ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 5, Issue, 04, pp. 4036-4042, April, 2015

International Journal of
DEVELOPMENT RESEARCH

Full Length Research Article

OPTIMIZATION OF INDOLE-3-ACETIC ACID PRODUCTION BY *BACILLUS SUBTILIS* TIB6 USING RESPONSE SURFACE METHODOLOGY

[1,*]Ton That Huu Dat, [2]Nguyen Thị Kim Cuc and [1]Pham Viet Cuong

[1]Mientrung Institute of Scientific Research, Vietnam Academy of Science and Technology
[2]Institute of Marine Biochemistry, Vietnam Academy of Science and Technology

ARTICLE INFO

Article History:
Received 07th January, 2015
Received in revised form
11th February, 2015
Accepted 04th March, 2015
Published online 29th April, 2015

Key words:
Bacillus subtilis,
Central Composite Design,
Indole-3-acetic acid,
Plackett-Burman Design,
Response Surface Methodology

ABSTRACT

The influence of carbon, nitrogen sources and tryptophan supplementation on indole-3-acetic acid (IAA) production of *Bacillus subtilis* TIB6 isolated from pepper rhizosphere, Tay Nguyen province of Viet Nam was investigated and obtained results showed that sucrose and yeast extract were the most favorable carbon and nitrogen sources for TIB6's IAA production, respectively, while the presence of L-tryptophan in culture medium stimulated the formation of IAA. The student's t-test of the Plackett-Burman design were used for screening significant factors affecting IAA producing capacity of TIB6 and obtained results showed that l-tryptophan, yeast extract concentrations and inoculation ratio were the main effecting parameters. By using central composite design (CCD) of response surface methodology (RSM), the culture conditions for TIB6 were optimized for maximum IAA production. The optimum values of variables were: L-tryptophan (0.088%), Yeast extract (0.82%) and inoculation ratio (1.65%). At these conditions, the maximum IAA production was estimated to be 120,846 mg/L.

INTRODUCTION

Plant growth promoting rhizobacteria play a crucial role in agriculture by increasing the exchange of plant nutrients and reducing the use of chemical fertilizers. There are several mechanisms by which rhizosphere bacteria may stimulate plant growth. One of the most commonly reported direct growth promotion mechanism by bacteria is the production of plant growth promoting compounds. IAA (indole-3-acetic acid) is known as the member of the group of phytohormones and is considered the most important native auxin (Ashrafuzzaman *et al*., 2009), involving in increasing the root growth and length as well as wide root surface area, which enables the plant to easily access to more nutrients from soil (Vessey, 2003). IAA is produced through l-tryptophan metabolism by bacteria, fungus and algae (Sarwar and Kremer, 1995; Stein *et al*., 1990; Finnie and Van Staden, 1985; Rifat *et al*., 2010). Bacteria from different genera (*Azospirillum, Enterobacter, Azotobacter, Klebsiella, Alcaligenes faecalis*), actinomycetes (*Streptomyces olivaceoviridis, Streptomyces rimosus*) and fungi (*Colletotrichum gloeosporioides, Ustilago maydis*) have shown to stimulate plant growth by the synthesis of IAA (Torres-Rubio *et al*., 2000; Reinekei *et al*., 2008; Khamna *et al*., 2010). Root exudates are natural source of l-tryptophan for rhizospheric microflora, which may promote IAA biosynthesis in the rhizosphere (Glick *et al*., 1995), thus IAA is considered as secondary metabolites (Ahmad *et al*., 2005). Apart from detecting and screening IAA producing microorganisms, optimization of cultural conditions for IAA production improvement of the isolated strain was essential. Before now, the classical optimization method was used popularly in which "one factor at a time" was studied, i.e. only one factor of a system being variable, whereas the others were fixed during each experiment and therefore, much trials were required (Sasirekha *et al*., 2012). Additionally, this laborious and time consuming method does not guarantee the determination of optimal conditions (Aravindan *et al*., 2007). Statistical approach for medium optimization would effectively solve these problems, which involves specific design of trials to minimize the error in determining the effect of variables (Krishnan *et al*., 1998). Statistical methods have benefits over conventional methods in predicting the accurate

Corresponding author: Ton That Huu Dat
Mientrung Institute of Scientific Research, Vietnam Academy of Science and Technology

results based on utilization of fundamental principles of statistics, randomization, and replication (Sasirekha *et al*., 2012). In this study, we applied statistical approach for optimization of culture conditions for IAA production of *Bacillus subtilis* TIB6 isolated from pepper rhizosphere, Tay Nguyen province of Vietnam

MATERIALS AND METHODS

Bacillus subtilis TIB6 strain

Bacillus subtilis TIB6 strain was isolated from pepper rhizosphere in Vietnam and the identification was carried out based on morphological, biochemical characteristics and 16S rDNA sequence analysis. Strain was maintained on nutrient agar slant at 4^0C and stored at Vietnam Academy of Science and Technology. Inoculum was prepared in nutrient broth (g/L): glucose 10, peptone 5, KH_2PO_4 0.3, $MgSO_4.7H_2O$ 0.5, NaCl 3 by transferring a loop of the bacteria from stock culture and incubated at 37^oC, shaking 200 rpm for 24 h.

