAB that can inhibit *C. albicans* was the highest among all test organisms. The inhibition index of antimicrobial activity of LAB isolates ranged between 0.21-0.31, 0.46-0.54, 0.28, 0.65, 0.17-0.82, and 0.06-0.28 for *B. subtilis*, *E. coli*, *M. luteus*, *S. aureus*, *C. albicans*, *A. niger*, respectively. Inhibition index of *L. plantarum* LIPI13-2-LAB060 and *L. plantarum* LIPI13-2-LAB061 showed the highest value of 0.82 for *C.albicans*. Only one isolate (*L. plantarum* LIPI13-2-LAB011) showed wide spectrum activity against four test organisms. Therefore, *L. plantarum* LIPI13-2-LAB011 was selected for further evaluation. The inhibition index of antimicrobial activity of *L. plantarum* LIPI13-2-LAB011 was 0.23, 0.65, 0.42, 0.12 against *B. subtilis*, *S. aureus*, *C. albicans*, and *A. niger*, respectively. Inhibition index of *L. plantarum* LIPI13-2-LAB011 for *S.aureus* was the highest compared to other test microbes, but when analysed by RSM CCD showed the model was not significant (data not shown). *C. albicans* was chosen as a target for optimization.

Optimization of medium for producing antimicrobial activity

A total of 30 experiments with different combination of variables were performed to examine the combined effect of four independent variables on antimicrobial activity from *L. plantarum* LIPI13-2-LAB011 against *C. albicans*. The CCD and experimental yields are shown in Table 3. The highest output of inhibition index observed was 2.108 at run 27 (Table 3). In order to determine the maximum inhibition index corresponding to the optimum levels of glucose, inoculum, temperature and NaCl, a quadratic model was proposed to calculate the optimum levels of these variables in producing antimicrobial activity.

Table 2. Antimicrobial activity[a] of LAB tested by disc diffusion assay

No.	Genus and Species Name	Code	Inhibition index against[b]:						
			Bs	*Ec*	*Ml*	*Sa*	*Ca*	*An*	*Af*
1	*Bifidobacterium psychraerophilum*	LIPI13-2-LAB041	-	-	-	-	-	0.21	-
2	*Enterococcus faecalis*	LIPI13-2-LAB078	-	-	-	-	-	0.14	-
3	*Enterococcus faecium*	LIPI13-2-LAB133	-	0.52	-	-	-	-	-
4	*Lactobacillus casei*	LIPI13-2-LAB128	0.31	-	-	-	0.29	-	-
5	*Lactobacillus dextrinicus*	LIPI13-2-LAB012	-	-	-	-	0.24	0.12	-
6	*Lactobacillus farciminis*	LIPI13-2-LAB129	-	-	-	-	0.19	-	-
7	*Lactobacillus fermentum*	LIPI13-2-LAB051	-	-	-	-	0.50	0.13	-
8	*Lactobacillus fermentum*	LIPI13-2-LAB168	-	-	-	-	0.17	-	-
9	*Lactobacillus mali*	LIPI13-2-LAB043	-	-	-	-	-	0.13	-
10	*Lactobacillus nagelii*	LIPI13-2-LAB199	-	-	-	-	0.43	-	-
11	*Lactobacillus paracasei*	LIPI13-2-LAB077B	-	0.46	-	-	0.50	0.14	-
12	*Lactobacillus paracasei*	LIPI13-2-LAB136	-	-	-	-	0.50	-	-
13	*Lactobacillus paracasei*	LIPI13-2-LAB142	-	-	-	-	0.57	-	-
14	*Lactobacillus paracasei*	LIPI13-2-LAB149	-	-	-	-	0.50	-	-
15	***Lactobacillus plantarum***	**LIPI13-2-LAB011**	**0.23**	**-**	**-**	**0.65**	**0.42**	**0.12**	**-**
16	*Lactobacillus plantarum*	LIPI13-2-LAB044	0.27	-	-	-	0.80	-	-
17	*Lactobacillus plantarum*	LIPI13-2-LAB050	-	-	-	-	0.50	0.13	-
18	*Lactobacillus plantarum*	LIPI13-2-LAB060	-	-	-	-	0.82	0.10	-
19	*Lactobacillus plantarum*	LIPI13-2-LAB061	-	-	-	-	0.82	0.24	-
20	*Lactobacillus plantarum*	LIPI13-2-LAB076	-	0.46	-	-	0.79	0.17	-
21	*Lactobacillus plantarum*	LIPI13-2-LAB077A	-	0.54	-	-	0.67	0.17	-
22	*Lactobacillus plantarum*	LIPI13-2-LAB087	-	0.52	-	-	0.72	0.14	-
23	*Lactobacillus plantarum*	LIPI13-2-LAB099	-	-	-	-	0.58	0.28	-
24	*Lactobacillus plantarum*	LIPI13-2-LAB130	-	-	-	-	0.19	-	-
25	*Lactobacillus plantarum*	LIPI13-2-LAB145	0.21	-	-	-	-	-	-
26	*Lactobacillus plantarum*	LIPI13-2-LAB147	-	-	-	-	0.50	-	-
27	*Lactobacillus plantarum*	LIPI13-2-LAB178	0.27	-	0.28	-	0.37	-	-
28	*Lactobacillus plantarum*	LIPI13-2-LAB184	-	-	-	-	0.62	-	-
29	*Lactobacillus plantarum*	LIPI13-2-LAB206	-	-	-	-	0.50	-	-
30	*Lactobacillus plantarum*	LIPI13-2-LAB210	0.23	0.52	-	-	-	-	-
31	*Lactobacllus fermentum*	LIPI13-2-LAB057	-	-	-	-	0.49	0.08	-
32	*Lactobacllus fermentum*	LIPI13-2-LAB073	-	-	-	-	0.36	0.14	-
33	*Lactobacllus fermentum*	LIPI13-2-LAB074	-	-	-	-	0.66	-	-
34	*Lactobacllus fermentum*	LIPI13-2-LAB084	-	0.47	-	-	-	-	-
35	*Lactobacllus fermentum*	LIPI13-2-LAB088	-	-	-	-	0.47	0.14	-
36	*Lactobacllus fermentum*	LIPI13-2-LAB100	-	-	-	-	0.33	0.28	-
37	*Lactobacllus fermentum*	LIPI13-2-LAB101	-	-	-	-	0.33	0.28	-
38	*Leuconostoc mesenteroides*	LIPI13-2-LAB049	-	-	-	-	0.28	0.13	-
39	*Leuconostoc mesenteroides*	LIPI13-2-LAB059	-	-	-	-	0.49	0.28	-
40	*Leuconostoc mesenteroides*	LIPI13-2-LAB067	0.26	-	-	-	0.42	0.14	-
41	*Leuconostoc mesenteroides*	LIPI13-2-LAB081	-	-	-	-	-	0.14	-
42	*Leuconostoc mesenteroides*	LIPI13-2-LAB221	-	0.54	-	-	-	-	-
43	*Pediococcus pentosaceus*	LIPI13-2-LAB122	-	-	-	-	0.29	-	-
44	*Tetragenococcus halophilus*	LIPI13-2-LAB019	-	-	-	-	-	0.12	-
45	*Tetragenococcus halophilus*	LIPI13-2-LAB024	-	-	-	-	-	0.14	-
46	*Tetragenococcus halophilus*	LIPI13-2-LAB027	-	-	-	-	-	0.14	-
47	*Tetragenococcus halophilus*	LIPI13-2-LAB033	-	-	-	-	-	0.14	-
48	*Tetragenococcus halophilus*	LIPI13-2-LAB038	-	-	-	-	-	0.06	-
49	*Tetragenococcus halophilus*	LIPI13-2-LAB042	-	-	-	-	-	0.21	-
50	*Tetragenococcus halophilus*	LIPI13-2-LAB102	-	-	-	-	-	0.28	-
51	*Tetragenococcus muriaticus*	LIPI13-2-LAB003	-	-	-	-	-	0.12	-
52	*Tetragenococcus muriaticus*	LIPI13-2-LAB006	-	-	-	-	-	0.06	-
53	*Weissella confusa*	LIPI13-2-LAB160	-	-	-	-	0.22	-	-
54	*Weissella confusa*	LIPI13-2-LAB170	-	-	-	-	0.20	-	-
55	*Weissella confusa*	LIPI13-2-LAB223	-	-	0.28	-	-	-	-

