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Optimization of antioxidant exopolysaccharidess production by *Bacillus licheniformis* in solid state fermentation

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ABSTRACT

Response surface methodology was applied to optimize physical and nutritional variables for the production of antioxidant exopolysaccharidess (EPSs) by *Bacillus licheniformis* UD061 in solid state fermentation with squid processing byproduct and maize cob meal used as a carbon and nitrogen source and solid matrix. The factors noted with Plackett–Burman design for optimization of EPSs production were NaCl, $MgSO_4 \cdot 7H_2O$, and moisture level. These factors were further optimized using Box–Behnken design and response surface methodology. Using this methodology, the quadratic regression model of EPSs production was built. Maximum EPSs production was obtained under the optimal conditions of 4.08 g L^{-1} NaCl, 0.71 g L^{-1} $MgSO_4 \cdot 7H_2O$, and 60.49% moisture level. A production of 14.68 mg gds^{-1}, which was well in agreement with the predicted value, was achieved by this optimized procedure.

1. Introduction

Microbial exopolysaccharides (EPSs) are high molecular weight polymers of monosaccharides (>20) and are secreted by microorganisms into the surrounding environment. EPSs have been wildly utilized as ingredients in food products, pharmacy, petroleum industry and emulsification of crude oil, hydrocarbons, vegetable, mineral oils and bioremediation agents in environment management system (Kuzma et al., 2012; Wang, Huang, Liang, Fang, & Wang, 2011). Although a vast number of bacterial extracellular polysaccharides have been reported over recent decades, and their composition, structure, biosynthesis and functional properties have been extensively studied, there is still a demand for novel EPSs that impart improved physical or chemical characteristics (Fang, Liu, Lu, Jiao, & Wang, 2013; Freitas, Alves, & Reis, 2011).

The oceans cover greater than 70% of the earth's surface. Taking this into account by volume, it represents more than 95% of the biosphere. Moreover, marine environments are massively complex and contain diverse assemblage of life forms, which occur in environments with extreme variations in pressure, salinity, and temperature. As a result, marine microorganisms have developed unique metabolic profiles, which led them to produce different kind of metabolites including EPSs, which are not produced by terrestrial microorganisms (Guezenneca et al., 2012; Freitas et al., 2011; Poli, Anzelmo, and Nicolaus1, 2010). Over the past decade, marine microorganisms have become recognized as an important and untapped resource for novel EPSs. Unfortunately, only few of those microorganisms have been commercially exploited because of low yields and high cost of EPSs production. Although mutation breeding was utilized to improve the yield of EPSs from several microorganisms, the yields of EPSs production are still low (Lima et al., 2008; Luo et al., 2009). EPSs production costs can be significantly reduced by using inexpensive raw materials, such as agro-industrial wastes.

Squid (*Loligo chinensis*) is one of the most important biological resources in world particularly in China, however its utilization ratio is not high. Industrial processing of squid for human consumption yields more than 30% by-products and only 70% edible fish products (Xu, Yu, Xue, Xue, & Ren, 2008). Moreover, the byproduct of squid processing were looked as worthless garbage and discarded without an attempt of recovery, and those by-products resulted in different level of environment pollution. The nutritional value of these waste products is almost identical to that of the edible parts (Nagai & Suzuki, 2000). Moreover, due to the rich nutrient composition, this waste stream has the potential for utilization as a nutrition source for microorganisms, resulting in the production of different groups of metabolites in solid state fermentation characteristics (Xu et al., 2008). Its utilization will not only reduces the pollution problems, but can also provide an additional income to the seafood industry (Bhaskar, Benila, Radha, & Lalitha, 2008).

Solid state fermentation has several advantages over conventional submerged fermentation such as use of agro industrial wastes as simpler substrates and making the downstream

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processing less time consuming and less expensive, which reduces metabolites production costs and, to some extent, solves environmental issues (Bhargav, Panda, Ali, & Javed, 2008; Mienda, Idi, & Umar, 2011). The squid processing byproducts have served as an excellent substrate for growth of various industrially important bacteria and fungi for production of important metabolites in solid-state fermentation. Therefore, by-products derived from squid processing industry were utilized to investigate their suitability for antioxidant exopolysaccharidess production in solid-state fermentation. Media components and culture conditions were found to have great influence on extracellular EPSs production. Optimizing process parameters can lead to the enhanced production of exopolysaccharidess through solid-state fermentation, which will cut the production cost.