Estimation of IAA production

The IAA produced was estimated by previously described method (Gordon and Weber, 1951). One ml of the cell-free supernatant was added to 4 ml of the Salkovski's reagent and this preparation was incubated for 30 minutes at room temperature in darkness. Presence of pink color pointed out IAA production. The amount of IAA produced was determined calorimetrically at 540 nm and calculated based on standard graph prepared from authentic IAA

Effects of precursor (l-tryptophan) and carbon, nitrogen sources on IAA production

Effect of nutritional factors on IAA production, such as l-tryptophan, carbon and nitrogen sources was investigated. The bacteria was cultivated in nutrient broth supplemented with 0.1 % tryptophan or 1% different carbon sources like Glucose, Galactose, Lactose, Mannitol, Sucrose and 0.5% different nitrogen sources such Soya bean, beef extract, Yeast extract, peptone and tryptone. After 24 hours cultivation at 37^oC and shaking 200 rpm, the amount produced IAA by TIB6 strain was determined.

Screening main parameters affecting on IAA production by Plackett-Burman design

Plackett-Burman design (PBD) is the most commonly used screening design due to its ability to estimate main effects with the same precision (Antony, 2008). It is a fractional factorial design with the advantage of minimizing the experimental runs from large number of variables to smaller most significant factors (El-Refai *et al*., 2010; Fang *et al*., 2010). In this design, N factors can be screened with N + 1 trial runs (Fang *et al*., 2010). In this study, the PBD were applied to evaluate the effect of 10 cultural factors which are considered to be important for IAA production. Each component was tested at two concentration levels, low and high. Table 1 shows the factors used for PBD with 12 experiments for assessing their effects on IAA production. All experiments were carried out in 250 ml Erlenmeyer flasks containing 100 ml media, shaking at 200 rpm.

Table 1. Coded and actual values of the variables used in PBD

Variables	Unit	Low level (-1)	High level (+1)
X1-Sucrose	%	0.1	2
X2-Yeast extract	%	0.1	1
X3-L-tryptophan	%	0.01	0.1
X4-KH_2PO_4	%	0.01	0.05
X5-$MgSO_4.7H_2O$	%	0.01	0.05
X6-NaCl	%	0.1	1
X7-Temperature	^{0}C	20	40
X8-pH	-	5	8
X9-Incubation period	Days	2	10
X10-Inoculation ratio	%	1	3

The PBD based on the first order model without interaction effect has been represented by Equation 1.

$$Y = \beta_0 + \beta_i X_i \quad (1)$$

Where Y is the response function, β_0 is the model intercept, i is the variable number, β_i is regression coefficient and X_i represents the independent variables. A 12-experimental run PBD consisting of the 10 factors at 2 levels was designed and the trials were carried out with triplicates. The standard design matrix with the responses is presented in Table 2.

The main effect of each of the variable is given in Equation 2.

$$E_{(Xi)} = (\sum M_{i+} - \sum M_{i-})/n \quad (2)$$

Where $E_{(Xi)}$ is the effect of the variables, $\sum M_{i+}$ is the summation of the response at high level, $\sum M_{i-}$ is the summation of the response at low level, n is the number of trials (Ndaliman, 2013). When the effect value is positive, the influence of the variable on IAA production is greater at higher concentration, conversely, if the effect value is negative, the influence of the variable is greater at lower concentration. The statistical analysis of the results was performed with the aid of statistical software package Design Expert 7.0 (Sasirekha *et al*., 2012).

Table 2. PBD with the responses for IAA production

Exp. No.	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	IAA (mg/L)
1	+	-	+	-	-	-	+	+	+	-	91.91
2	+	+	-	+	-	-	-	+	+	+	105.29
3	-	+	+	-	+	-	-	-	+	+	118.43
4	+	-	+	+	-	+	-	-	-	+	111.82
5	+	+	-	+	+	-	+	-	-	-	93.73
6	+	+	+	-	+	+	-	+	-	-	110.51
7	-	+	+	+	-	+	+	-	+	-	101.19
8	-	-	+	+	+	-	+	+	-	+	105.26
9	-	-	-	+	+	+	-	+	+	-	79.98
10	+	-	-	-	+	+	+	-	+	+	81.81
11	-	+	-	-	-	+	+	+	-	+	87.23
12	-	-	-	-	-	-	-	-	-	-	74.94

Optimization for IAA production using Response Surface Methodology

In order to determine the optimal level of each important independent variable, a central composite design (CCD) of response surface method had been used, which allows to estimate a full quadratic model for each response (Adinarayana and Ellaiah, 2002; Beg *et al*., 2002; Kristo *et al*.,

2003; Chang *et al*., 2006). The CCD in 20 runs was adopted (Table 4) for the experiment containing 3 key effect factors at 5 levels each (Table 3). The relationship of the independent variables and the responses was represented in the second-order polynomial equation 4.

