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Comparison of Statistical Methods for Optimization of Salts in Medium for Production of Carboxymethylcellulase of *Bacillus amyloliquefaciens* DL-3 by a Recombinant *E. coli* JM109/DL-3

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The optimal concentrations of salts in medium for cell growth and the production of carboxymethylcellulase (CMCase) by a recombinant *E. coli* JM109/DL-3 were established using two statistical methods: orthogonal array method (OAM) and response surface method (RSM). The analysis of variance (ANOVA) of data based on OAM indicated that K_2HPO_4 gave maximum sum of square (*S*) and percentage contribution (*P*) for cell growth as well as production of CMCase. The optimal concentrations of K_2HPO_4, NaCl, $MgSO_4 \cdot 7H_2O$, and $(NH_4)_2SO_4$ in medium for cell growth extracted by Qualitek-4 (W32b) Software were 10.0, 1.0, 0.2, and 0.6 g/l, respectively, whereas those for the production of CMCase by *E. coli* JM109/DL-3 were 5.0, 1.0, 0.4, and 0.6 g/l. The analysis of variance (ANOVA) resulting from RSM indicated that a highly significant salt for cell growth was K_2HPO_4 ("probe>F" less than 0.0001), whereas K_2HPO_4 and $MgSO_4 \cdot 7H_2O$ were significant for the production of CMCase. The optimal concentrations of K_2HPO_4, NaCl, $MgSO_4 \cdot 7H_2O$, and $(NH_4)_2SO_4$ for cell growth extracted by Design Expert Software were 7.44, 1.08, 0.22, and 0.88 g/l, respectively, whereas those for production of CMCase were 5.84, 0.69, 0.28, and 0.54 g/l. The optimal concentrations of salts and their influences on cell growth and production of CMCase extracted by OAM were almost the same as those by RSM. Production of CMCase by a recombinant *E. coli* JM109/DL-3 under optimized concentration of salts was 1.93 times higher than that by *Bacillus amyloliquifaciens* DL-3.

Key words : Carboxymethylcellulase, *E. coli* JM109, optimization, orthogonal array method, response surface method, salts

Introduction

Conversion of cellulosic materials to fermentable sugars represents a major challenge in global efforts to utilize renewable resources [2,3]. The complete enzymatic hydrolysis of cellulosic materials for production of fermentable sugars needs at least three different types of cellulases; endoglucanase (carboxymethylcellulase), exocellobiohydrolase (avicelase), and β-glucosidase [28]. The enzymatic saccharification of lignocellulosic materials for the production of ethanol was performed by commercial cellulases, in which the major cellulase was carboxymethylcellulase (CMCase) [25,26]. A major restriction in enzymatic saccharification of cellulosic biomass for the production of fermentable sugars is low productivity and the cost of cellulases [23].

Most commercial cellulases have been produced by *Aspergillus* and *Trichoderma* species with solid-state cultures [7]. Many studies on types of strains, culture conditions, and substrates for production of cellulases have been reported [12,15]. However, there have been few reports on optimization of mineral salts in the medium for production of cellulases. Genes encoding cellulases of *Trichoderma reesei* was cloned and expressed in *Aspergillus oryzae* [24]. The production of cellulases by recombinant *A. oryzae* was several to hundreds times higher than *T. reesei*. The full-length gene encoding the CMCase of *B. amyloliquefaciens* DL-3 was cloned in *E. coli* JM109 in the previous report [17].

The optimization of culture conditions by the traditional one-factor-at-a-time method requires a considerable amount of work and time. An alternate strategy is a statistical approach such as orthogonal array method (OAM) and response surface method (RSM), involving the minimum number of experiments for a large number of factors [4,22]. In this study, OAM and RSM were used to optimize concentrations of salts in medium for production of CMCase

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of *B. amlyloliquefaciens* DL-3 by a recombinant *E. coli* JM109/DL-3 and results from two methods were compared.

Materials and Methods

Bacterial strain and medium

E. coli JM109/DL-3 was used to produce carboxymethylcellulase (CMCase) in this study. The open reading frame (ORF) of the cloned CMCase gene of *B. amyloliquefaciens* DL-3 consists of 1497 nucleotides encoding a protein of 499 amino acids with a predicted molecular weight of 55,118 Da [17]. *E. coli* JM109/DL-3 was grown at 37℃ in Luria-Bertani (LB) broth supplemented with 50 μg/ml ampicillin.