We have previously reported a solvent tolerant strain of *Bacillus licheniformis* UD061 which secretes antioxidant exopolysaccharidess, but the yield of exopolysaccharidess was relatively low in our previous work, therefore, we improved the production of antioxidant extracellular polysaccharides with UV and DES mutagenetic treatments and organic solvent stress treatments (Fang et al., 2013). The objectives of present study were to enhance the production of antioxidant extracellular polysaccharides by *B. licheniformis* UD061 in solid-state fermentation with squid processing byproduct and maize cob meal used as carbon and nitrogen sources and solid matrix by response surface methodology. These results demonstrated viable approach for utilization of the squid processing byproduct by solid-state fermentation for the production of antioxidant extracellular polysaccharides and offer significant benefit due to low cost and abundant availability of squid processing byproduct during polysaccharides production.

2. Materials and methods

2.1. Materials

The squid processing byproduct acquired from local squid processing unit was rinsed and homogenised using a Biohomogenizer at high speed for 1 min, then dried, ground in a mill Wiley type in the particle size of approximately 2 mm, placed in polyethylene bag and stored at −20 °C for not longer than 1 month. Maize cobs obtained from local farm were cut into small pieces, air-dried, powdered in a grinder and sieved through 2 mm mesh screens.

2.2. Measurement of chemical composition

Moisture content, ash, and crude lipid content was determined according to the method described by Chaijan, Jongjareonrak, Phatcharat, Benjakul, and Rawdkuen (2010). Nitrogen (N) contents of fish muscle samples were determined with Cui's procedure (2013). The N content was multiplied with 6.25 to estimate the crude protein of these samples. Each determination was performed in triplicate.

2.3. Microorganism and their cultivation

Strain *B. licheniformis* UD061 was used in the present study (Fang et al., 2013). The strain was maintained on modified 2216E medium agar slants having composition (per liter aged seawater): 5 g tryptone, 1 g yeast extract, 0.1 g $FePO_4$, agar 2.0 (pH 7.0). After incubation at 25 °C for 36 h, the slants were stored at 4 °C, and sub-cultured every 2 months.

2.4. Inoculum preparation and solid state fermentation

Seed cultures were prepared by transferring a loop of lawn from the fully-grown slant into a 250 mL flask containing 50 mL of 2216E medium and incubated at 25 °C for 36 h. Four grams of squid processing byproduct and one gram of maize cob meal were taken in 250 ml Erlenmeyer flasks and to this, a predetermined quantity of mineral salts medium (composition in g L^{-1}: NaCl 40, KH_2PO_4 30, K_2HPO_4 10, $MgSO_4{\cdot}7H_2O$ 5, $MnSO_4{\cdot}H_2O$ 4, Tween 80 10) was added and mixed thoroughly. The initial pH of the medium was adjusted to 7.0 by 1 N NaOH or 1 N HCl, and autoclaved at 121 °C for 20 min. Moisture content of the solid medium was adjusted to 50% by increasing the quantity of distilled water before autoclaving. After cooling to room temperature, the flasks were inoculated with 2% inoculum (0.8 OD at 600 nm) under sterile conditions. After incubation at 26 °C for 6 days, contents were used for polysaccharides extraction and estimation of yield of antioxidant extracellular polysaccharides. Unless specified otherwise, these fermentation conditions were maintained throughout the study. All the experiments were conducted in triplicates and the results described are mean of three replicates.

2.5. Polysaccharides extraction

The extracellular polysaccharides of fermented matter were extracted in water bath at 80 °C with distilled water (ratio of water to fermented matter (ml g^{-1}) was 10:1) for 3 h and pretreated with 80% ethanol twice to remove oligosaccharides and small molecules. This water extracted sample was transferred to a dialysis bag and was dialyzed against 2 l of distilled water under magnetic stirring for 2 h, and then concentrated to one-fifth of initial volume using a rotary evaporator at 55 °C under vacuum. The resulting sample was mixed with four volumes of anhydrous ethanol, stirred vigorously and placed at 4 °C for overnight. Then the solution was centrifuged at 10,000 × *g* for 15 min, washed three times with dehydrated ethanol, and the precipitate was collected as crude EPSs. The extract was weighted with a balance after freeze-drying. Total EPSs concentrations in the ethanol extracts were measured by using the phenol–sulfuric acid method (Ravella et al., 2010).