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1 x_1 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{22} x_2 x_2 + \beta_{23} x_2 x_3 + \beta_{33} x_3 x_3 \quad (4)$$

Where Y is the response; β_0 is the intercept; β_1, β_2, β_3 are linear coefficients; β_{11}, β_{22}, β_{33} are squared coefficients; β_{12}, β_{13}, β_{23} are interaction coefficients. The analysis of variance (ANOVA) was used to value the effects and determine regression coefficients of model. The response surface plots were adopted to represent the effect of the independent variables on IAA production. These response curves were then used to predict the optimum level of the factors (Pham *et al*., 1998; Kwak *et al*., 2006).

Table 3. Experimental range of the three significant variables used in Central composite design for IAA production

Independent variables	Levels				
	-α	-1	0	+1	+α
A: L-tryptophan (%)	0.046	0.06	0.08	0.1	0.114
B: Yeast extract (%)	0.33	0.5	0.75	0.1	1.17
C: Inoculation period (%)	0.66	1.0	1.5	2.0	2.34

Table 4. Experimental design and responses of IAA production in Central composite design

Observations	A	B	C	IAA production (mg/L)
1	-1	-1	-1	102.13
2	1	-1	-1	111.41
3	-1	1	-1	109.52
4	1	1	-1	115.63
5	-1	-1	1	109.34
6	1	-1	1	114.75
7	-1	1	1	112.91
8	1	1	1	114.88
9	-α	0	0	104.24
10	+α	0	0	116.45
11	0	-α	0	108.92
12	0	+α	0	116.89
13	0	0	-α	111.12
14	0	0	+α	118.21
15	0	0	0	120.31
16	0	0	0	118.24
17	0	0	0	118.72
18	0	0	0	120.34
19	0	0	0	119.67
20	0	0	0	120.53

RESULTS AND DISCUSSION

Effect of L-tryptophan on IAA production

Obtained results showed that l-tryptophan stimulated IAA production of *B. subtilis* TIB6. TIB6 produced 76.4 ± 2.1 mg/L IAA when 0.1% tryptophan was added into culture medium compared with 28.5 ± 2.8 mg/L IAA in control (without tryptophan). It means that strain *B. subtilis* TIB6 probably synthesized IAA through l-tryptophan dependent pathways. Tryptophan is believed to be the primary precursor for the formation of IAA in plants and rhizobacterium (Monteiro *et al*., 1988).

The role of tryptophan in the enhancement of IAA production also reported in several earlier reports (Chopade *et al*., 2008; Khalid *et al*., 2004). Maximum IAA production of 170µg/ml was obtained when 0.5% l-tryptophan was used for culturing *Pseudomonas* sp. (Balaji *et al*., 2012), while IAA production of *Klebsiella* increased with an increasing of tryptophan up to 0.2%. Similarly, strains *Pseudomonas aeruginosa* produced IAA at 5 fold higher than control (without tryptophan) when culture medium was supplemented with 0.1 g/l-tryptophan (Chaiharn and Lumyong, 2010). IAA synthesis of many bacteria was improved when tryptophan was added into culture medium (El- Khawas and Adachi, 1999; Ali *et al*., 2010). However, excessive concentration of l-tryptophan may also have adverse effect on IAA production (Nita *et al*., 2011). This phenomenon may be due to release of IAA degrading enzymes as reported earlier for *Rhizobium* species (Datta and Basu, 2000).

Effect of carbon sources on IAA production

Strain TIB6 can use all investigated carbon sources for growth and IAA synthesis. In culture medium with glucose and sucrose (1%), TIB6 synthesized 76.3 ± 2.7 and 78.3 ± 3.2 mg/L IAA, respectively. Meanwhile, in medium supplemented with other carbon sources, TIB6 produced less amount of IAA (Table 5). The studies regarding correlation between utilization different carbon sources and production of IAA of *Rhizobium* showed that different *Rhizobium* strains prefer different carbon source. The *Rhizobium* strains 12, 16 and 18 required sucrose, whereas *Rhizobium* strain 13 and *Rhizobium* sp. from Cajanus Cajan preferred glucose for maximum production of IAA (Datta and Basu, 2000; Sridevi and Mallaiah, 2007). Sucrose was also the best carbon source for the growth and IAA production of *Acetobacter diazotrophicus* and *Pantoea agglomerans* (Apine and Jadhav, 2011; Nita *et al*., 2011).