Production of CMCase by *E. coli* JM109/DL-3

Starter cultures were prepared as described in the previous report [17]. The main culture was carried out in a medium containing 58 g/l rice bran, 5.0 g/l tryptone, 5.0 g/l K_2HPO_4, 1.0 g/l NaCl, 0.2 g/l $MgSO_4 \cdot 7H_2O$, and 0.6 g/l $(NH_4)_2SO_4$ at 37℃ for 3 d under aerobic conditions.

Experimental design using orthogonal array method

Design of experiments (DOE) was performed based on orthogonal array method for optimization of four slats - K_2HPO_4, NaCl, $MgSO_4 \cdot 7H_2O$, and $(NH_4)_2SO_4$ - in the medium using Qualitek-4 (W32b) Software (Nutek, Inc., Bloomfield Hills, USA), which was used for automatic design of experiments, analysis of results, and calculation of interactions among different factors [22]. The L_{16} (4^4) orthogonal array experiment used in this study had 4 factors and each factor had 4 different levels. These trials were done in three replicates. Results from optimization for production of CMCase were statistically analyzed using one-way analysis of variance (ANOVA).

Experimental design using response surface method

The K_2HPO_4 (X_1), NaCl (X_2), $MgSO_4 \cdot 7H_2O$ (X_3), and $(NH_4)_2SO_4$ (X_4) were chosen as the independent variables and each variable was designated as −1, 0, and 1, respectively. Cell growth (Y_1, g/l) and CMCase activity (Y_2, U/ml) were used as a dependent output variable. The total number of experiments was 30 ($=2^k+2k+6$), where k is the number of independent variables [18]. Values of cell growth and production of CMCase were taken as the responses of the design experiment. Statistical analysis of the model was performed to evaluate the analysis of variance (ANOVA). A multiple regression analysis of the data was carried out with the statistical software, Design-Expert Version 7.1.6 (Stat-Ease Inc., Minneapolis, USA).

Analytical methods

Dry cells weight was measured as described in the previous report [12]. Activity of the CMCase produced by *E. coli* JM109/DL-3 was determined based on the release of reducing sugar from CMC using the 3,5-dinitrosalicylic acid (DNS) method, as described in the previous report [14]. Glucose (Sigma-Aldrich, UK) was used to prepare a calibration curve. One unit of each CMCase was defined as the amount of enzyme that released 1 μmol of glucose equivalent per minute under the assay condition.

Results and Discussion

Optimization of salts by one-factor-at-a-time method

The optimal concentrations of K_2HPO_4, NaCl, $MgSO_4 \cdot 7H_2O$, and $(NH_4)_2SO_4$ in the medium were investigated using 'one-factor-at-a time' experiments. Composition of basic medium and culture conditions for 'one-factor-at a time' experiment were 58.0 g/l rice bran, 5.0 g/l tryptone, initial pH of 7.1, and temperature of 37℃. The optimal concentrations of K_2HPO_4, NaCl, $MgSO_4 \cdot 7H_2O$, and $(NH_4)_2SO_4$ for cell growth were 7.5, 1.0, 0.25, and 1.0 g/l, respectively, whereas those for the production of CMCase by *E. coli* JM109/DL-3 were 5.0, 1.0, 0.25, and 0.5 g/l, as shown in Fig. 1. The optimal concentrations of four salts for cell growth were different from those for production of CMCase. The optimal concentrations of four salts for cell growth and the production of CMCase by *B. amyloliquefaciens* DL-3 were 5.0, 1.0, 0.2, and 0.6 g/l, respectively [12].