2.6. Antioxidant extracellular polysaccharides extraction

2.6.1. Determination of reducing power of EPSs

The reducing power was determined as described previously (Song et al., 2008). The reaction mixtures contained 2.5 ml phosphate buffer (pH 6.6, 0.2 M), 2.5 ml potassium ferricyanide (1%, w/v) and the EPS (5–250 mg/l). After incubation at 50 °C for 20 min, 2.5 ml of trichloroacetic acid (10%, w/v) was added to the mixture for terminating the reaction, and then centrifuged at 5000 × *g* for 10 min. The supernatant (5 ml) was mixed with 0.5 ml ferric chloride (0.1%, w/v), and the absorbance was read spectrophotometrically at 700 nm. A higher absorbance indicates a higher reducing power. Deionized water and ascorbic acid were used as the blank and control, respectively.

2.6.2. Superoxide anion scavenging activity

The superoxide anion scavenging activity of EPSs was determined as described previously (Stewart & Bewley, 1980). The reaction mixture contained each 1.0 ml of NBT solution (156 μmol/L of NBT in 0.1 M phosphate buffer, pH 7.4), NADH solution (468 μmol/L of NADH in 0.1 M phosphate buffer, pH 7.4) and EPS solution (5–250 mg/l). 1.0 ml of PMS solution (60 μmol/L PMS in 0.1 M phosphate buffer, pH 7.4) was added to the reaction mixture at 25 °C for 5 min. Absorbance was noted at 560 nm was measured. Deionized water and ascorbic acid BHT were used as the blank and control, respectively. The scavenging activity was determined as: superoxide radical (%) = $(1 - A_{sample}/A_{blank}) \times 100$.

Table 1
Variables and test levels for Plackett–Burman experiment.

Run order	Coded variable level											EPSs (mg gds^{-1})	
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	Observed	Predicted
1	3	3.5	0.5	0.4	0.3	0.8	8	8	25	1	65	9.16 ± 0.29	8.44
2	5	2.5	0.5	0.8	0.3	0.8	8	8	30	5	55	8.13 ± 0.33	8.38
3	3	3.5	0.5	0.4	0.6	1.6	8	8	25	5	55	6.86 ± 0.25	6.91
4	5	2.5	1.5	0.4	0.3	0.8	6	8	30	1	55	7.33 ± 0.29	6.77
5	3	2.5	0.5	0.4	0.6	1.6	6	6	30	1	55	7.02 ± 0.30	6.81
6	3	2.5	1.5	0.4	0.3	1.6	8	8	30	1	65	8.89 ± 0.26	8.56
7	5	3.5	1.5	0.8	0.6	1.6	8	8	30	5	65	12.62 ± 0.32	11.90
8	5	3.5	0.5	0.4	0.6	0.8	6	8	30	5	65	9.31 ± 0.27	9.94
9	5	3.5	1.5	0.4	0.6	0.8	8	6	25	1	55	8.05 ± 0.23	8.00
10	3	3.5	1.5	0.4	0.3	1.6	6	6	30	5	65	7.55 ± 0.25	8.68
11	3	3.5	1.5	0.8	0.6	0.8	8	6	30	1	55	7.51 ± 0.22	7.90
12	5	2.5	0.5	0.4	0.3	1.6	8	6	25	5	55	7.28 ± 0.25	7.86
13	5	3.5	1.5	0.8	0.3	1.6	6	8	25	1	55	7.69 ± 0.22	8.93
14	5	3.5	0.5	0.8	0.3	1.6	6	6	25	1	65	12.88 ± 0.39	11.78
15	3	3.5	0.5	0.8	0.3	0.8	6	6	30	5	55	8.05 ± 0.29	7.23
16	5	2.5	0.5	0.8	0.6	1.6	8	6	30	1	65	11.95 ± 0.32	12.22
17	5	2.5	1.5	0.4	0.6	0.8	6	6	25	5	65	10.12 ± 0.33	9.45
18	3	2.5	1.5	0.8	0.6	1.6	6	8	25	5	55	8.13 ± 0.27	7.26
19	3	2.5	1.5	0.8	0.3	0.8	8	6	25	5	65	8.82 ± 0.29	9.14
20	3	2.5	0.5	0.8	0.6	0.8	6	8	25	1	65	8.27 ± 0.22	9.44