Table 5. Effect of carbon source on IAA production of TIB6

Carbon source	Glucose	Sucrose	Mannitol	Galactose	Lactose
IAA (mg/L)	76.3±2.7	78.3±3.2	63.5±2.3	68.7±2.3	71.2±2.9

± standard error.

Effect of nitrogen sources on IAA production

Effect of nitrogen source on IAA production of TIB6 strain was assessed and experimental results pointed out that IAA production reached a peak of 79.1 ± 3.4 µg/ml when yeast extract was used as nitrogen source. Like carbon sources, bacteria utilize dissimilar nitrogen sources for maximum IAA production. *Rhizobium* can utilize both organic and inorganic nitrogen sources (Sridevi and Mallaiah, 2007), while *Pseudomonas* sp. prefers yeast extract (Balaji, 2011).

Table 6. Effect of nitrogen source on IAA production of TIB6

Nitrogen source	Soya bean	Beef extract	Yeast extract	Peptone	Tryptone
IAA (mg/L)	65.7±2.6	70.1±2.9	79.1±3.4	75.9±3.2	63.9±3.1

± standard error.

Beef extract was the most favorite nitrogen source for *Pantoea agglomerans* PVM (Apine and Jadhav, 2011), and NH_4Cl was found to be the most suitable nitrogen source for IAA production of *Acetobacter diazotrophicus* L1 (Nita *et al*., 2011).

Screening of main factors effecting IAA production by Plackett-Burman design

PBD experiments showed that IAA production by TIB6 varied widely, ranging from 74.94 to 118.43 µg/ml. Sucrose, L-tryptophan, yeast extract, KH_2PO_4, $MgSO_4$ concentration, and inoculation ratio showed positive effects, while NaCl, temperature, pH and incubation period have shown negative effects on IAA production. The Student's t-test and p-value were used to determine the significance of model at 95% confidence level. On the basis of the Student's t-test and the calculated p-values, l-tryptophan, yeast extract concentration and inoculation ratio were identified as the three significant components affecting IAA production. Based on the PB design, the effect of independent variables on IAA production is given by the first order linear model as equation 5 (actual units). The regression coefficient of the model (R^2 = 0.9993) close to 1.0, implying that the given model is well fitted with the trial results. Analysis of variance (ANOVA) given in table 8 showed that the main effect of the independent variables was valid with 93.7% confidence level.

$$IAA = 72.3 + 2.46X1 + 13.1X2 + 215X3 + 123X4 + 72.3X5 - 3.15X6 - 0.332X7 - 0.097X8 - 0.102X9 + 4.8X10 \quad (5)$$

Table 7. Estimated effects and coefficients for analysis of PBD

Variables	Main effect	Coefficients	t-value	p-value
Constant		96.8417	283.44	0.002
X1	4.6733	2.3367	6.84	0.092
X2	11.7767	5.8883	17.23	0.037
X3	19.3567	9.6783	28.33	0.022
X4	5.4067	2.7033	7.91	0.080
X5	2.8900	1.4450	4.23	0.148
X6	-2.8367	-1.4183	-4.15	0.150
X7	-6.6400	-3.3200	-9.72	0.065
X8	-0.2900	-0.1450	-0.42	0.744
X9	-0.8133	-0.4067	-1.19	0.445
X10	9.5967	4.7983	14.04	0.045

Table 8. ANOVA for linear model for effect of independent variables on IAA production

Source	DF	Sum of squares	Mean square	F-value	p-value
Main effects	10	2153.32	215.33	153.72	0.063
Residual error	1	1.40	1.40		
Total sum of squares	11	2154.72			

Plackett-Burman design can be used to find the significant variables in a system and allow them to be ranked in order of importance and to decide which one is to be investigated further so as to determine the optimum values (Liu *et al*., 2003). Sasirekha *et al*. (2012) used Plackett-Burman design for screening the most significant effects influencing IAA production by *Pseudomonas aeruginosa*, and yeast extract, tryptophan and EDTA were identified as significant components.