Optimization of salts using orthogonal array method

The simultaneous effect of K_2HPO_4, NaCl, $MgSO_4 \cdot 7H_2O$, and $(NH_4)_2SO_4$ in the medium on cell growth and the production of CMCase by *E. coli* JM109/DL-3 was investigated using L_{16} (4^4) orthogonal array design, as shown in Table 1. Cell growths and the productions of CMCase by *E. coli* JM109/DL-3 from sixteen different conditions ranged from 2.35 to 2.56 g/l and from 306.3 to 398.9 U/ml, as shown in Table 2. The analysis of variance (ANOVA) for experimental results obtained by L_{16} (4^4) orthogonal array

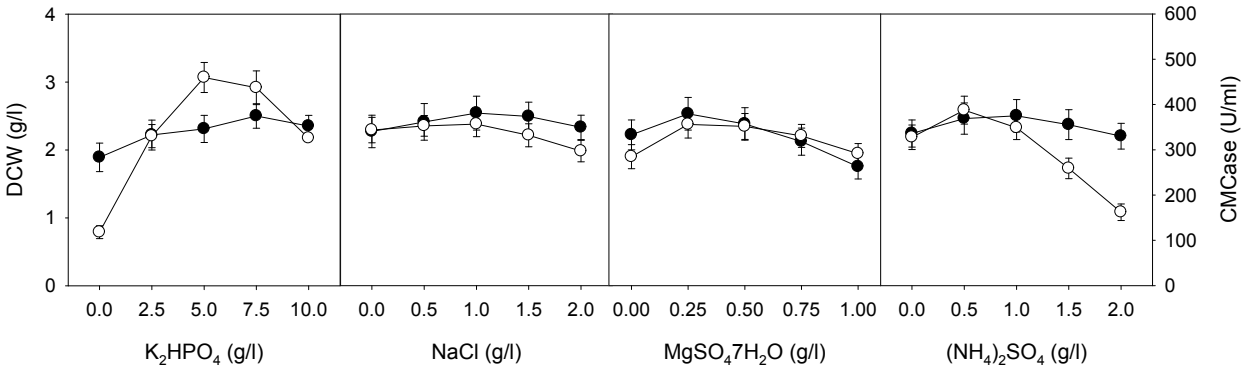

Fig. 1. Effect of four salts in the medium on cell growth and production of CMCase by *E. coli* JM109/DL-3 (●, Dry cell weight and ○, CMCase activity).

Table 1. Factors and their levels in the orthogonal array experiment using Qualitek-4 (W32b) software based on orthogonal array method

Factor	Level 1	Level 2	Level 3	Level 4
K_2HPO_4 (g/l)	2.5	5.0	7.5	10.0
NaCl (g/l)	0.5	1.0	1.5	2.0
$MgSO_4 \cdot {}_{7H2}O$ (g/l)	0.1	0.2	0.4	0.8
$(NH_4)_2SO_4$ (g/l)	0.3	0.6	1.2	2.4

design indicated optimal levels of each salt for cell growth and production of CMCase, as shown in Table 3. The order of each salt's effect (contribution percent) on cell growth was found to be K_2HPO_4, $MgSO_4 \cdot {}_{7H2}O$, NaCl, and $(NH_4)_2SO_4$, whereas that on production of CMCase was K_2HPO_4, $(NH_4)_2SO_4$, $MgSO_4 \cdot 7H_2O$, and NaCl, as shown in Fig. 2. Potassium phosphate was the most significant factor for cell growth as well as the production of CMCase by *E. coli* JM109/DL-3. The optimal concentrations of K_2HPO_4, NaCl, $MgSO_4 \cdot 7H_2O$, and $(NH_4)_2SO_4$ in the medium for cell growth extracted by Qualitek-4 (W32b) software were 10.0, 1.0, 0.2, and 0.6 g/l, respectively, whereas those for the production of CMCase by *E. coli* JM109/DL-3 were 5.0, 1.0, 0.4, and 0.6 g/l. The expected maximal cell growth and production of CMCase were 2.58 g/l and 413.8 U/ml.

Optimization of salts using response surface method

The effects of K_2HPO_4, NaCl, $MgSO_4 \cdot 7H_2O$, and $(NH_4)_2SO_4$ in the medium on cell growth and the production of CMCase by *E. coli* JM109/DL-3 were also investigated using response surface method (RSM). Levels of K_2HPO_4,

Table 2. Simultaneous effect of mineral salts in the medium on cell growth and the production of CMCase by *E. coli* JM109/DL-3 designed using Qualitek-4 (W32b) software based on L_{16} (4^4) orthogonal array experiment