X_1 NaCl (g L^{-1}), X_2 KH_2PO_4 (g L^{-1}), X_3 K_2HPO_4 (g L^{-1}), X_4 $MgSO_4{\cdot}7H_2O$ (g L^{-1}), X_5 $MnSO_4$ (g L^{-1}), X_6 Tween 80 (g L^{-1}), X_7 Initial pH, X_8 incubation time (h), X_9 incubation temperature (°C), X_{10} inoculum size (%) and X_{11} moisture level (%).

2.6.3. Hydroxyl radical scavenging assay by EPS

The hydroxyl radical scavenging activity was measured by the method of Liu et al. (2009). The reaction mixture contained 1 ml of 0.75 mM 1,10-phenanthroline, 1.5 ml of 0.15 M sodium phosphate buffer (pH 7.4), 1 ml of 0.75 mM $FeSO_4$, 1 ml of H_2O_2 (0.01%, v/v) and 0.5 ml of the EPS (5–250 mg/l). After incubation at 37 °C for 30 min, the absorbance of the EPS was measured at 560 nm. Deionized water and ascorbic acid were used as the blank and control, respectively. The scavenging activity was determined as: hydroxyl radical (%) = $(A_{sample} - A_{blank})/(A_0 - A_{blank}) \times 100$, where A_0 was the absorbance of the deionized water instead of H_2O_2 and sample in the assay system.

2.7. Screening of significant factor using Plackett–Burman designing

Plackett–Burman experimental design, based on the following first-order model with Eq. (1), is a powerful method applied to reflect the relative importance of various medium components and cultivation parameters (Chen, Chen, Chen, Xu, & Jin, 2011).

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i \quad (1)$$

where Y represents the response (i.e., EPSs yield), β_0 is the model intercept, β_i is the linear coefficient, X_i is the level of the independent variable, and k is the number of involved variables.

Based on the result of the optimization of physical and nutritional variables on the production of the EPSs by *B. licheniformis* UD061 in solid state fermentation with one-factor-at-a-time method (data not shown), PB designing was employed to evaluate the important factors affecting the EPSs production by solid-state fermentation. Eleven physical and nutritional variables, i.e. NaCl, KH_2PO_4, K_2HPO, $MgSO_4{\cdot}7H_2O$, $MnSO_4$, Tween 80, initial pH, incubation time, incubation temperature, inoculum size and moisture level were selected to study their effect on EPSs production. This design was developed by the JMP software package (version 10.0.2, SAS Institute Inc., Cary, NC). The eleven independent variables were organized according to the Plackett–Burman design matrix. For each variable, a high (+1) and low (−1) level was tested (Table 1). Response value was measured in terms of EPSs activity.

2.8. Response surface methodology

Based on the analysis result of the Plackett–Burman design experiment, three variables, namely NaCl, initial $MgSO_4$, and moisture level were considered to have significant effect on EPSs production and thus used for further optimization by response surface methodology. Then, the response surface methodology was used to optimize the screened variables for EPSs production based on Box–Behnken design. A total of 17 runs were formulated using the statistical software package 'Design Expert 7.0' to optimize the range and levels of chosen variables. Regression analysis was performed on the data obtained from the design experiments with software package 'Design Expert' software (Version 7.0, Stat-Ease Inc., Minneapolis, USA).