Optimization for IAA production using response surface methodology

CCD has been adopted in determination of optimum cultural conditions and in the analysis of the interaction of three main variables for IAA production by bacteria. All the trials were carried out with triplicates and the average IAA production given in Table 4 was subjected to multiple linear regression analysis using Design-Expert 7.0.0 software. The effect of l-tryptophan, yeast extract concentration and inoculation ratio on IAA production was described in the form of second order polynomial model in coded units.

$$IAA\ (mg/L) = 119.66 + 3.17A + 2.10B + 1.84C - 0.83AB - 1.00AC - 0.99BC - 3.47A^2 - 2.57B^2 - 1.95C^2 \quad (6)$$

Where Y is IAA production (mg/L), A is l-tryptophan concentration (%), B is yeast extract concentration (%) and C is inoculation ratio (%). The analysis of variance (ANOVA) for second order polynomial model was given in Table 9. The statistical significance of the second-order polynomial model was assessed by F-test ANOVA. The model F-value of 57.03 implied the model was significant. P-value less than 0.05 will indicate that the model terms are significant at α=0.05. In this case, the model had a very low probability value (p-value < 0.0001 < α=0.05), implying that the model was highly significant and adequate to represent the relationship between IAA production (µg/ml) and l-tryptophan, yeast extract concentration and inoculation ratio. Lack-of-fit of model had a high probability value (p-value = 0.385 > α=0.05), which showed that lack of fit is insignificant. Similarly, the lack of fit F-value of 1.32 implied the lack of fit was not significant relative to the pure error. Non-significant lack of fit demonstrated that the model was well fit.

The R^2 value of model represents the correlation between observed and predicted values. In this study, R^2 = 0.9809% showed good correlation between observed and predicted values and proved that model could explain 98.09% of variability in the response and only 1.91% of the total variance could not be explained by the model. The Pred R-squared of 0.9058 was in reasonable agreement with the Adj R-squared of 0.9637. Adeq Precision measures the signal to noise ratio and a ratio greater than 4 is desirable. In this case, a ratio of 24.707 indicated an adequate signal. The coefficient of variation (CV) indicates the degree of precision with which the experiments are compared. A lower value of coefficient of variation shows the experiments conducted are precise and reliable (Box, 1978). In the present case, a low CV (0.9%) pointed out that the experiments performed are reliable.

Table 9. Analysis of variance (ANOVA) for second order polynomial model of IAA production

Factors	DF	Sum of Squares	Mean Square	F-value	P-value
Model	9	539.6666	59.96295	57.02756	< 0.0001
Lack of fit	5	5.976582	1.195316	1.316964	0.3850
Pure error	5	4.53815	0.90763		
Total	19	550.1813			

CV = 0.9%; R^2 = 0.9809; Pred-R^2= 0.9058; Adj-R^2 = 0.9637; Adeq Precision = 24.707

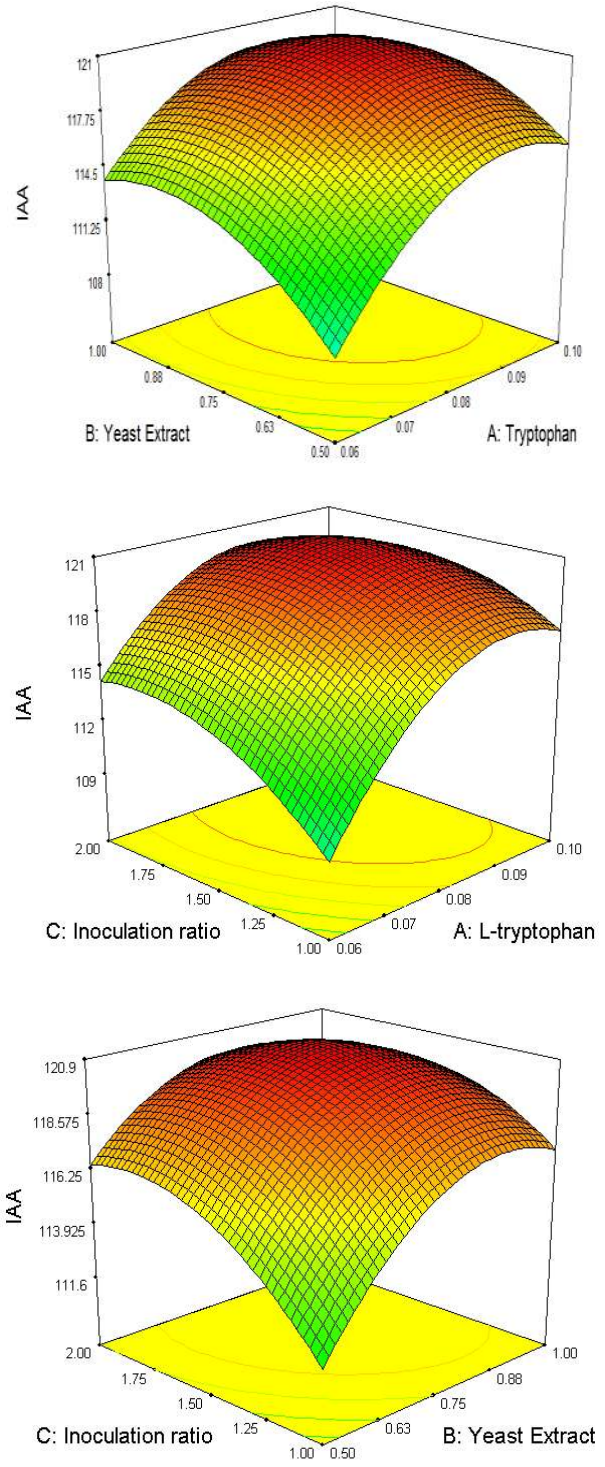

Fig. 1. Optimization for IAA production using response surface method in 3D surface plot