Run	K_2HPO_4 (g/l)	NaCl (g/l)	$MgSO4 \cdot 7H_2O$ (g/l)	$(NH_4)_2SO_4$ (g/l)	DCW (g/l)	CMCase (U/ml)
1	2.5	0.5	0.1	0.3	2.35	311.3
2	2.5	1.0	0.2	0.6	2.46	346.2
3	2.5	1.5	0.4	1.2	2.43	324.3
4	2.5	2.0	0.8	2.4	2.36	306.3
5	5.0	0.5	0.2	1.2	2.41	386.6
6	5.0	1.0	0.1	2.4	2.36	360.8
7	5.0	1.5	0.8	0.3	2.39	391.2
8	5.0	2.0	0.4	0.6	2.40	398.9
9	7.5	0.5	0.4	2.4	2.41	367.0
10	7.5	1.0	0.8	1.2	2.45	372.0
11	7.5	1.5	0.1	0.6	2.43	364.1
12	7.5	2.0	0.2	0.3	2.41	361.5
13	10.0	0.5	0.8	0.6	2.51	364.3
14	10.0	1.0	0.4	0.3	2.54	353.9
15	10.0	1.5	0.2	2.4	2.56	328.7
16	10.0	2.0	0.1	1.2	2.50	306.6

Table 3. Analysis of variance (ANOVA) of cell growth and the production of CMCase by *E. coli* JM109/DL-3 analyzed using Qualitek-4 (W32b) software based on L_{16} (4^4) orthogonal array experiment

	Factor	Degree of freedom (*f*)	Sums of squares (*S*)	Variance (*V*)	F-ratio (*F*)	Pure sum (*S'*)	Contribution (*P*, %)	Optimal level
DCW	K_2HPO_4	3	0.05	0.02	111.28	0.05	77.36	4
	NaCl	3	0.00	0.00	10.67	0.00	6.78	2
	$MgSO4 \cdot 7H_2O$	3	0.01	0.00	13.13	0.01	8.51	2
	$(NH_4)_2SO_4$	3	0.00	0.00	6.47	0.00	3.84	2
	Other/error	3	0.00	0.00	-	-	3.51	-
	Total	15	0.06	-	-	-	100.00	-
CMCase	K_2HPO_4	3	9321.45	3107.15	93214524.2	9321.45	70.78	2
	NaCl	3	559.83	186.61	5598314.82	559.83	4.25	2
	$MgSO4 \cdot 7H_2O$	3	1602.65	534.22	16026479.9	1602.65	12.17	3
	$(NH_4)_2SO_4$	3	1684.90	561.63	16848953.7	1684.90	12.79	2
	Other/error	3	0.00	0.00	-	-	0.00	-
	Total	15	13168.83	-	-	-	100.00	-

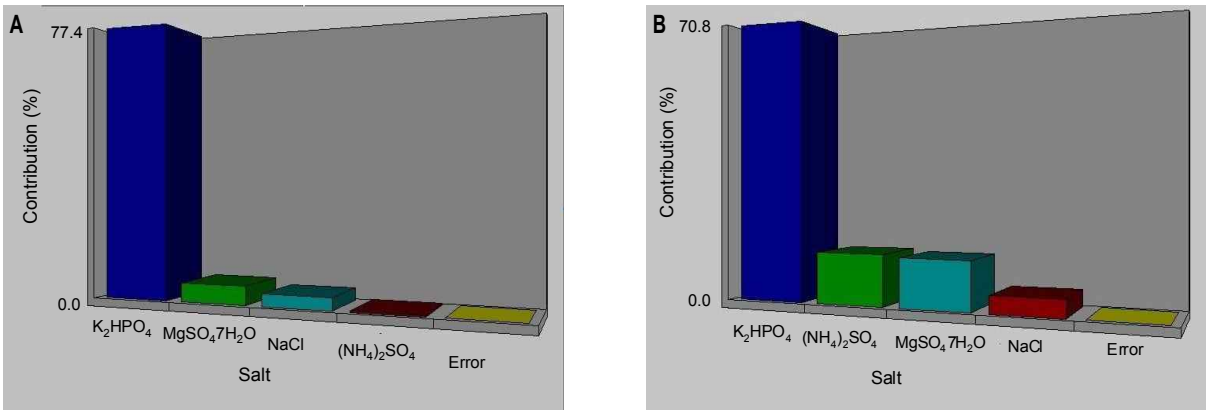

Fig. 2. The percentage contributions of four salts for cell growth (A) and the production of CMCase (B) by *E. coli* JM109/DL-3 analyzed using Qualitek-4 (W32b) software. X-axis shows factors and Y-axis shows percentage contributions of each factor.

