Statistical Optimization of Growth Medium for the Production of the Entomopathogenic and Phytotoxic Cyclic Depsipeptide Beauvericin from *Fusarium oxysporum* KFCC 11363P

Lee, Hee-Seok[1], Hyuk-Hwan Song[1], Joong-Hoon Ahn[2], Cha-Gyun Shin[3], Gung Pyo Lee[4], and Chan Lee[1]*

[1]Department of Food Science and Technology, BET Research Institute, Chung-Ang University, Ansung 456-756, Korea
[2]Department of Bioscience & Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701, Korea
[3]Department of Biotechnology, BET Research Institute, Chung-Ang University, Ansung 456-756, Korea
[4]Department of Applied Plant Science, Chung-Ang University, Ansung 456-756, Korea

The production of the entomopathogenic and phytotoxic cyclic depsipeptide beauvericin (BEA) was studied in submerged cultures of *Fusarium oxysporum* KFCC 11363P isolated in Korea. The influences of various factors on mycelia growth and BEA production were examined in both complete and chemically defined culture media. The mycelia growth and BEA production were highest in *Fusarium* defined medium. The optimal carbon and nitrogen sources for maximizing BEA production were glucose and $NaNO_3$, respectively. The carbon/nitrogen ratio for maximal production of BEA was investigated using response surface methodology (RSM). Equations derived by differentiation of the RSM model revealed that the production of BEA was maximal when using 108 mM glucose and 25 mM $NaNO_3$.

Keywords: Cyclodepsipeptide, beauvericin, response surface methodology, medium optimization, *Fusarium oxysporum*

Cyclic peptides are important classes of biologically active peptides that have been isolated from bacteria, fungi, and plants, and as prepared by synthetic methods [9, 17, 29, 31]. They have numerous pharmaceutical applications, including as enzyme inhibitors, antifungal and antibacterial agents, immunomodulating substances, and anticancer drugs [8, 12]. *Fusarium* genera produce biologically active cyclic depsipeptides such as beauvericin (BEA) and enniatins (ENs). Owing to their ionophoric characteristics, BEA and ENs exert many biological effects on animal systems by altering ion transport across membranes, such as by disrupting their cationic selectivity [15, 18, 20]. These basic mechanisms lead to a large variety of biological

actions, including antimicrobial, insecticidal, and strong cytotoxic activities in several invertebrate, rodent, cattle, and human cell lines [4, 7, 10]. These observations prompted us to investigate the significance of such cyclic depsipeptides in biological systems.

BEA and ENs contain an alternating sequence of three N-methyl-L-amino acids (N-methyl-L-phenylalanine, BEA; N-methyl-L-valine, N-methyl-L-leucine, or N-methyl-L-isoleucine, ENs) and three D-α-hydroxyisovaleric acids in their molecular structure. Several BEA analogs have been reported [5, 11], and they have the following structures: three N-methyl-L-phenylalanines and three hydroxylic acids are condensed to form a cyclic structure, and the hydroxylic acid is 2-hydroxy-3-methylpentanoic acid or 2-hydroxy-3-methyl-butanoic acid. Other types of BEA include allobeauvericins A and B, as found by Nilanonta *et al.* [27], and allobeauvericin C.

BEA was first reported to be produced by certain entomopathogenic fungi, such as *Beauveria bassiana* Vuill [16] and *Paecilomyces fumosoroseus* [3], and some of its phytotoxic properties have also been reported recently [30]. BEA exerts many biological effects on insects [16] and animals owing to its ionophoric activity [33]. It also acts as an antibiotic against several Gram-positive bacteria [16], and insecticidal activity against various insects including mosquito larvae [15], brine shrimp [16], blowfly [14], Colorado potato beetle [15], and *Schizaphis graminum* [13]. These observations suggest that BEA could be useful as a herbicide. BEA also exhibits antiplasmodial, antimalarial, and antimycobacterial activities [27].

We recently isolated the *F. oxysporum* KFCC 11363P (Korea Federation of Culture Collections, KFCC) strain that produces various cyclic depsipeptides, including BEA and ENs H, I, and MK1688 [32]. In the present study, we optimized the culture conditions for the maximal production of BEA and its utilization by selecting the

*Corresponding author
Phone: 82-31-670-3035; Fax: 82-31-676-8865;
E-mail: chanlee@cau.ac.kr

culture media and determining appropriate carbon and nitrogen sources. Furthermore, the response surface methodology (RSM) statistical technique was used to determine the optimal carbon/nitrogen ratio for maximizing BEA production.