[a]Antimicrobial activity detected as inhibition index

[b]Bs: *B. subtilis*, Ec: *E. coli*, ML: *M. luteus*, Sa: *S. aureus*, Ca: *C. albicans*, An: *A. niger*, Af: *A.flavus*

Table 3. Central Composite Design (CCD) and experimental antimicrobial activity yield of *L. plantarum* LIPI13-2-LAB011 against *C. Albican.*

No.	Variables*				
	X_1	X_2	X_3	X_4	Inhibition index
1	1	2	30	3	0.850
2	2	2	30	3	1.075
3	1	4	30	3	0.575
4	2	4	30	3	1.408
5	1	2	40	3	0.808
6	2	2	40	3	0.892
7	1	4	40	3	0.600
8	2	4	40	3	0.358
9	1	2	30	5	0.658
10	2	2	30	5	0.908
11	1	4	30	5	0.750
12	2	4	30	5	0.883
13	1	2	40	5	0
14	2	2	40	5	0.167
15	1	4	40	5	0.292
16	2	4	40	5	0.217
17	0.5	3	35	4	0.217
18	2.5	3	35	4	1.200
19	1.5	1	35	4	1.350
20	1.5	5	35	4	1.975
21	1.5	3	25	4	0.892
22	1.5	3	45	4	0.167
23	1.5	3	35	2	2.058
24	1.5	3	35	6	0.725
25	1.5	3	35	4	1.508
26	1.5	3	35	4	1.925
27	**1.5**	**3**	**35**	**4**	**2.108**
28	1.5	3	35	4	1.767
29	1.5	3	35	4	1.608
30	1.5	3	35	4	1.900

* X_1: glucose (%), X_2: inoculum (%), X_4: temperature (°C), X_4: NaCl (%)

The ANOVA summary is shown in Table 4. The model *F*-value of 5.526 implied that the model was highly significant and there was only a 0.0011 chance that a model *F*-value could occurred due to noise. The fitness of the model was examined by determination coefficient ($R^2 = 0.84$), which implied that the sample variation of more than 84% was attributed to the variables and only less than 16% of the total variance could not be explained by the model.