3. Results and discussion

3.1. Composition of squid processing byproduct

The proximate composition of squid meat is 75–84% water, 13–22% crude protein, and 0.1–2.7% lipids (Sikorski & Kolodziejska, 1986). Although the squid-processing byproduct has higher moisture and lower protein than that of squid meat, it is a waste of biological resources as majority of squid by-products were dumped as wastes. Moreover, this material is discarded many times without undergoing further treatment, causing environmental pollution and ecological problems. Thus, ecologically and economically, the recovery and use of high-value by-products from these materials is very beneficial (Castro-Cesena, Sanchez-Saavedra, & Marquez-Rocha, 2012)

3.2. Screening of significant variables using a Plackett–Burman design

The Plackett–Burman design is a powerful method to select the significant variables from various medium components and cultivation parameters. The 20 run Plackett–Burman design was used to screen among eleven independent variables, which have main effects on EPSs production of *B. licheniformis* UD061 (Table 1). The statistical significance of the model coefficients was determined by Student's *t*-test (Table 2). As shown in Table 2, NaCl, $MgSO_4{\cdot}7H_2O$

Table 2
Estimated effect, regression coefficient and corresponding t and P values for Plackett–Burman design experiment.

Model term	Coefficients	Standard error	t ratio	P values
Intercept	8.78	0.24	35.92	<0.0001[a]
NaCl	0.74	0.24	3.04	0.0160[a]
KH_2PO_4	0.19	0.24	0.78	0.4559
K_2HPO_4	−0.12	0.24	−0.50	0.6325
$MgSO_4{\cdot}7H_2O$	0.64	0.24	2.61	0.0310[a]
$MnSO_4$	0.20	0.24	0.82	0.4336
Tween 80	0.31	0.24	1.27	0.2396
Initial pH	0.15	0.24	0.62	0.5552
Incubation time (h)	−0.13	0.24	−0.52	0.6160
Incubation temperature (°C)	0.06	0.24	0.24	0.8138
Inoculum size (%)	−0.11	0.24	−0.43	0.6774
Moisture level (%)	1.17	0.24	4.81	0.0013[a]

[a] Indicates model terms are significant.

and moisture level appeared to be the most significant variables affecting EPSs production. Moisture level was found to be the most influencing factor followed by NaCl and $MgSO_4{\cdot}7H_2O$, in the medium. So, these three independent variables were selected for further optimization by response surface methodology.

3.3. Response surface methodology

The three significant factors influencing EPSs production were further optimized using Box–Behnken design of RSM. A total of 17 runs with different combination of NaCl, $MgSO_4{\cdot}7H_2O$ and moisture level were designed (Table 3). The observed and predicted responses of the twenty experiments are given in Table 3.

The values of regression coefficients were calculated, and the fitted equation (in terms of coded values) for predicting EPSs production (Y) was as given below regardless of the significance of the coefficients:

$$Y = 14.57 + 0.29X_1 + 0.30X_2 + 0.54X_3 + 0.26X_2X_3 - 1.91X_1^2 - 2.24X_2^2 - 2.85X_3^2$$

where Y is the predicted EPSs yield, X_1 is NaCl, X_2 is $MgSO_4{\cdot}7H_2O$, and X_3 is moisture level.

The analysis of variance (ANOVA) was conducted to test the significance of the fit of the second-order polynomial equation for the experimental data as shown in Table 4. The fit of the model was checked by the coefficient of determination R^2, which was calculated to be 0.9984, indicating that 99.84% of the variability in the response could be explained by the model. The model F-value of 489.42 implied that the model was significant. It indicates a good agreement between experimental and predicted values and implies that the mathematical model is very reliable for hydrogen production in the present study. The P-values are used as a tool to check significance of each variable, which also indicated the interaction strength between each independent. The larger the magnitude of t-test and smaller the P-value, the more significant is the corresponding coefficient (Li et al., 2009). In this case, seven model terms (X_1, X_2, X_3, X_2X_3, X_1^2, X_2^2 and X_3^2) were significant model terms at 99% of probability level for EPSs production. Significant interactions were noted between $MgSO_4{\cdot}7H_2O$ and moisture level. The other interactions among variables were not significant (Table 5).

Table 3
Experimental design and results of Box–Behnken optimisation experiment.