MATERIALS AND METHODS

Cultivation of *Fusarium* Isolate

F. oxysporum KFCC 11363P was isolated from soil in Korea and identified as *F. oxysporum* according to the morphological criteria and synoptic keys of Nelson *et al.* [26]. *F. oxysporum* KFCC 11363P [32] was cultivated in potato dextrose agar (PDA), and spore suspensions were prepared for submerged cultures grown on PDA for 7 days at 25°C and aseptically filtered through sterile filter paper No. 1 (Whatman, Maidstone, United Kingdom) to remove mycelial debris. Approximately 10^5 spores/ml were inoculated in 100 ml of culture medium in a 250-ml Erlenmeyer flask, and the culture was incubated at 25°C with shaking at 120 rpm for several days.

Analysis of Beauvericin in Submerged Cultures of *F. oxysporum* KFCC 11363P

BEA was analyzed as described by Moretti *et al.* [24] with minor modifications. The liquid culture of *F. oxysporum* KFCC 11363P including mycelia was extracted twice with a double volume of chloroform [1]. The bottom layer was evaporated to dryness, and the residue was resuspended in methanol (high-performance liquid chromatography [HPLC] grade) and subjected to HPLC for the analysis of BEA. The extract in methanol was filtered through an MF3 filter (pore size, 0.5 μm; Advantec MFS, Pleasanton, CA, U.S.A.) before HPLC purification. HPLC-grade water and organic solvents (J.T. Baker, Phillipsburg, NJ, U.S.A.) were used throughout the procedure. A C18 column (0.46×25 cm, Shiseido, Tokyo, Japan) was used for the analysis of cyclic depsipeptides. HPLC was performed for 30 min at a constant flow rate (1 ml/min) with acetonitrile:water (70:30, v/v) as the eluent. Peaks were detected at 210 nm.

Selection of Culture Medium

For the optimization of BEA production, *F. oxysporum* KFCC 11363P was cultivated in the following ten types of complete and chemically defined culture media at 25°C and 120 rpm (amounts are listed per liter):

1. Potato dextrose broth (PDB): infused potato, 200 g; and dextrose, 20 g.

2. Malt extract broth (MB): malt extract base, 6 g; maltose, 1.8 g; dextrose, 6 g; and yeast extract, 1.2 g.

3. Yeast and malt extract broth (YMB): malt extract base, 3 g; yeast extract, 3 g; peptone, 5 g; and dextrose, 10 g.

4. Fungi corn steep and molasses medium (FCM): molasses, 3 g; and corn steep liquor, 3 g.

5. Fungi nutrient medium (FNM): glucose, 20 g; peptone, 5 g; yeast extract, 10 g; and corn steep liquor, 10 g.

6. *Fusarium* defined medium (FDM): sucrose, 25 g; NaNO₃, 4.25 g; NaCl, 5 g; MgSO₄·7H₂O, 2.5 g; KH₂PO₄, 1.36 g; FeSO₄·7H₂O, 0.01 g; and ZnSO₄·7H₂O, 0.0029 g.

7. Nash and Snyder medium (NSM): peptone, 15 g; KH₂PO₄, 1 g; and MgSO₄·7H₂O, 0.5 g.

8. *Fusarium* basal medium (FBM): D-galactose, 20 g; L-asparagine, 2 g; KH₂PO₄, 1 g; KCl, 0.5 g; and MgSO₄·7H₂O, 0.5 g.

9. Czapek-Dox medium (CzD): sucrose, 30 g; NaNO₃, 3 g; KH₂PO₄, 1 g; KCl, 0.5 g; and FeSO₄·7H₂O, 0.01 g.

10. Modified Czapek-Dox medium (MCzD): dextrose, 20 g; KH₂PO₄, 0.5 g; NaNO₃, 2 g; MgSO₄·7H₂O, 0.5 g; yeast extract, 1 g; and 1 ml of 1% FeSO₄·7H₂O.

Optimization of Fermentation Conditions

The production of BEA was optimized by using various carbon and nitrogen sources and varying the carbon/nitrogen ratio in the culture conditions. After selecting the actual culture medium, 7 carbon sources (galactose, sucrose, sorbitol, maltose, glucose, lactose, and fructose) and 15 nitrogen sources (polar amino acids: L-valine, L-alanine, L-leucine, isoleucine, and phenylalanine; nonpolar amino acids: serine, glutamine, asparagine, histidine, and glycine; and inorganic nitrogen sources: $(NH_4)_2SO_4$, KNO_3, $NaNO_3$, $NH_4H_2PO_4$, and NH_4Cl) were employed. RSM was applied for further optimization of the carbon/nitrogen ratio. Design Expert (version 6, State-Ease Inc., Minneapolis, MN, U.S.A.) software was used to generate the experimental designs and for statistical analyses and application of regression models.