In the analysis of regression (Table 4), the term of X_3 (temperature), X_4 (NaCl), X_1^2 (glucose), X_3^2, X_4^2 term (P <0.05) were statistically significant on antimicrobial activity. Since the model showed insignificant lack of fit, the response was sufficiently explained by the regression equation. On the basis of *p*-values which are more than 0.05, the generated regression relationship between inhibition index (Y) and four variables (Table 4 and 5) were represented in Eq. (2).

$$Y = 1.80 - 0.218\ X_3 - 0.223X_4 - 0.344\ X_1^2 - 0.388\ X_3^2 - 0.173X_4^2 \quad \text{(Eq.2)}$$

Where Y is the predicted inhibition index yield, X_1 is glucose, X_3 is temperature and X_4 is NaCl. The values in this equation were derived from coefficient of intercept and significant variables (Table 5).

Table 4. ANOVA for *Response Surface Quadratic* (X_1,glucose; X_2, inoculum; X_3,Temperature; X_4, NaCl)

Sources	Sum of Squares	df	Mean Square	*F*-Value	*P* > F	Note
Model	**9.834**	**14**	**0.702**	**5.526**	**0.0011**	*
X_1	0.465	1	0.465	3.659	0.0751	
X_2	0.040	1	0.040	0.312	0.5849	
X_3	1.137	**1**	1.137	8.942	**0.0092**	
X_4	1.196	**1**	1.196	9.407	**0.0078**	
X_1X_2	0.000	1	0.000	0.003	0.9577	
X_1X_3	0.142	1	0.142	1.117	0.3074	
X_1X_4	0.011	1	0.011	0.089	0.7698	
X_2X_3	0.017	1	0.017	0.136	0.7179	
X_2X_4	0.075	1	0.075	0.587	0.4553	
X_3X_4	0.101	1	0.101	0.797	0.3861	
X_1^2	3.239	**1**	3.239	25.478	**0.0001**	
X_2^2	0.303	1	0.303	2.384	0.1434	
X_3^2	4.137	**1**	4.137	32.547	**< 0.0001**	
X_4^2	0.820	**1**	0.820	6.448	**0.0227**	
Residual	1.907	15	0.127			
Lack of Fit	1.663	10	0.166	3.412	0.0939	ns
Pure Error	0.244	5	0.049			
Cor Total	11.741	29				

* = significant
ns =not significant

The relationship between X_3 (temperature), X_4 (NaCl) and the inhibition index yield is illustrated as a three dimensional representation of the response surfaces and two-dimensional contour plots generated by the model in Figure 1. From the response surface plots (a), it is easy and convenient to understand the interaction between two factors and also to locate their optimum levels. Figure 1 (a) and (b) reveal that the inhibition index yield increased with an increase in the amounts of the NaCl (X_4) up to an optimum (maximum inhibition index yield) beyond which there was a decrease. NaCl (X_4) had a similar trend to temperature (X_3). Relationship between X_1 (glucose), X_3 (temperature) and relationship between X_1 (glucose), X_4 (NaCl) and the

inhibition index yield are illustrated as a three dimensional representation of the response surfaces (a) and two-dimensional contour plots (b) generated by the model (Figure 2 and 3). The interaction term X_1, X_3, X_4 with a positive regression coefficient in Eq. (2) provided evidence that the inhibition index yield was favored by an increase in the factor interactions up to a value beyond which there was a decline. The optimized concentrations of glucose, inoculum, temperature, and NaCl were 1.63 %, 3.03%, 33.74°C, and 3.4%, respectively, with the maximum predicted of inhibition index of 1.916, which increased 3.56-fold compared to that of obtain in medium before optimization processes (inhibition index of 0.42). This result is confirmed when the optimum concentration of nutrition was used as the inhibition index increased by 3.12-fold.

Table 5. Regression coefficients and significance of response surface quadratic (X_1,glucose; X_2, inoculum; X_3,Temperature; X_4, NaCl)

Factor	Coefficient Estimate	df	Standard Error	95%CI Low	95%CI High
Intercept	**1.803**	**1**	0.146	1.492	2.113
X_1	0.139	1	0.073	-0.016	0.294
X_2	0.041	1	0.073	-0.114	0.196
X_3	**-0.218**	1	0.073	-0.373	-0.063
X_4	**-0.223**	1	0.073	-0.378	-0.068
X_1X_2	-0.005	1	0.089	-0.195	0.185
X_1X_3	-0.094	1	0.089	-0.284	0.096
X_1X_4	-0.027	1	0.089	-0.217	0.163
X_2X_3	-0.033	1	0.089	-0.223	0.157
X_2X_4	0.068	1	0.089	-0.122	0.258
X_3X_4	-0.080	1	0.089	-0.270	0.110
X_1^2	**-0.344**	1	0.068	-0.489	-0.199
X_2^2	-0.105	1	0.068	-0.250	0.040
X_3^2	-0.388	1	0.068	-0.533	-0.243

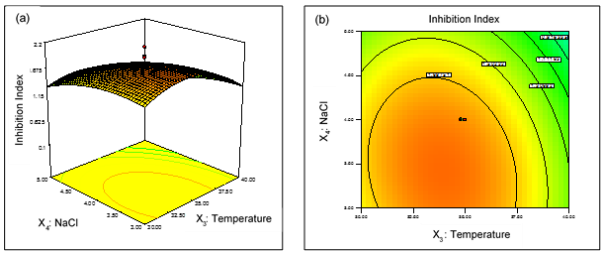

Figure 1. Response surface (a) and contour plots (b) for the effects temperature (X_3) and NaCl (X_4) on inhibition index.