The obtained 3D surface plots represent the effect of each of the three factors and their interactive influence on IAA production. The surface plots (Figure.1) showed that lower and higher level of l-tryptophan, yeast extract and inoculation ratio had less significant effect on the IAA production and increasing of IAA production was observed at middle level of l-tryptophan, yeast extract, as well as inoculation ratio. Results of optimization for culture conditions of IAA production based on response surface method indicated that predicted maximum IAA production of 120.846 (mg/L) was obtained using L-tryptophan concentration of 0.088%, yeast extract concentration of 0.82% and inoculation ratio of 1.65%. The comparison of predicted (120.846 mg/L) and experimental values (121.35 mg/l) showed good correlation between them, implying that the empirical model derived from RSM can be used to adequately describe the relationship between the cultural conditions and the IAA production. Response surface method has already been successfully adopted for optimization of media and culture conditions in many cultivation process for the production of primary and secondary metabolites (Shirai *et al*., 2001; Boyaci, 2005), such as amino acids (Jyohi *et al*., 2005; Xiong *et al*., 2005), ethanol (Carvalho *et al*., 2003), flavoring compound (acetoin) (Xian *et al*., 2007), enzyme (Rao *et al*., 1993), IAA (Swain and Ray, 2008), and biomas (Sreekumar and Soundarajan, 2010). Swain and Ray (2008) applied RSM-CCD for optimization of IAA production in SSF, and showed that optimum parameters for IAA production were incubation period (6 days), pH (7.0) and moisture holding capacity (70%).

Conclusion

The influence of carbon, nitrogen sources and tryptophan supplementation on IAA production of the TIB6 strain was studied and the experimental results revealed that sucrose and yeast extract were the most favorable carbon and nitrogen sources for TIB6's IAA producing capability, respectively. Besides, the presence of L-tryptophan in culture medium increased amount of IAA produced by TIB6 strain. Plackett-Burman design was used for determination of main culturing factors affecting IAA production by *B.subtilis* TIB6 and obtained results showed that L-tryptophan, yeast extract concentration and inoculation ratio were parameters exhibited significant effect on IAA production. These variables were further optimized using RSM with CCD design and the comparison of predicted and experimental values of IAA production showed good correlation between them. The optimum culture parameters for maximum IAA production (121.35 mg/L) of TIB6 were l-tryptophan (0.088%), yeast extract (0.82%) and inoculation ratio (1.65%).

Acknowledgement

This work was financially supported by Tay Nguyen 3rd Program of Technological and Scientific Development, Vietnam Academy of Science & Technology for the project: *Improving and technological transfer of POLYFA-TN3 product for soil reclamation in Tay Nguyen.* N° Code: TN3/C10.

REFERENCES

Adinarayana, K. and Ellaiah, P. 2002. Response surface optimization of the critical medium components for the production of alkaline protease by a newly isolated *Bacillus* sp. *J. Pharm Sci*, 5, 272-276.

Ahmad, F. I. Ahmad and M. S. Khan. 2005. Indole acetic acid production by the indigenous isolates of *Azotobacter* and *Fluorescent Pseudomonas* in the presence and absence of Tryptophan. *Turkish Journal of Biology*, 29, 29-34.

Ali, B., Sabri, A. N. and Hasnain, S. 2010. Rhizobacterial potential to alter auxin content and growth of *Vigna radiata* (L).*World J Microbiol Biotechnol*, 26, 1379-1384.

Antony, J. 2008. *Design of Experiments for Engineers and Scientists*. (Butterworth Heinemann, U.K), pp. 48.

Apine, O. A. and Jadhav, J. P. 2011. Optimization of medium for indole-3-acetic acid production using *Pantoea agglomerans* strain PVM. *Journal of Applied Microbiology,* 110(5), 1235-44.

Aravindan Rajendran, Meikandhan Thirugnanam and Viruthagiri Thangavelu, 2007. Statistical evaluation of medium components by Plackett-Burman experimental design and kinetic modeling of lipase production by *Pseudomonas fluorescens*. *Indian Journal of Biotechnology*, 6, 469-478.

Ashrafuzzaman, M., F. A. Hossen, M. R. Ismail, M. A. Hoque, M. Z. Islam, S. M. Shahidullah and S. Meon, 2009. Efficiency of Plant Growth promoting Rhizobacteria (PGPR) for the Enhancement of Rice Growth. *African Journal of Biotechnology*, 8, 1247-1252.