Central composite design (CCD) with a quadratic model was employed. The two independent variables were the carbon source (x_1) and the nitrogen source (x_2), with each having three levels: 1, 0, and +1. A total of 11 combinations (including three replicates of the center point, each signed with the coded value of 0) were chosen in random order according to a CCD configuration for two factors. The experiments performed in terms of the coded (x) and actual (X) levels of variables are listed in Table 1. The production of BEA represented the measured response function (y). These values were related to the coded variables $(x_i, i=1$ and 2) by the following second-degree polynomial:

$$Y=B_0+B_iX_i+B_{ii}X_i^2+B_{ij}X_iX_j \tag{1}$$

Analysis of variance (ANOVA) tables were generated, and regression coefficients of the individual linear, quadratic, and interaction terms were determined. The statistical significances of all

Table 1. Effect of carbon/nitrogen ratio on dependent variable.

Experiment number	Independent variable (mM)		Dependent variable (g/l)
	Carbon source	Nitrogen source	BEA
	X_1 (x_1)	X_2 (x_2)	Y
1	150 (+1)	200 (+1)	0.11
2	95 (0)	200 (+1)	0.32
3	95 (0)	112.5 (0)	0.39
4	95 (0)	112.5 (0)	0.41
5	40 (−1)	200 (+1)	0.11
6	95 (0)	112.5 (0)	0.27
7	150 (+1)	112.5 (0)	0.34
8	95 (0)	25 (−1)	0.41
9	40 (−1)	25 (−1)	0.21
10	150 (+1)	25 (−1)	0.39
11	40 (−1)	112.5 (0)	0.13

terms in the polynomial were determined based on the F value at a probability (p) of 0.05. The regression coefficients were then used in statistical calculations to generate contour maps from the regression models.

RESULTS AND DISCUSSION

Selection of Culture Medium

The fermentation kinetics of *F. oxysporum* KFCC 11363P in various complete and chemically defined culture media were shown in Figs. 1 and 2. The mycelia level was maximal at the 11th, 11th, 12th, 11th, and 9th days after inoculation in PDB, MB, FCM, FNM, and YMB, respectively. In FCM, FNM, and YMB, the pH increased to 9.0 during cultivation, whereas it increased to only 6.5 in both PDB and MB. In the case of chemically defined media, the mycelia level was maximal at the 3rd, 9th, 12th, 7th, and 6th days of cultivation in NSM, FBM, CzD, MCzD, and FDM, respectively. Changes in pH during cultivation could be

divided into three groups: it increased to 9.0 in NSM and FDM, remained unchanged at near 3.5 in FBM and MCzD, and decreased to 5.0 at the 6th day in CzD before increasing to 7.0 from the 8th day.