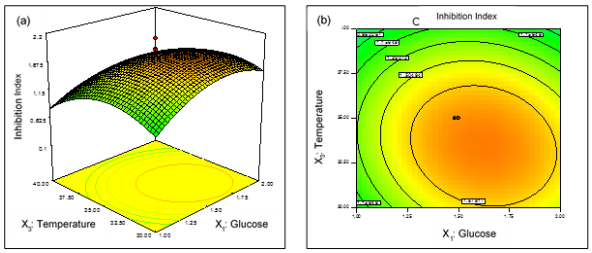

Figure 2. Response surface (a) and contour plots (b) for the effects glucose (X_1) and temperature (X_3) on inhibition index.

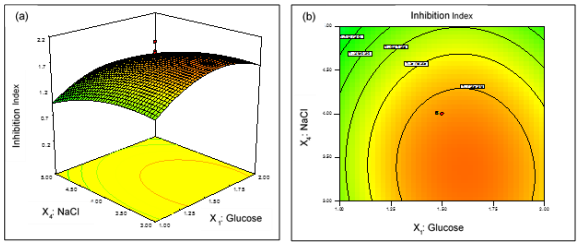

Figure 3. Response surface (a) and contour plots (b) for the effects glucose (X_1) and NaCl (X_4) on inhibition index.

Discussion

Intensive isolation and identification of LAB from fermented foods in Indonesia during the last 10 years resulted in the accumulation of LAB isolates and need to explore for its potential. This study is a dedication on further evaluation of LAB isolates for its ability to inhibit spoilage/food born pathogenic microorganisms present in food such as *B. subtilis*, *E. coli*, *M. luteus*, *S. aureus*, *C. albicans*, *A. niger*, and *A. flavus*. Those spoilage/food born pathogenic microbes were chosen as a model targets to screen LAB for its ability to have antimicrobial properties. *B. subtilis* could cause ropiness – a sticky, stringy consistency due to its ability in producing long-chain polysaccharides in spoiled bread dough (Pepe *et al*., 2003). *E. coli* is shown to cause diarrhea, cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), and other clinical infections such as neonatal meningitis and pneumonia (Vincent *et al*., 2010). Cases of bacteremia, endocarditis, ventriculitis, peritonitis, pneumonia, endophthalmitis, keratolysis and septic arthritis have been reported as a disease caused by the *Micrococcus* Species (Von Eiff *et al*., 1996). *S. aureus* can cause staphylococcal food poisoning via the

production of enterotoxin. *S. aureus* is also a transient pathogenic microorganism of the scalded skin syndrome, bacteremia, toxic shock syndrome, and infections on medical implants (Foster, 2005). *C. albicans* can cause superficial infections such as oropharyngeal candidiasis (thrush) and vulvovaginal candidiasis (vaginal candidiasis). Oral candidiasis is common in elderly denture wearers (Darwazeh *et al.*, 1990*).*

The nature of *Aspergillus* sp. (including *A. niger* and *A. flavus*) presence in nature and large amounts of aspergillus conidia (spores) being released into the air currents and available for inhalation, making the human pulmonary exposure to these organisms inevitable. *Aspergillus* species have emerged as important causes of morbidity and mortality in immunocompromised patients. Invasive aspergillosis currently constitutes the most common cause of infectious pneumonic mortality in patients undergoing HSCT (hematopoietic stem cell transplantation) and is an important cause of opportunistic respiratory and disseminated infection in other immunocompromised patients (Walsh *et al*., 2008; Person *et al*., 2010).

Wide ranges mode of action have been described for bacteriocins or antimicrobial, such as enzyme activity modulation, spores inhibition outgrowth and formation of pores in cell membrane. Most bacteriocins or antimicrobe interact with anionic lipids that are abundantly present in the membranes, and consequently initiate the formation of pores in the membranes of susceptible cells (Šušković *et al.*, 2010).

In this study, 42.6% of 129 LAB isolates show significant inhibition to test organisms with varying degrees. However, for the purpose of the study, only one isolate was selected, e.g. *Lactobacillus plantarum* LIPI13-2-LAB011 due to its broad spectrum nature relative to the other LAB species tested here.

Many studies show that LAB have antimicrobial activity. Spoilage or contamination of food caused by microbial spoilage can eventually be prevented by the addition of antimicrobial-producing LAB. Noordiana *et al*. (2013) have isolated and screened LAB strains (P1S1 and P3S3) from threadfin salmon and grass shrimp samples and found that they exhibited inhibitory effects against *Salmonella typhimurium*, *Listeria monocytogenes*, *E. coli* and *B. cereus*. They also stated that the antagonistic activity may have been due to the presence of organic acid, hydrogen peroxide and bacteriocin, which act as antibacterial substances. Desniar *et al*. (2013) reported that LAB from bekasam (Indonesian fermented fish) inhibited growth of *L. monocytogenes, S. typhimurium, E. coli, B. cereus* and *S. aureus.* The highest inhibition zone and inhibition index was on *S. aureus*. However, culture supernatant of the LAB isolates did not produce inhibition zone at neutral pH. Their result indicated that inhibition activity of LAB isolated from Indonesian bekasam was from organic acids, and those organic acids are probably the main preservative factor in the bekasam.