Balaji, N., S. S. Lavanya, S. Muthamizhselvi and K. Tamilarasan, 2012. Optimization of fermentation condition for indole acetic acid production by *Pseudomonas* species. *International Journal of Advanced Biotechnology and Research*, 3, 797-803.

Beg, Q. K., Saxena, R. K. and Gupta, R. 2002. Kinetic constants determination for an alkaline protease from *Bacillus mojavensis* using response surface methodology. *Biotechnol. Bioeng*, 78, 289-295.

Box, G. E. P., Hunter, W. G. and Hunter, J. S. 1978. Statistics for Experimenters (John Wiley and Sons, New York), pp. 291.

Boyaci, I. H. 2005. A new approach of determination of enzyme kinetic constants using response surface methodology, *Biochem Eng J*, 25, 55-62.

Carvalho, J. C. M., Vitolo, M., Sato, S. and Aquarone, E. 2003. Ethanol production by *Saccharomyces cerevisiae* grown in sugarcane blackstrap molasses through a feed batch process: optimization by response surface methodology. *Appl Microbiol Biotechnol,* 110, 151-164.

Chaiharn, M. and S. Lumyong, 2011. Screening and optimization of indole-3-acetic acid production and phosphate solubilization from rhizobacteria aimed at improving plant growth. *Curr. Microbiol*, 62(1), 173-181.

Chang, Y. C., Lee, C. L. and Pan, T. M. 2006. Statistical optimization of media components for the production of *Antrodia cinnamomea* AC0623 in submerged cultures. *Appl. Microbiol. Biotechnol*, 72, 654-661.

Chopade, B. A., S. B. Huddedar, A. M. Shete, J. N. Tilekar, S. D. Ghore and D. D. Dhavale, *United States* 7, 341, 868 (Drinker, Biddle & Reath LLP) 11th of March 2008.

Datta, C. and Basu, P. S. 2000. Indole acetic acid production by Rhizobiumspecies from root nodules of a leguminous shrub, *Cajanus cajan*. *Microbiol. Res*, 155, 123-127.

El-Khawas, H. and Adachi, K. 1999. Identification and quantification of auxins in culture media of *Azospirillumand Klebsiella* and their effect on rice roots. *Biol. Fert. Soils*, 28, 377-381.

El-Refai, HA., E. R. El-Helow, M. A. Amin, L. A. Sallam and H. A. Salem, 2010. Application of multifactorial experimental design for optimization of biotin production by a *Rhizopus Nigricans* strain. *Journal of American Science*, 6, 179-187.

Fang, H., C. Zhao and Y. Y. Song, 2010. Optimization of enzymatic hydrolysis of steam-exploded corn stover by two approaches: response surface methodology or using cellulose from mixed cultures of *Trichoderma Reesei* RUT-C30 and *Aspergillus Niger* NLO2. *Bioresource Technology*, 101, 4111-4119.

Finnie, J. F. and Van Staden, J. 1985. Effect of seed weed concentrate and applied hormones on in vitro cultured tomato roots. *J. Plant Physiol,* 120, 215-222.

Glick, B. R., D. M. Karaturovic and P. C. Newell, 1995. A novel procedure for rapid isolation of plant growth promoting *Pseudomonads*. *Canadian Journal of Microbiology*, 41(6), 533-536.

Gordon, S. N. and Weber, R. P. 1951. Colorimetric estimation of indole acetic acid. *Plant Physiology*, 26, 192-195.

Jyohi, A. N., Sasikiran, K., Nambisan, B. and Balagopalan, C. 2005. Optimization of glutamic acid production from cassava starch factory residues using *Brevibacterium divaricatum. Process Biochem*, 40, 3576-3579.

Khalid, A., M. Arshad and Z. A. Zahir, 2004. Screening plant growth-promoting Rhizobacteria for improving growth and yield of wheat. *Journal of Applied Microbiology*, 96, 473-480.

Khamna, S., Yokota, A., Peberdy, J. F. and Lumyong, S. 2010. Indole-3-acetic acid production by *Streptomyces* sp. isolated from some Thai medicinal plant rhizosphere soils. *Eur. Asia. J. Biosci,* 4, 23-32.

Kristo, E., Biliaderis, C. G. and Tzanetakis, N. 2003. Modeling of the acidification process and rheological properties of milk fermented with a yogurt starter culture using response surface methodology. *Food Chem,* 83, 437-446.

Kwak, K. O., Jung, S. J., Chunga, S. Y., Kangc, C. M., Huhd, Y. I. and Baea, S. O. 2006. Optimization of culture conditions for CO_2 fixation by a chemoautotrophic microorganism, strain YN-1 using factorial design. *Biochem. Eng. J*, 31, 1-7.