The maximal productions of BEA by *F. oxysporum* KFCC 11363P in complete and chemically defined culture media are summarized in Table 2. BEA was maximal at the 8th and 25th days of cultivation in YMB and PDB (means of 0.12 and 0.11 g/l, respectively). Replacement of YMB by MB and FNM decreased BEA production by about 40% (to 0.047 g/l) and 37%, respectively. The production of BEA was low in FCM (0.008 g/l). In chemically defined media, the BEA level was highest on the 6th day of cultivation in FDM (mean of 0.17 g/l), and the final production rate of BEA in FDM was about three- to four-fold higher than that in FBM and MCzD. Replacement of FDM by CzD reduced the production by 20%, and BEA did not appear in NS. These observations indicate that the production of BEA from *F. oxysporum*
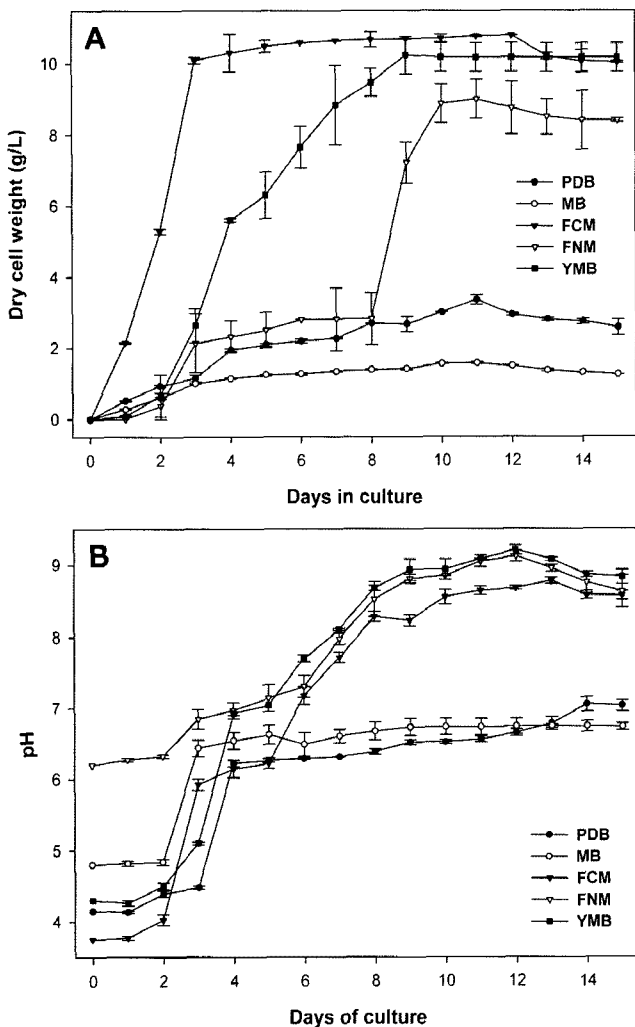

Fig. 1. Growth of *F. oxysporum* KFCC 11363P (A) and changes in pH (B) during cultivation in complete culture media.
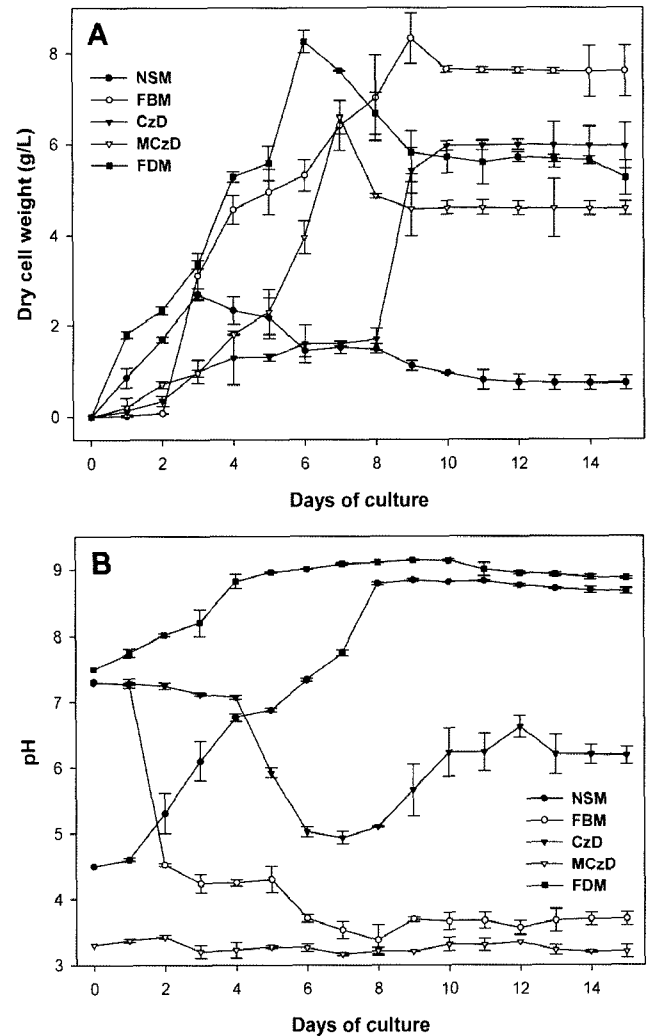

Fig. 2. Growth of *F. oxysporum* KFCC 11363P (A) and changes in pH (B) during cultivation in chemically defined culture media.

Table 2. Production of BEA in different liquid culture media.