Hwanhelm *et al*. (2011) studied that *Streptococcus salivarius* LD219, *Enterococcus faecalis* LPS04, *E. faecalis* LPS17 and *E. faecalis* LPS18, which were isolated from plasom (Thai fermented fish), had a good antimicrobial activity against foodborne pathogens (*Salmonella* sp., *S. aureus* and *E. coli*) as well as a good acidifying activity. Cizeikiene *et al*. (2013) stated that *L. sakei* KTU05-6, *Pediococcus acidilactici* KTU05-7, *P. pentosaceus* KTU05-8, KTU05-9 and KTU05-10 strains produced organic acids and bacteriocins-like inhibitory substances (BLIS) against pathogenic microorganisms (*Bacillus, Pseudomonas, Listeria, Escherichia, Fusarium culmorum, Penicillium chrysogenum, A. fumigatus, A.versicolor, P. expansum, A. niger, Debaryomyces hansenii* and *C. parapsilosis* genera in various degrees) in the food industry.

Our study shows that *L. plantarum* LIPI13-2-LAB011 has a broad spectrum that could inhibit four testing microorganisms and shows activity against Gram-positive bacteria i.e B. *subtilis* and *S. aureus* and fungi i.e *C. albicans* and *A. niger*. The strain was isolated from terasi (fermented shrimp) obtained from Sukawati Traditional Market in Bali, Indonesia using MRS medium pH 6.0. *L. plantarum* is a type of facultative anaerobic bacteria that can control the growth of microbial pathogens by producing organic acid, hydrogen peroxide, diacetyl and bacteriocin (Dalié *et al*., 2010). The ability of *L. plantarum* strain to display antimicrobial activity against pathogen has been published in several reports, for example *L. plantarum* MiLAB 14 produces hydroxylated fatty acids with strong antifungal effects in MRS medium, with total inhibitory

effects of hydroxy fatty acids in the range of 10 to >100 μg.mL^{-1} against several moulds and yeasts (Magnusson, 2003). Antifungal produced by *L. plantarum* DR 1-6-2 on MRS Agar medium against *Aspergillus fumigatus* and *A. flavus* by plate assay method (aerobic condition) had a percentage of inhibition of 41% and 32 %, respectively (Rohmatussolihat, 2013). This strain also produced antimicrobial that work against *Penicillium* by deep tube technique (anaerobic condition) (Rohmatussolihat, 2013). *Lactobacillus plantarum* ST16PA produced bacteriocin, which actively inhibited the growth of *Enterobacter, Enterococcus, Lactobacillus, Pseudomonas, Streptococcus, Staphylococcus,* and *Listeria (*Todorov *et al*., 2011). *Lactobacillus plantarum* LB-B1 from a traditionally fermented dairy product koumiss produced a bacteriocin active against *Listeria, Lactobacillus, Streptococcus, Enterococcus, Pediococcus and Escherichia* (Xie *et al*., 2011). *Lactobacillus plantarum* 21B was isolated from sourdough had antifungal activity. Growth of *A. niger* FTDC3227 in bread started with *Lactobacillus plantarum* 21B and *Sacharomyces cerevisiae* 141 could be delayed after 7 days of storage, while in bread started with *Lactobacillus brevis* and *S. cerevisiae* only after 2 days of storage (Lavermicocca *et al*., 2000).

In producing these antimicrobial compounds, it is interesting to evaluate the effect of nutrient composition of medium used. Evaluation of the nutritional and environmental requirements for microorganism to produce optimum antimicrobial should be an important stage in developing and determining the overall economic feasibility of its bioprocess. Environmental factors such as temperature, pH and media composition influence the level of production of antimicrobial substances. Nutrients in the production of antimicrobial substances and the different types of supplements added to the growth media at different concentrations could affect the quantity and ability to inhibit pathogenic bacteria (Widyaningsih *et al*., 2017). Optimization of cultural medium is a very important aspect in the field of food microbiology and fermentation (Mu *et al*., 2009). The RSM has been a popular and effective method to solve multivariate problems and optimize several responses in many types of experiment (Wang & Liu, 2008).

In this study, optimum glucose concentration at 1.63% increase the antimicrobial activity of *L. plantarum* LIPI13-2-LAB011 against *C. Albicans*. Todorov (2008) reported that *L. plantarum* AMA-K produced bacteriocin/antimicrobial AMA-K that inhibits the growth of *Enterococcus* sp., *E. coli, Klebsiella pneumoniae* and *Listeria* sp. The production of bacteriocin AMA-K of 100 % is stimulated when cells are grown in medium supplemented with 3 % glucose. Glucose is main source of carbon, nutrients that are needed for growth of microorganisms. Bogaert and Naidu (2000) and Nelson & Cox, (2008) stated that antimicrobial activity of LAB is mainly caused by organic acids produced through glucose catabolism (Embden Meyerhoff Parnas pathway). Vatanyoopaisarn *et al*. (2011) studied that the use of media without glucose inhibited the production of organic acids as antimicrobial substances.

This study unveiled that concentration of inoculum added in the culture media were statistically has no significant effect to the antimicrobial activity against *C. albicans*. This result is similar to Himelbloom *et al*. (2001) that different concentration of inoculum had no significant effect on the production of bacteriocin by *L. acidophilus* LF221 and *Candida piscicola* A9b. On the other hand, Taheri *et al*. (2012) studied that inoculum level significantly affected bacteriocin production by *L. plantarum* ST1. Maximum bacteriocin activity and stability (640 AU/ml) were obtained at the lowest inoculum level (0.01%). The difference in results may be due to the type of bacterial strains, composition of medium production used, and also incubation conditions used in growing the inoculum.