Table 4. Process variables used central composite design (CCD) with actual factor levels corresponding to coded factor levels using Design Expert software based on response surface method

Variables	Coded levels			
	Symbol	-1	0	1
K_2HPO_4 (g/l)	X_1	2.5	5.0	7.5
NaCl (g/l)	X_2	0.5	1.0	1.5
$MgSO_4 \cdot 7H_2O$ (g/l)	X_3	0.1	0.2	0.3
$(NH_4)_2SO_4$ (g/l)	X_4	0.3	0.6	0.9

NaCl, $MgSO_4 \cdot 7H_2O$, and $(NH_4)_2SO_4$ were as shown in Table 4. The results of central composite design (CCD) experiments consisted of experimental data to investigate effects of four independent variables, as shown in Table 5. Cell growths and the productions of CMCase by *E. coli* JM109/DL-3 from 30 different conditions ranged from 2.34 to 2.50 g/l and from 328.6 to 406.2 U/ml.

The analysis of variance (ANOVA) of cell growth indicated that the model term of X_1 was highly significant ("probe>F" less than 0.0001) and those of X_3, X_2^2, and X_3^2 were significant ("probe>F" less than 0.0500) for cell growth of *E. coli* JM109/DL-3, as shown in Table 6. Multiple regression analysis of the experimental data gave the following second-order polynomial equation in terms of coded factors (1):

$$Y_1 = 2.46 + 0.039X_1 + 0.010X_2 + 0.015X_3 + 0.015X_4$$

Table 5. Central composite design and determined response values using Design Expert software based on response surface method

Run	X_1	X_2	X_3	X_4	Y_1 (g/l)	Y_2 (U/ml)
1	5.0	1.0	0.2	0.0	2.40	378.2
2	2.5	0.5	0.3	0.9	2.41	356.9
3	2.5	0.5	0.1	0.9	2.38	333.8
4	5.0	2.0	0.2	0.6	2.41	381.1
5	5.0	1.0	0.2	0.6	2.46	396.0
6	7.5	0.5	0.3	0.3	2.45	383.5
7	0.0	1.0	0.2	0.6	2.36	305.6
8	5.0	1.0	0.2	0.6	2.44	401.2
9	2.5	0.5	0.3	0.3	2.38	356.9
10	2.5	1.5	0.1	0.3	2.36	328.6
11	2.5	1.5	0.1	0.9	2.40	328.6
12	7.5	1.5	0.3	0.3	2.47	378.3
13	5.0	1.0	0.2	1.2	2.46	375.0
14	5.0	1.0	0.2	0.6	2.45	392.6
15	7.5	0.5	0.3	0.9	2.48	383.5
16	2.5	1.5	0.3	0.3	2.40	351.7
17	5.0	1.0	0.2	0.6	2.44	406.2
18	2.5	1.5	0.3	0.9	2.43	351.7
19	5.0	0.0	0.2	0.6	2.39	392.7
20	5.0	1.0	0.2	0.6	2.46	394.3
21	7.5	1.5	0.3	0.9	2.50	378.3
22	5.0	1.0	0.2	0.6	2.48	388.8
23	7.5	0.5	0.1	0.3	2.41	360.5
24	2.5	0.5	0.1	0.3	2.34	333.8
25	5.0	1.0	0.0	0.6	2.39	357.5
26	5.0	1.0	0.4	0.6	2.45	401.3
27	7.5	0.5	0.1	0.9	2.45	360.5
28	10.0	1.0	0.2	0.6	2.56	362.5
29	7.5	1.5	0.1	0.9	2.47	355.3
30	7.5	1.5	0.1	0.3	2.43	355.3

$$+ 0.002X_1X_2 - 0.002X_1X_3 - 0.002X_1X_4 + 0.002X_2X_3 + 0.002X_2X_4 - 0.004X_3X_4 - 0.002X_1^2\ 1 - 0.017X_2^2 - 0.012X_3^2 - 0.010X_4^2 \quad (1)$$