Medium	*DCW (g/l)	BEA (g/l)	Specific production (g/g DCW*)
PDB	3.4	0.11	0.03
MB	1.6	0.04	0.03
FCM	10.8	0.008	0.0007
FNM	9.0	0.05	0.006
YMB	10.2	0.12	0.01
NS	1.5	ND	–
FBM	8.1	0.03	0.004
CzD	6.0	0.14	0.02
FDM	8.1	0.2	0.03
MCzD	4.6	0.05	0.01

*: Dry cell weight.

was maximal in FDM on the 6th day of cultivation. Fukuda et al. [11] reported that the maximal concentrations of BEAs D, E, and F produced by Beauveria sp. FKI-1366 were 9.9, 11.1, and 3.3 mg/l, respectively, on the 6th day of fermentation in liquid culture medium, which are much lower than the level of BEA (mean of 0.17 g/l) obtained in the present study in the culture of F. oxysporum in FDM. Madry et al. [21] reported that prolonging the cultivation did not further enhance the production of biological active compounds already obtained after about 4 days. Consistent with this, we found that more than 90% of biologically active compounds were extractable from the mycelia on the 4th day of fermentation.

Selection of the Most Suitable Carbon and Nitrogen Sources

The influence of several nutritional factors was tested in FDM, for it is well known that production of cyclic peptides may be severally effected by the quality and quantity of, for example, carbon, nitrogen, or phosphate supply [22]. In the defined FDM, production of cyclic peptides such as enniatins and beauvericin was studied intensively with carbon and nitrogen sources [1, 21]. The influence of several other nutritional factors like phosphate and trace element concentrations were only moderate [21].

Table 3. Production of BEA with different carbon sources.

	*DCW (g/l)	BEA (g/l)	Specific production (g/g DCW)
Control (sucrose)	9.5	0.17	0.018
Galactose	9.6	0.30	0.031
Sorbitol	5.5	0.04	0.007
Maltose	11.6	0.17	0.014
Glucose	7.0	0.30	0.043
Lactose	4.8	0.01	0.002
Fructose	7.2	0.11	0.015

Carbon and nitrogen sources for the production of beauvericin were optimized in FDM (Tables 3 and 4). In the most previous study related to optimization of carbon and nitrogen sources, sucrose and NaNO$_3$ were tested for the possibility of their substitution with other carbon or nitrogen sources [1, 21, 22]. Replacing sucrose with maltose as the carbon source in FDM did not decrease the final BEA yield, whereas replacing it with glucose or galactose increased BEA production by about 75%, and substituting fructose reduced BEA production by about 37%, as shown in Table 3. Sorbitol and lactose were very poor substrates for both mycelia growth and BEA production. Specific productions of beauvericin using glucose and galactose were 0.043 g/g dry cell weight (DCW) and 0.031 g/g DCW, respectively. Therefore, sucrose was substituted with glucose in FDM for further experiment to produce a high level of beauvericin.

The effects of using several nonpolar amino acids as the nitrogen source on the production of BEA are compared in Table 4. The highest yield of BEA was obtained with L-alanine (0.06 g/l) as the sole source of nonpolar amino acid. Replacing NaNO$_3$ with L-valine decreased BEA production by about 71%. Leucine and phenylalanine were very poor substrates for both mycelia growth and BEA production. The effects of polar amino acids on the production of BEA are listed in Table 4. Replacing NaNO$_3$ with glutamine decreased BEA production by about 35%. Table 4 also compares five inorganic nitrogen sources. It is clear that the presence of ammonium ions adversely affects the production of BEA by F. oxysporum KFCC 11363P. The yield of BEA was highest (0.17 g/l) with NaNO$_3$ as the

Table 4. Production of BEA with different nitrogen sources.

	*DCW (g/l)	BEA (g/l)	Specific production (g/g DCW)
Control (NaNO$_3$)	9.5	0.17	0.018
Nonpolar amino acids			
L-Valine	10.0	0.05	0.005
L-Alanine	10.4	0.06	0.006
L-Leucine	11.1	0.01	0.0001
Isoleucine	8.3	0.02	0.002
Phenylalanine	11.3	0.01	0.0001
Polar amino acids			
Serine	6.5	0.04	0.006
Glutamine	9.0	0.10	0.029
Asparagine	10.0	0.07	0.006
Histidine	6.1	0.04	0.022
Glycine	4.2	0.09	0.006
Inorganic nitrogen sources			
(NH$_4$)$_2$SO$_4$	8.7	0.01	0.001
KNO$_3$	4.9	0.11	0.043
NH$_4$H$_2$PO$_4$	8.7	0.01	0.001
NH$_4$Cl	10.2	0.03	0.003

sole source of inorganic nitrogen, and specific production of beauvericin using $NaNO_3$ was 0.018 g/g DCW. Therefore, $NaNO_3$ was applied as a single source of nitrogen for further experiment to produce a high level of beauvericin.