In this study, optimum temperature for antimicrobial production against *C. albicans* from *L. plantarum* LIPI13-2-LAB011 was at 33.74°C. Results were not different from those carried out by Kumar *et al*. (2012) that effective temperature for bacteriocin production by *L. casei* LA-1 ranged from 33.5 to 34.5 °C. Martinez *et al.* (2013) reported that the optimal temperature range of *L. plantarum* ST71KS isolated from homemade goat feta cheese in producing antimicrobial against *Listeria monocytogenes* strains 603 and 607 was between 30-37 °C. While, Malheiros *et al*. (2015) reported that higher amounts of

bacteriocin as antimicrobial from *L. casei* were produced when the incubation temperature was either 25 °C or 30 °C. The difference in results may be due to the type of bacterial strains and composition of medium production used.

Optimum concentration of NaCl for antimicrobial production against *C. albicans* from *L. plantarum* LIPI13-2-LAB011 is 3.4%. According to Noordiana *et al*. (2013) the medium supplemented with NaCl (1%) significantly enhanced the antibacterial activity as shown by the P1S1 and P3S3 strains against *E. coli*, *Staphylococcus typhimurium* and *L. monocytogenes*. The results were the same as those expressed by Settanni *et al*. (2008), that the presence of NaCl (1- 2%) improves LAB growth and increases bacteriocin activity.

In conclusion, out of 129 LAB isolates, 55 isolates (42.6%) show significant inhibition against test organisms with varying degrees and 74 isolates (57.4%) did not show inhibition. Only one isolate (*L. plantarum* LIPI13-2-LAB011) shows wide spectrum activity against four test organisms. Therefore, *L. plantarum* LIPI13-2-LAB011 was selected for optimization of antimicrobial production using CCD-RSM.

The optimized medium composition was 1.63% glucose, 3.03% inoculum, 33.74 °C temperature, 3.4% NaCl, with the maximum product of inhibition index (1.916) after 48 h fermentation, which corresponded to the results of RSM optimization.

Acknowledgements

This project was funded by SATREPS Project ”Development of Internationally Standardized Microbial Resources Center As A Core of Biological Resources Center to Promote Life Science Research and Biotechnology (JST-JICA), Program 2011-2015. The authors acknowledge Ayu Syafitri for laboratory work.

References

Al-Allaf, M. A. H., Al-Rawi, A. M. M., & Al-Mola, A. T. (2009). Antimicrobial activity of lactic acid bacteria isolated from minced beef meat against some pathogenic bacteria. *Iraqi Journal of Veterinary Sciences, 23*(3), 115-117.

Badis, A., Guetarni, D., Boudjema, B. M., Henni, D. E., & Kihal, M. (2004). Identification and technological properties of lactic acid bacteria isolated from raw goat milk of four Algerian races. *Food Microbiology, 21*(5), 579-588.

Balouiri, M., Sadiki, M., & Ibnsouda S.K. 2016. Methods for in vitro evaluating antimicrobial activity:Areview. *Journal of Pharmaceutical Analysis, 6*, 71–79.

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., & Escaleira, L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta, 76*(5), 965-977.

Bogaert, J., & Naidu, A. (2000). *Natural Food Antimicrobial System*. Florida (US): CRC Pr.

Bromberg, R., Moreno, I., Zaganini, C. L., Delboni, R. R., & Oliveira, J. D. (2004). Isolation of bacteriocin-producing lactic acid bacteria from meat and meat products and its spectrum of inhibitory activity. *Brazilian Journal of Microbiology, 35*(1-2), 137-144.

Chauhan, K., Trivedi, U., & Patel, K. C. (2007). Statistical screening of medium components by Plackett–Burman design for lactic acid production by *Lactobacillus* sp. KCP01 using date juice. *Bioresource Technology, 98*(1), 98-103.

Cizeikiene, D., Juodeikiene, G., Paskevicius, A., & Bartkiene, E. (2013). Antimicrobial activity of lactic acid bacteria against pathogenic and spoilage microorganism isolated from food and their control in wheat bread. *Food Control, 31*(2), 539-545.

Dalié, D. K. D., Deschamps, A. M., & Richard-Forget, F. (2010). Lactic acid bacteria–Potential for control of mould growth and mycotoxins: A review. *Food Control, 21*(4), 370-380.

Darwazeh, A. M. G., Lamey, P. J., Samaranayake, L. P., MacFarlane, T. W., Fisher, B. M., Macrury, S. M., & MacCuish, A. C. (1990). The relationship between colonisation, secretor status and in-vitro adhesion of *Candida albicans* to buccal epithelial cells from diabetics. *Journal of Medical Microbiology, 33*(1), 43-49.

Desniar, M. Rusmana, I., Suwanto, A. & Mubarik, N. R. (2013). Characterization of lactic acid bacteria isolated from an Indonesian fermented fish (bekasam) and their antimicrobial activity against pathogenic bacteria. *Emirates Journal of Food and Agriculture*, 489-494.

Foster, T. J. (2005). Immune evasion by staphylococci. *Nature Reviews Microbiology, 3*(12), 948.

Gálvez, A., Abriouel, H., López, R. L., & Omar, N. B. (2007). Bacteriocin-based strategies for food biopreservation. *International Journal of Food Microbiology, 120*(1-2), 51-70.

Gálvez, A., López, R. L., Abriouel, H., Valdivia, E., & Omar, N. B. (2008). Application of

bacteriocins in the control of foodborne pathogenic and spoilage bacteria. *Critical Reviews in Biotechnology, 28*(2), 125-152.

Ghrairi, T., Frere, J., Berjeaud, J. M., & Manai, M. (2008). Purification and characterisation of bacteriocins produced by *Enterococcus faecium* from Tunisian rigouta cheese. *Food Control, 19*(2), 162-169.