The regression equation obtained from analysis of variance (ANOVA) indicated that the multiple correlation coefficient of R^2 is 0.9330. The model can explain 93.30% variation in the response. The model *F*-value of 14.93 implied that this model was significant. The value of the adjusted coefficient (Adj. R^2=0.8705) was very high to advocate for a high significance of this model [13]. The predicted coefficient (Pred. R^2=0.7515) was in reasonable agreement with the Adj. R^2 of 0.8705. From the statistical results obtained, it was shown that the above models were adequate to predict cell growth of *E. coli* JM109/DL-3 within the range of variables studied. The optimal conditions of K_2HPO_4, NaCl, $MgSO_4 \cdot 7H_2O$, and $(NH_4)_2SO_4$ for cell growth extracted by Design Expert Software were 7.4,4 1.08, 0.22, and 0.88 g/l, respectively. The maximum cell growth of 2.51 g/l was predicted by the model.

The ANOVA of the production of CMCase also indicated that the model terms of X_1, X_3, and X_1^2 were highly significant for the production of CMCase by *E. coli* JM109/DL-3. Multiple regression analysis of the experimental data gave the following second-order polynomial equation (2).

$$Y_2=397.72 + 13.63X_1 - 2.70X_2 + 11.33X_3 - 0.27X_4 + 0.03X_1X_3 - 18.11X_1^2 - 4.90X_2^2 - 6.77X_3^2 - 7.47X_4^2 \quad (2)$$

The regression equation obtained from the ANOVA indicated that the multiple correlation coefficient of R^2 is 0.9437. The model can explain 94.37% variation in the response. The model *F*-value of 17.95 implied that this model was significant. The value of the adjusted determination coefficient (Adj. R^2=0.8911) is also very high to advocate for

Table 6. Parameter estimates and analysis of variance (ANOVA) of the design for cell growth and production of CMCase by *E. coli* JM109/DL-3

	Source of variation	Degree of freedom	Sum of squares	Mean squares	*F*-value	Probe>F
Cell growth	Model	14	0.061	0.004	14.93	<0.0001
	X_1	1	0.036	0.036	123.9	<0.0001
	X_2	1	0.002	0.002	7.53	0.0151
	X_3	1	0.006	0.006	19.48	0.0005
	X_4	1	0.006	0.006	19.48	0.0005
	X_1^2	1	0.000	0.000	0.23	0.6394
	X_2^2	1	0.007	0.007	25.70	0.0001
	X_3^2	1	0.004	0.004	12.52	0.0030
	X_4^2	1	0.002	0.002	7.69	0.0142
	Error	5	0.002	0.000	-	-
	Total	29	0.066	-	-	-
MCase	Model	14	17791.720	1270.840	17.95	<0.0001
	X_1	1	4455.370	4455.370	62.94	<0.0001
	X_2	1	174.960	174.960	2.47	0.1368
	X_3	1	3082.670	3082.670	43.55	<0.0001
	X_4	1	1.710	1.710	0.02	0.8787
	X_1^2	1	8996.220	8996.220	127.09	<0.0001
	X_2^2	1	658.000	658.000	9.30	0.0081
	X_3^2	1	1258.210	1258.210	17.77	0.0007
	X_4^2	1	1531.730	1531.730	21.64	0.0003
	Error	5	127.85	25.570	-	-
	Total	29	18853.550	-	-	-

a high significance of this model. The predicted determination of coefficient of 0.7049 is in reasonable agreement with the Adj. R^2 of 0.8911. The optimal concentrations of K_2HPO_4, NaCl, $MgSO_4 \cdot 7H_2O$, and $(NH_4)_2SO_4$ for production of CMCase were 5.84, 0.69, 0.28, and 0.54 g/l, respectively. The maximum production of CMCase of 404.6 U/ml was predicted by the model. The experimentally determined values of cell growth and the production of CMCase by *E. coli* JM109/DL-3 were in close agreement with the statistically predicted ones, confirming the model's authenticity, as shown in Fig. 3. The points clustered around the diagonal line which indicated the good fit of the model.