Audhya and Russel [2] and Minasyan *et al.* [23] reported that the optimal nitrogen source was NH_4NO_3 in surface cultures of *F. sambucinum*. Madry *et al.* [21] reported that the optimal carbon and nitrogen sources for maximizing the production of cyclic depsipeptides, ENs, by *F. oxysporum* were sucrose and $NaNO_3$. In addition, ammonium ions inhibited the growth of *F. oxysporum* [21] and reduced EN levels during fermentation. From these results, the optimal carbon and nitrogen sources for maximal production of BEA were determined as glucose and $NaNO_3$, respectively.

In the defined FDM, the influence of several other nutritional factors like phosphate and trace element concentrations was only moderate. Inorganic phosphate concentrations could be varied between 1 and 100 mM without a dramatic effect on cyclic depsipeptide including enniatin and beauvericin production [21]. Additionally, the concentrations of several other inorganic salts such as NaCl, KCl, $CaCl_2$, and $ZnSO_4$ had only moderate effects on the fermentation characteristics, except $CaCO_3$, which reduced cyclic depsipeptide production [1, 21]. Therefore, carbon and nitrogen sources such as glucose and $NaNO_3$ were employed to study C/N ratio using RSM technology for further experiment.

Optimization of Carbon/Nitrogen Ratio by Response Surface Methodology

RSM has been successfully applied to foods, chemicals, and biological processes [19, 28], and its theoretical and practical applications have been widely reviewed and shown to be very effective for analyzing the production of both antibiotics and industrial enzymes [6, 25, 34]. Our screening experiments using different carbon and nitrogen sources in the fermentation medium revealed that they significantly influenced BEA production, with
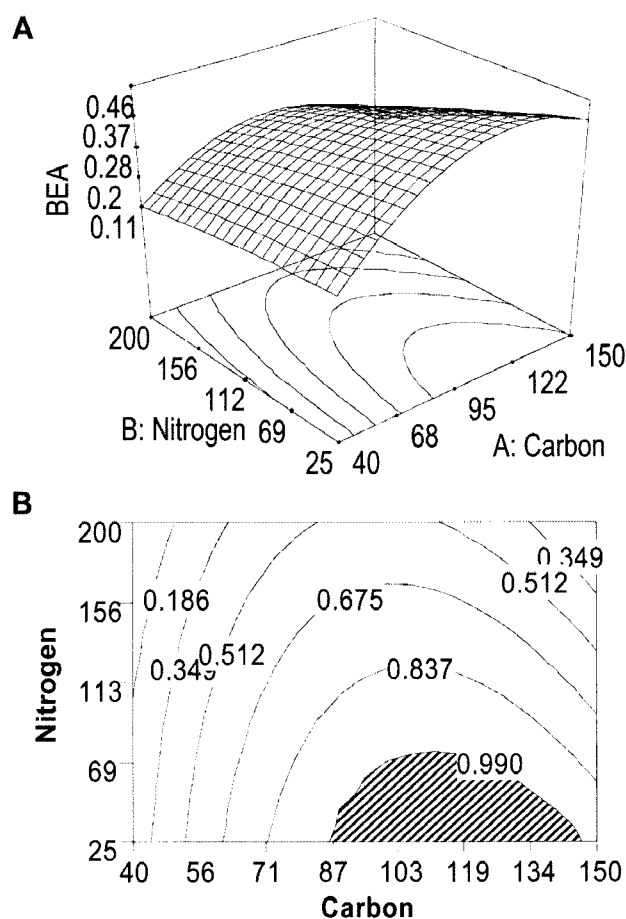
Table 5. ANOVA table for the production of BEA in terms of coded values of variables.

	Sum of squares	Degrees of freedom	Mean of squares	F
Model	0.128	5	0.026	6.234
X_1	0.025	1	0.025	6.174
X_2	0.037	1	0.037	8.967
X_1^2	0.051	1	0.051	12.414
X_2^2	0.0004	1	0.0004	0.087
$X_1 \times X_2$	0.008	1	0.008	1.97
R^2		0.86		

P less than 0.05.
X_1: Carbon source.
X_2: Nitrogen source.