Himelbloom, B., Nilsson, L., & Gram, L. (2001). Factors affecting production of an antilisterial bacteriocin by *Carnobacterium piscicola* strain A9b in laboratory media and model fish systems. *Journal of Applied Microbiology, 91*(3), 506-513.

Hwanhlem, N., Buradaleng, S., Wattanachant, S., Benjakul, S., Tani, A., & Maneerat, S. (2011). Isolation and screening of lactic acid bacteria from Thai traditional fermented fish (Plasom) and production of Plasom from selected strains. *Food Control, 22*(3-4), 401-407.

Kadariya, J., Smith, T. C., & Thapaliya, D. (2014). *Staphylococcus aureus* and staphylococcal food-borne disease: an ongoing challenge in public health. *BioMed Research International*, Article ID 827965, 9 pages.

Khurana, S., Kapoor, M., Gupta, S., & Kuhad, R. C. (2007). Statistical optimization of alkaline xylanase production from *Streptomyces violaceoruber* under submerged fermentation using response surface methodology. *Indian Journal of Microbiology, 47*(2), 144-152.

Kumar, M., Jain, A. K., Ghosh, M., & Ganguli, A. (2012). Statistical optimization of physical parameters for enhanced bacteriocin production by *L. casei*. *Biotechnology and Bioprocess Engineering, 17*(3), 606-616.

Lavermicocca, P., Valerio, F., Evidente, A., Lazzaroni, S., Corsetti, A., & Gobbetti, M. (2000). Purification and characterization of novel antifungal compounds from the sourdough *Lactobacillus plantarum* strain 21B. *Applied and Environmental Microbiology, 66*(9), 4084-4090.

Leal-Sánchez, M. V., Jiménez-Díaz, R., Maldonado-Barragán, A., Garrido-Fernández, A., & Ruiz-Barba, J. L. (2002). Optimization of bacteriocin production by batch fermentation of *Lactobacillus plantarum* LPCO10. *Applied and Environmental Microbiology, 68*(9), 4465-4471.

Liong, M. T., & Shah, N. P. (2005). Production of organic acids from fermentation of mannitol, fructooligosaccharide and inulin by a cholesterol removing *Lactobacillus acidophilus* strain. *Journal of Applied Microbiology, 99*(4), 783-793.

Magnusson, J. (2003). Antifungal activity of lactic acid bacteria (Doctoral dissertation, Uppsala: Department of Microbiology, Swedish University of Agricultural).

Malheiros, P. S., Sant'Anna, V., Todorov, S. D., & Franco, B. D. (2015). Optimization of growth and bacteriocin production by *Lactobacillus sakei* subsp. *sakei* 2a. *Brazilian Journal of Microbiology, 46*(3), 825-834.

Martinez, R. C. R., Wachsman, M., Torres, N. I., LeBlanc, J. G., Todorov, S. D., & de Melo Franco, B. D. G. (2013). Biochemical, antimicrobial and molecular characterization of a noncytotoxic bacteriocin produced by *Lactobacillus plantarum* ST71KS. *Food Microbiology, 34*(2), 376-381.

Martins, N., Ferreira, I. C., Barros, L., Silva, S., & Henriques, M. (2014). Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment. *Mycopathologia, 177*(5-6), 223-240.

Miyadoh, S., & Otoguro M. 2004. Workshop on Isolation Method and Clasification of Actinomycetes. Bogor (ID): LIPI Bioteknologi.

Mu, W., Chen, C., Li, X., Zhang, T., & Jiang, B. (2009). Optimization of culture medium for the production of phenyllactic acid by *Lactobacillus* sp. SK007. *Bioresource Technology, 100*(3), 1366-1370.

Nelson, D.L., & Cox, M.M. (2008). *Lehninger: Principles of Biochemistry*, fifth edition. Madison Avenue (NY): WH Freeman Comp.

Noordiana, N., Fatimah, A. B., & Mun, A. S. (2013). Antibacterial agents produced by lactic acid bacteria isolated from Threadfin Salmon and Grass Shrimp. *International Food Research Journal, 20*(1), 117-124.

Pepe, O., Blaiotta, G., Moschetti, G., Greco, T., & Villani, F. (2003). Rope-producing strains of *Bacillus* spp. from wheat bread and strategy for their control by lactic acid bacteria. *Applied and Environmental Microbiology, 69*(4), 2321-2329.

Person, A. K., Chudgar, S. M., Norton, B. L., Tong, B. C., & Stout, J. E. (2010). *Aspergillus niger*: an unusual cause of invasive pulmonary aspergillosis. *Journal of Medical Microbiology, 59*(7), 834-838.

Rajendran, A., & Thangavelu, V. (2007). Sequential optimization of culture medium composition for extracellular lipase production by *Bacillus sphaericus* using statistical methods. *Journal of Chemical Technology and Biotechnology, 82*(5), 460-470.

Rohmatussolihat. (2013). Seleksi dan optimasi bakteri asam laktat penghasil senyawa antikapang (Magister Tesis, Bogor: Institut Pertanian Bogor).

Saranraj, P. (2012). Microbial spoilage of bakery products and its control by preservatives. International *Journal of Pharmaceutical & Biological Archive, 3*(1), 38-48.

Saranya, S., & Hemashenpagam, N. (2011). Antagonistic activity and antibiotic sensitivity of lactic acid bacteria from fermented dairy

products. *Advances in Applied Science Research, 2*(4), 528-534.