In the conventional one-factor-at-a-time method for optimizing fermentation conditions, one independent variable is changed while all others are held at constant

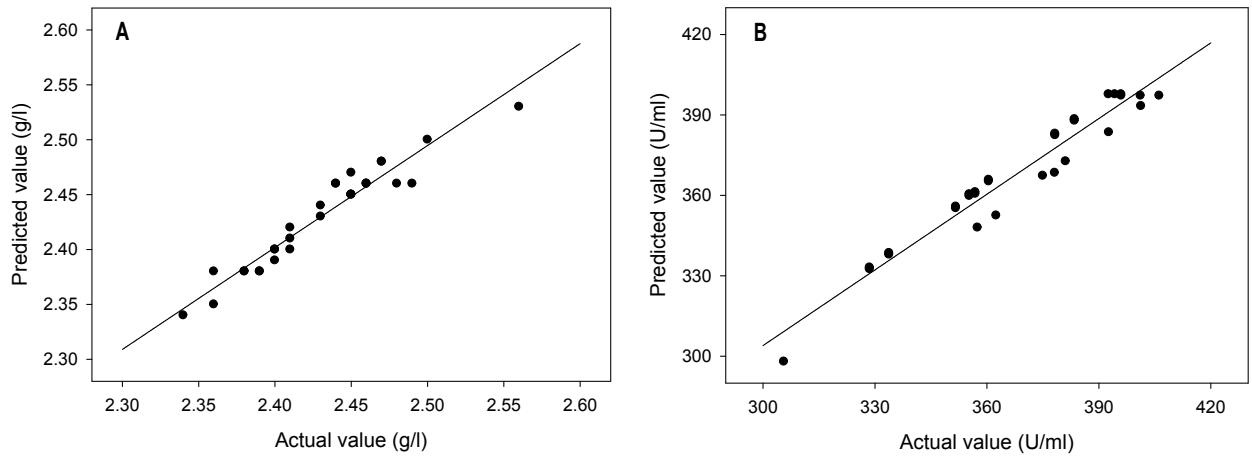

Fig. 3. Distribution of experimentally determined values versus statistically predicted ones of cell growth (A) and production of CMCase (B) by *E. coli* JM109/DL-3.

Table 7. Comparison of optimal concentrations of salts estimated by statistical methods for cell growth and production of CMCase by *E. coli* JM109/DL-3 and *B. amyloliquefaciens* DL-3

Optimal concentration	*E. coli* JM109/DL-3						*B. amyloliquefaciens* DL-3	
	One-factor-at-a-time method		Orthogonal array method		Response surface method		One-factor-at-a-time method[1)]	
	DCW	CMCase	DCW	CMCase	DCW	CMCase	DCW	CMCase
K_2HPO_4 (g/l)	7.5	5.0	10.0	5.0	7.44	5.84	5.0	5.0
NaCl (g/l)	1.0	1.0	1.0	1.0	1.08	0.69	1.0	1.0
$MgSO_4 \cdot 7H_2O$ (g/l)	0.25	0.25	0.2	0.4	0.22	0.28	0.2	0.2
$(NH_4)_2SO_4$ (g/l)	1.0	0.5	0.6	0.6	0.88	0.54	0.6	0.6
Maximal production	2.54 g/l	399.0 U/ml	2.58 g/l	413.8 U/ml	2.51 g/l	404.6 U/ml	2.96 g/l	211.0 U/ml

[1)]Previous repot [17]

levels. This one-dimensional evaluation is tedious and time-consuming and it usually does not lead to the determination of optimal conditions, mainly due to ignoring interactions [1]. However, a full factorial design results in a large number of experiments [21]. As a solution, fractional factorial experimental designs, including Placket-Burman design, OAM, and RSM have been introduced, which reduce the number of tests while giving reliable results [27]. Taguchi's design uses a special set of arrays called orthogonal arrays, which gives the minimal number of experiments, and provides full information on all factors that affect the performance parameter [21]. RSM employing the central composite design (CCD) is the collection of mathematical and statistical techniques, which can be used to identify the effect of individual variables, evaluate the relative significance, and expert optimal conditions [8].