Fig. 3. Surface (A) and contour (B) plots of BEA production versus carbon and nitrogen concentrations.

this being maximal when using glucose and $NaNO_3$, respectively. The experimental data of the carbon/nitrogen ratio for the production of BEA is presented in Table 1, and the ANOVA table is given in Table 5. The derived second-order polynomial was $-0.1895142 + 0.0111428X_1 + 0.0003410X_2 - 0.0000468X_1^2 - 0.0000015X_2^2 - 0.0000094X_1X_2$. The statistical analyses indicated that the proposed model yielded high values of R^2 for all the responses. The R^2 value for the production of BEA was 0.86. To aid visualization, a surface plot of the production of BEA versus the carbon and nitrogen concentrations is shown in Fig. 3. This figure indicates the interaction between the concentrations of carbon and nitrogen sources in the production of BEA, which was maximal when glucose and $NaNO_3$ were present at concentrations of about 108 and 25 mM, respectively. The model predicted a maximum response of 0.46 g/l for this condition, and the actual production was 0.42 g/l; the closeness of these values demonstrates the validity of the response model. The final yield at optimal carbon/nitrogen ratio determined by RSM increased about 2.5 fold when it was compared in FDM. These results indicate that the carbon/nitrogen ratio plays a significant role in maximizing the production of BEA.

Given that *F. oxysporum* KFCC 11363P is receiving increasing attention as a valuable resource, it is desirable to determine the culture conditions that provide optimal yields. The focus in the present study on the effect of various parameters on mycelia growth and BEA production revealed the optimum carbon and nitrogen sources, and their concentrations.

Acknowledgment

REFERENCES

1. Audhya, T. K. and D. W. Russell. 1973. Spectrophotometric determination of enniatin A and valinomycin in fungal extracts by ion complexation. *Anal. Lett.* **6:** 265–274.

2. Audhya, T. K. and D. W. Russell. 1974. Production of enniatins by *Fusarium sambucinum:* Selection of high-yield conditions from liquid surface cultures. *J. Gen. Microbiol.* **82:** 181–190.

3. Bernardini, M., A. Carilli, G. Pacioni, and B. Santurbano. 1975. Isolation of beauvericin from *Paecilomyces fumosoroseus. Phytochemistry* **14:** 1865.

4. Calò, L., F. Fornelli, R. Ramires, S. Nenna, A. Tursi, M. F. Caiaffa, and L. Macchia. 2004. Cytotoxic effects of the mycotoxin beauvericin to human cell lines of myeloid origin. *Pharmacol. Res.* **49:** 73–77.

5. Castella, G., G. P. Munkvold, P. Imerman, and W. G. Hyde. 1999. Effect of temperature, incubation period and substrate on production of fusaproliferin by *Fusarium subglutinans* ITEM 2404. *Nat. Toxins* **7:** 129–132.

6. Chauhan, B. and R. Gupta. 2004. Application of statistical experimental design for optimization of alkaline protease production from *Bacillus* sp. PGR-14. *Process Biochem.* **39:** 2115–2122.

7. Deol, B. S., D. D. Ridley, and P. Singh. 1978. Isolation of cyclodepsipeptides from plant pathogenic fungi. *Aust. J. Chem.* **31:** 1397–1399.

8. Fairlie, D. P., G. Abbenante, and D. R. March. 1995. Macrocyclic peptidomimetics: Forcing peptides into bioactive conformations. *Curr. Med. Chem.* **2:** 654–686.

9. Faulkner, D. J. 1988. Marine natural products. *Nat. Prod. Rep.* **5:** 613–663.

10. Fostso, J., J. F. Leslie, and J. S. Smith. 2002. Production of beauvericin, moniliformin, fusaproliferin, and fumonisins B1, B2, and B3 by fifteen ex-type strains of *Fusarium* species. *Appl. Environ. Microbiol.* **68:** 5195–5197.

11. Fukuda, T., M. Arai, Y. Yamaguchi, R. Masuma, H. Tomoda, and S. Omura. 2004. New beauvericins, potentiators of antifungal miconazole activity, produced by *Beauveria* sp. FKI-1366. II. Structure elucidation. *J. Antibiot.* **57:** 117–124.

12. Fusetani, N., T. Sugawara, S. Matsunaga, and H. Hirota. 1991. Orbiculamide A: A novel cytotoxic cyclic peptide from a

marine sponge *Theonella* sp. *J. Am. Chem. Soc.* **113:** 7811–7812.

13. Ganassi, S., A. Moretti, A. M. Bonvicini-Pagliai, A. Logrieco, and M. A. Sabatini. 2002. Effects of beauvericin on *Schizaphis graminum (Aphididae). J. Invertebr. Pathol.* **80:** 90–96.