Trials	X_1	X_2	X_3	EPSs (U/gds)	
				Observed	Predicted
1	3	0.6	60	10.03 ± 0.26	9.88
2	5	0.6	60	10.35 ± 0.31	10.36
3	3	0.8	60	10.39 ± 0.30	10.38
4	5	0.8	60	10.91 ± 0.25	11.06
5	3	0.7	55	9.01 ± 0.31	9.09
6	5	0.7	55	9.52 ± 0.26	9.44
7	3	0.7	65	9.86 ± 0.19	9.94
8	5	0.7	65	10.82 ± 0.27	10.74
9	4	0.6	55	8.83 ± 0.22	8.90
10	4	0.8	55	9.05 ± 0.25	8.98
11	4	0.6	65	9.39 ± 0.30	9.46
12	4	0.8	65	10.65 ± 0.29	10.58
13	4	0.7	60	14.52 ± 0.32	14.57
14	4	0.7	60	14.69 ± 0.35	14.57
15	4	0.7	60	14.57 ± 0.29	14.57
16	4	0.7	60	14.65 ± 0.33	14.57
17	4	0.7	60	14.43 ± 0.36	14.57

X_1 NaCl ($g\,L^{-1}$), X_2 $MgSO_4{\cdot}7H_2O$ ($g\,L^{-1}$) and X_3 moisture level (%).

Table 4
Analysis of variance (ANOVA) for the fitted quadratic polynomial model for optimization of EPSs production.

Source	SS	DF	MS	F-value	Prob > F
Model	82.88	9	9.21	553.62	<0.0001
Lack of fit	0.15	5	0.030	2.79	0.1711
Pure error	0.043	4	0.011		
Total	83.01	16			

$R^2 = 0.9977$; $R_{Adj}^2 = 0.9959$; $R_{Pre}^2 = 0.9892$; SS, sum of squares; DF, degrees of freedom; and MS, mean square.

The optimum level of each variable and the effect of their interactions on EPSs production were studied by plotting three-dimensional response surfaces and two-dimensional contours against any two independent variables, while the other variables were kept constant at the level of zero (Fig. 1). Fig. 1(a_1 and b_1), which gives the interaction between NaCl and $MgSO_4{\cdot}7H_2O$, shows that lower and higher levels of both NaCl and $MgSO_4{\cdot}7H_2O$ did not lead to higher EPSs yield. Roberson and Firestone (1992) and Seesuriyachan et al. (2012) also reported that the presence of NaCl led to an accumulation of low molecular weight carbohydrates. Fig. 1(a_2 and b_2) shows that EPSs yield increased evidently with increasing of moisture level from 55 to 61%, but beyond 61%, the yield of EPSs decreased slowly as the moisture level ascended. The initial moisture content is a critical factor for solid-state fermentation processes because this variable has influence on growth, biosynthesis and secretion of different metabolites. The control of this parameter could be used to modify microbial metabolic production and its excretion. As the optimal value of moisture content depends on both the microorganism and the solid matrix used, for economical production, the microorganism should be grown in optimal moisture levels either for maximizing the growth or metabolite production depending on the application. Generally, microorganisms, when cultivated on agro industrial residues during SSF, grows best when the substrate moisture content is commonly between 50 and 75% (Perez-Guerra, Torrado-Agrasar, Lopez-Macias, & Pastrana, 2003). It can be seen from Fig. 1(a_3 and b_3) that the yield of EPSs increased evidently with increasing of

Table 5
Results of regression analysis of the second-order polynomial model for optimization of EPSs production.

Factor	Coefficient estimate	Standard error	F-value	$P > F$[a]
Intercept	14.57	0.065	553.62	<0.0001
X_1	0.29	0.052	31.21	0.0006
X_2	0.30	0.052	33.69	0.0005
X_3	0.54	0.052	108.66	<0.0001
X_2X_3	0.26	0.073	12.65	0.0068
X_1^2	−1.91	0.071	722.36	<0.0001
X_2^2	−2.24	0.071	986.19	<0.0001
X_3^2	−2.85	0.071	1605.71	<0.0001

[a] Statistically significant at 99% of probability level.