Settanni, L., Valmorri, S., Suzzi, G., & Corsetti, A. (2008). The role of environmental factors and medium composition on bacteriocin-like inhibitory substances (BLIS) production by *Enterococcus mundtii* strains. *Food Microbiology, 25*(5), 722-728.

Šušković, J., Kos, N., Beganović, Pavunc, A.L., Habjanić, K., & Matosić, S. (2010). Antimicrobial activity - The most important property of probiotic and starter lactic acid bacteria. *Food Technol Biotechnol 48* (3) 296-307.

Taheri, P., Samadi, N., Ehsani, M. R., Khoshayand, M. R., & Jamalifar, H. (2012). An evaluation and partial characterization of a bacteriocin produced by *Lactococcus lactis* subsp *lactis* ST1 isolated from goat milk. Brazilian *Journal of Microbiology, 43*(4), 1452-1462.

Tan, P., Peh, K., Gan, C., & Liong, M. T. (2014). Bioactive dairy ingredients for food and non-food applications. *Acta Alimentaria, 43*(1), 113-123.

Todorov, S. D. (2008). Bacteriocin production by *Lactobacillus plantarum* AMA-K isolated from Amasi, a Zimbabwean fermented milk product and study of the adsorption of bacteriocin AMA-K to *Listeria* sp. *Brazilian Journal of Microbiology, 39*(1), 178-187.

Todorov, S. D., Prévost, H., Lebois, M., Dousset, X., LeBlanc, J. G., & Franco, B. D. (2011). Bacteriocinogenic *Lactobacillus plantarum* ST16Pa isolated from papaya (*Carica papaya*)—From isolation to application: Characterization of a bacteriocin. *Food Research International, 44*(5), 1351-1363.

Valerio, F., De Bellis, P., Lonigro, S. L., Visconti, A., & Lavermicocca, P. (2008). Use of *Lactobacillus plantarum* fermentation products in bread-making to prevent *Bacillus subtilis* ropy spoilage. *International Journal of Food Microbiology, 122*(3), 328-332.

Vatanyoopaisarn, S., Prapatsornwattana, K., Kuhakongkeat, T., & Phalakornkule, C. (2011). Potential use of lactic acid bacteria with bacteriocin-like activity against *Staphylococcus aureus* as dual starter cultures in Thai fermented sausage" Sai Krok Prew.". *International Food Research Journal, 18*(2), 697-704.

Vincent, C., Boerlin, P., Daignault, D., Dozois, C. M., Dutil, L., Galanakis, C., Reid-Smith, R. J., Tellier, P. P., Tellis, P. A., Ziebell, K., & Manges, A. R. (2010). Food reservoir for *Escherichia coli* causing urinary tract infections. *Emerging Infectious Diseases, 16*(1), 88.

Von Eiff, C., Kuhn, N., Herrmann, M., Weber, S., & Peters, G. (1996). *Micrococcus luteus* as a cause of recurrent bacteremia. *The Pediatric Infectious Disease Journal, 15*(8), 711-713.

Walsh, T. J., Anaissie, E. J., Denning, D. W., Herbrecht, R., Kontoyiannis, D. P., Marr, K. A., Morrison, V. A., Segal, B. H., Steinbach, W. J., Stevens, D. A., & van Burik, J. A. (2008). Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. *Clinical Infectious Diseases, 46*(3), 327-360.

Wang, Z. W., & Liu, X. L. (2008). Medium optimization for antifungal active substances production from a newly isolated *Paenibacillus* sp. using response surface methodology. *Bioresource Technology, 99*(17), 8245-8251.

Wessels, S., Axelsson, L., Hansen, E. B., De Vuyst, L., Laulund, S., Lähteenmäki, L., Lindgren, S., Mollet, B., Salminen, S., & von Wright, A. (2004). The lactic acid bacteria, the food chain, and their regulation. *Trends in Food Science & Technology, 15*(10), 498-505.

Widyaningsih, S., Trianto, A., Radjasa, O.K., Wittriansyah K. (2018). Antibacterial activity symbiotic fungi of marine sponge *Axinella* sp., *Aspergillus Sydowii* on four growth medium. *IOP Conf. Series: Earth and Environ Science 116*, 1-9.

Xie, Y., An, H., Hao, Y., Qin, Q., Huang, Y., Luo, Y., & Zhang, L. (2011). Characterization of an anti-Listeria bacteriocin produced by *Lactobacillus plantarum* LB-B1 isolated from koumiss, a traditionally fermented dairy product from China. *Food Control, 22*(7), 1027-1031.

Yang, E., Fan, L., Jiang, Y., Doucette, C., & Fillmore, S. (2012). Antimicrobial activity of bacteriocin-producing lactic acid bacteria isolated from cheeses and yogurts. *Amb Express, 2*(1), 48.

Yu, L., Lei, T., Ren, X., Pei, X., & Feng, Y. (2008). Response surface optimization of l-(+)-lactic acid production using corn steep liquor as an alternative nitrogen source by *Lactobacillus rhamnosus* CGMCC 1466. *Biochemical Engineering Journal, 39*(3), 496-502.

Yuan, L. L., Li, Y. Q., Wang, Y., Zhang, X. H., & Xu, Y. Q. (2008). Optimization of critical medium components using response surface methodology for phenazine-1-carboxylic acid production by *Pseudomonas* sp. M-18Q. *Journal of Bioscience and Bioengineering, 105*(3), 232-237.