Liu, C., Liu, Y., Liao, W., Wen, Z. and Chen, S. 2003. Application of statistically based experimental designs for the optimization of nisin production from whey. *Biotechnol. Lett,* 25, 877-882.

Monteiro, A. M., Crozier, A. and Saanberg, G. 1988. The biosynthesis and conjugation of indole-3- acetic acid in germinating seeds and seedlings of *Dalbergia dolichopetala*. *Planta,* 174, 561-568.

Ndaliman, M. B., A. A. Khan, M. Y. Ali and Z. Wahid, 2013. Determination of Influential Factors on EDMed Surface Properties Using Plackett-Burman Design. *World Applied Sciences Journal,* 21, 88-93.

Nita B. Patil, Milind Gajbhiye, Sangita S. Ahiwale, Aparna B. Gunjal and Balasaheb P. Kapadnis. 2011. Optimization of Indole 3-acetic acid (IAA) production by *Acetobacter diazotrophicus* L1 isolated from Sugarcane. *International Journal of Environmental Sciences,* 2(1), 307-314.

Pham, P. L., Taillandier, P., Delmas, M. and Strehaiano, P. 1998. Optimization of a culture medium for xylanase production by *Bacillus* sp. using statistical experimental designs. *W. J. Microbiol. Biotechnol*, 14, 185-190.

Rao, P. V., Jayaraman, K., Lakshmanan, C. M. 1993. Production of lipase by *Candida rugosa* in solid-state fermentation, medium optimization and effect of earation. *Process Biochem*, 28, 391-395.

Reinekei, G., Heinze, B., Schirawski, J., Buettner, H., Kahmann, R. and Bassei, C. W. 2008. Indole-3-acetic acid

(IAA) biosynthesis in the smut fungus *Ustilago* may disand its relevance for increased IAA levels in infected tissue and host tumor formation. *Mol Plant Pathol,* 9, 39-355.

Rifat Hayat, Safdar Ali, Ummay Amara, Rabia Khalid and Iftikhar Ahmed, 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. *Ann Microbiol,* 60, 579- 598.

Sarwar, M. and R. J. Kremer, 1995. Determination of bacterially derived auxins using a micro plate method. *Lett. Appl. Microbiol*. 147, 282-285.

Sasirekha, B., S. Shivakumar and S. B. Sullia, 2012. Statistical optimization for improved indole-3-acetic acid (iaa) production by *Pseudomonas aeruginosa* and demonstration of enhanced plant growth promotion. *Journal of Soil Science and Plant Nutrition*, 12(4), 863-873.

Shirai, K., Guerrero, I., Huerta, S., Saucedo, G., Castillo, A., Gorzalez, R. O. and Hall, G. M. 2001. Effect of initial glucose concentration and inoculum level of lactic acid bacteria in shrimp waste ensilation. *Enz Microbiol Technol,* 28, 446-452.

Shridevi, M. and Mallaiah, K. V. 2007. Bioproduction of indole acetic acid by Rhizobium strains isolated from root nodules of green manure crop. *Sesbania sesban* (L.) Merr. *Iran J Biotechnol*, 5, 178-182.

Sreekumar, G. and Soundarajan Krishnan, 2010. Enhanced biomass production study on probiotic *Bacillus subtilis* SK09 by medium optimization using response surface methodology. *African Journal of Biotechnology*, 9(45), 8078-8084.

Stein, A., Fortin, J. A. and Vallee, G. 1990. Enhanced rooting of *Picea mariana* cuttings by ecto mycorrhizal fungi. *Can J Bot,* 68, 492-498.

Swain, M. R. and Ray, R. C. 2008. Optimization of cultural conditions and their statistical interpretation for production of indole-3-acetic acid by *Bacillus subtilis* CM5 using cassava fibrous residue. *Journal of Scientific and Industrial Research*, 67, 622-628.

Torres-Rubio, M. G., Valencia-Plata, S.A., Bernal-Castillo, J. and Martinez-Nieto, P. 2000. Isolation of enterobacteria, *Azotobacter* sp. and *Pseudomonas* sp. producers of indole-3-acetic acid and siderophores, from Colombian rice rhizosphere. *Rev Latinoam Microbiol*, 42, 171-176.

Vessey, J. K. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*, 255, 571-586.

Xian, J. Z., Lin, P. H., Qin, J. Y. and Xu, P. 2007. Statistical optimization of medium composition for enhanced acetoin production from molases and soybean meal hydrolysate. *Appl Microbiol Biotechnol*, 74, 61-68.

Xiong, C., Shouwen, C., Ming, S. and Ziniu, Y. 2005. Medium optimization by response surface methodology for poly-Y-glutamic acid production using dairy manure as the basic of a solid substrate. *Appl Microbiol Biotechnol,* 69, 390-396.

****