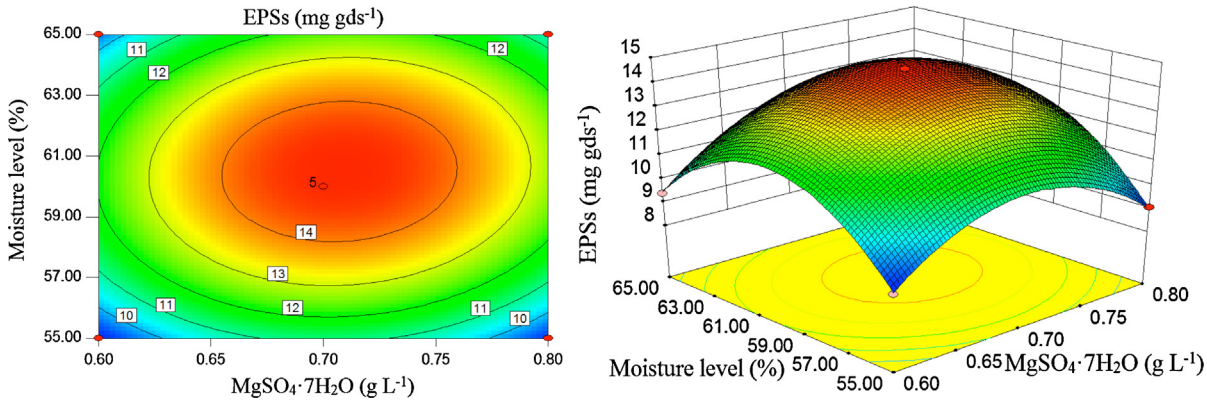

Fig. 1. The 3D-plot and 2D-projection showing the interaction between $MgSO_4{\cdot}7H_2O$ and moisture level.

Table 6-
Antioxidant activities in vitro of EPS of different strains.

Strain	Reducing power (A_{700})	Superoxide anion scavenging (%)	Hydroxyl radical scavenging (%)
UD061[b]	0.35 ± 0.03	42.55 ± 3.18	50.91 ± 3.79
UD061[a]	0.34 ± 0.04	42.53 ± 3.02	51.06 ± 3.68

[a] After optimization.
[b] Before optimization.

$MgSO_4{\cdot}7H_2O$ from 0.6 to 0.7 g L^{-1}, but beyond 0.7 g L^{-1}, the yield of EPSs decreased as the $MgSO_4{\cdot}7H_2O$ ascended.

As analysis from the present study, the model predicted a maximum EPSs production could be achieved when the NaCl, $MgSO_4{\cdot}7H_2O$, and moisture level were set at 4.08, 0.71 and 60.49%, respectively. The maximum predicted value of EPSs yield was 14.62 mg/gds.

3.4. Verification of the models

The suitability of the model equation for predicting the optimum response values was tested by using the selected optimal conditions. Under these suggested conditions, the mean value of the EPSs yield was 14.68 mg gds^{-1}, which was in agreement with the predicted value. It was about 1.32-fold increase compared with that using the original medium and fermentation conditions (6.33 mg gds^{-1}). Hence, the model developed in the current study is accurate and reliable for predicting the production of EPSs by *B. licheniformis* UD061 in solid-state fermentation.

3.5. Antioxidant activity of EPSs

Antioxidant activity is one of the main characters of exopolysaccharidess from *B. licheniformis* UD061. The antioxidant activity of the exopolysaccharides acquired before and after the optimization were investigated to see whether alteration of the media and cultural conditions change the antioxidant activity of the exopolysaccharides. The antioxidant activities of the exopolysaccharidess from the *B. licheniformis* UD061 before or after optimization were compared and the results indicated that the antioxidant activities did not change significantly (Table 6).

Media and cultural conditions not only significantly affect the yield of the exopolysaccharides, but also affect the quality of exopolysaccharides including molecular weight composition and distribution due to change in the bioactivity of the exopolysaccharides. Hence, the antioxidant activity of the exopolysaccharides may be changed if media and cultural conditions were optimized to acquire the high yield of production.

4. Conclusions

The present study lead to the optimization of key process parameters for increased EPSs yield in solid state fermentation with squid processing byproduct and maize cob and solid matrix with Plackett–Burman and RSM designs. Response surface analysis revealed that the maximum EPSs yield of 14.68 mg gds^{-1} was obtained under the optimal conditions of 4.08 g L^{-1} NaCl, 0.71 g L^{-1} $MgSO_4{\cdot}7H_2O$, and 60.49% moisture level. Thus our results suggested that the squid processing byproduct could be used to produce EPSs in solid-state fermentation, therefore reducing the production cost of EPSs, decreasing possible environmental problems, as well as adding economic value to squid processing byproduct.

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