The significant factors on cell growth and production of CMCase based on data from OAM were coincided with those from RSM. Moreover, two methods indicated that the optimal concentrations of four salts for cell growth were different from those for production of CMCase. The optimal concentrations of salts in the medium for cell growth were reported to be different from those for the production of pullulan by *A. pullulan* [6]. Moreover, the optimal concentrations of salts for cell growth and production of pullulan varied with concentrations of carbon and nitrogen sources [5]. Utilization rates of substrates were increased by the optimized concentration of salts, which resulted in enhanced cell growth and production of pullulan [20]. Two statistical methods also indicated that potassium phosphate was the most significant factor for cell growth and production of CMCase. Potassium phosphate is one of the major salts in the medium for productions of microbial polysaccharides and enzymes as well as a well-known ingredient in buffer solutions [11,16,19]. Sodium chloride was reported to be used as a physiological modulator of biosynthetic pathway of biopolymers [10]. Magnesium sulfate added to medium assisted spore germination and initial growth of *A. fisheri*, which resulted in 1.9 fold increased production of xylanase [19]. Higher cell growth and production of key enzymes for the production of 1,3-propanediol were obtained when ammonium chloride was added in the medium [9]. Potassium phosphate seemed to act as a mineral salt for cell growth of *P. aquimaris* LBH-10 as well as a pH stabilizer in the medium, which could enhance the production of CMCase [17]. Ammonium sulfate used in this study seems to be mainly used as a nitrogen source for cell growth.

The optimal concentrations of salts in the medium for cell growth and the production of CMCase by *P. aquimaris* LBH-10 were established using OAM and RSM in this study and compared with previously used concentrations of salts, as shown in Table 7. Production of CMCase by a recombinant *E. coli* JM109/DL-3 under optimized concentration of salts was 1.93 times higher than that by *B. amlyloliquifaciens* DL-3. The optimal concentrations of salts and their influences on cell growth and production of CMCase extracted by OAM were almost the same as those by RSM. However, each method gave its own detailed information such as sum of squares, mean square, and F value on effects of salts on cell growth and production of CMCase. The number of combination at an experiment can be minimized by reduced levels of factors using Qualitek-4 Software based on OAM, whereas the predicted optimal conditions can be extracted using Design Expert Software based on RSM.

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초록 : *Bacillus amyloliquefaciens* DL-3의 carboxymethylcellulase를 재조합 균주 *E. coli* JM109/DL-3에서 생산하는 배지의 염 농도를 최적화하기 위한 통계학적 실험 방법의 비교

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재조합 균주인 *E. coli* JM109/DL-3를 사용하여 carboxymethylcellulase를 생산하기 위한 배지의 최적 염 농도를 orthogonal array method (OAM)과 response surface method (RSM) 등과 같은 통계학적인 방법으로 확립하고 그 결과를 비교하였다. OAM에 기초를 한 Qualitek-4 Software를 사용하여 실험을 계획하고, 그 결과를 분석한 결과는 K_2HPO_4가 균체의 생장 및 carboxymethylcellulase의 생산에 미치는 영향이 가장 크다는 사실을 확인하였다. 균체의 생육에 최적인 K_2HPO_4, NaCl, $MgSO_4 \cdot 7H_2O$ 및 $(NH_4)_2SO_4$의 농도는 10.0, 1.0, 0.2 및 0.6 g/l이었으나, carboxymethylcellulase의 생산에 최적인 각 염들의 농도는 각각 5.0, 1.0, 0.4 및 0.6 g/l이었다. RSM에 기초를 한 Design-Expert Software를 사용하여 실험을 계획하고, 그 결과를 분석한 결과는 K_2HPO_4가 균체의 생장 및 carboxymethylcellulase의 생산에 가장 중요한 인자라는 사실을 확인하였다. 균체의 생장에 최적인 K_2HPO_4, NaCl, $MgSO_4 \cdot 7H_2O$ 및 $(NH_4)_2SO_4$의 농도는 7.44, 1.08, 0.22 및 0.88 g/l이었으나, carboxymethylcellulase의 생산에 최적인 각 염들의 농도는 각각 5.84, 0.69, 0.28 및 0.54 g/l이었다. 기본적으로 OAM에 기초한 software를 사용하여 얻은 결과는 RSM에 기초한 software를 사용하여 얻은 결과와 유사하였다. 최적 조건에서 재조합 균주 *E. coli* JM109/DL-3이 생산하는 carboxymethylcellulase의 생산은 *B. amyloliquifacience* DL-에 비하여 1.92배 증가하였다.