14. Grove, J. F. and M. Pople. 1980. The insecticidal activity of beauvericin and the enniatin complex. *Mycopathologia* **70:** 103–105.

15. Gupta, S., B. Krasnoff, N. L. Underwood, J. A. A. Renwick, and D. W. Roberts. 1991. Isolation of beauvericin as an insect toxin from *Fusarium semitectum* and *Fusarium moniliforme* var. *subglutinans. Mycopathologia* **115:** 185–189.

16. Hamill, R. L., C. E. Higgens, H. E. Boaz, and M. Gorman. 1969. The structure of beauvericin, a new depsipeptide antibiotic toxic to *Artemia salina. Tetrahedr. Lett.* **49:** 4255–4258.

17. Jeong, D. H., K. D. Park, S. H. Kim, K. R. Kim, S. W. Choi, J. T. Kim, K. H. Cho, and J. H. Kim. 2004. Identification of *Streptomyces* sp. Producing antibiotics against phytopathogenic fungi and its structure. *J. Microbiol. Biotechnol.* **14:** 212–215.

18. Lin, Y., J. Wang, X. W. S. Zhou, L. L. P. Vrijmoed, and E. B. G. Jones. 2002. A novel compound enniatin G from the mangrove fungus *Halosarpheia* sp. from the South China Sea. *Aust. J. Chem.* **55:** 225–227.

19. Linko, S. and L. C. Zkong. 1991. Central composite experimental in the optimization of lignin peroxidase production in shake cultures by free and immobilized *Phanerochaete chrysosporium. Bioproc. Eng.* **6:** 43–48.

20. Logrieco, A., A. Rizzo, R. Ferracane, and A. Ritieni. 2002. Occurrence of beauvericin and enniatins in wheat affected by *Fusarium avenaceum* head blight. *Appl. Environ. Microbiol.* **68:** 82–85.

21. Madry, N., R. Zocher, and H. Kleinkauf. 1983. Enniatin production by *Fusarium oxysporum* in chemically defined medium. *Eur. J. Appl. Microbiol. Biotechnol.* **17:** 75–79.

22. Martin, J. F. and A. L. Demain. 1980. Control of antibiotic biosynthesis. *Microbiol. Rev.* **44:** 230–251.

23. Minasyan, A. E., D. N. Cherminskii, and I. A. Ellanskaya. 1978. Synthesis of enniatin B by *Fusarium sambucinum. Mikrobiologiya* **47:** 67–71.

24. Moretti, A., A. Logrieco, A. Bottalico, A. Ritieni, G. Randazzo, and P. Corda. 1995. Beauvericin production by *Fusarium subglutinans* from different geographical areas. *Mycol. Res.* **99:** 282–286.

25. Murat, E. 2004. Optimization of medium composition for actinorhodin production by *Streptomyces coelicolor* A3(2) with response surface methodology. *Process Biochem.* **39:** 1057–1062.

26. Nelson, P. E., T. A. Toussoun, and W. F. Marasas. 1983. *Fusarium species: An Illustrated Manual for Identification.* The Pennsylvannia State University Press.

27. Nilanonta, C., M. Isaka, P. Kittakoop, S. Trakulnaleamsai, M. Tanticharoen, and Y. Thebtaranonth. 2002. Precursor-directed biosynthesis of beauvericin analogs by the insect pathogenic fungus *Paecilomyces tenuipes* BCC1614. *Tetrahedron.* **58:** 3355–3360.

28. Jagannadha Rao, K. and C. H. Kim. 2000. Statistical optimization of medium for the production of recombinant hirudin from *Saccharomyces cerevisiae* using response surface methodology. *Process Biochem.* **35:** 639–647.

29. Rim, S. O., J. H. Lee, W. Y. Choi, S. K. Hwang, S. J. Suh, I. J. Lee, I. K. Rhee, and J. G. Kim. 2005. *Fusarium proliferatum* KGL0401 as a new gibberellin-producing fungus. *J. Microbiol. Biotechnol.* **15:** 809–814.

30. Sagakuchi, M., A. Moretti, E. Endo, Y. Matsuda, H. Toyoda, and S. Ouchi. 2000. An approach to the use of plant sensitivity for simple detection of mycotoxins. *In: Proceedings of the First Asian Conference of Plant Pathology. Kuala Lumpur, Malaysia.*

31. Selvaraj, T., C. Padmanabhan, Y. J. Jeong, and H. Kim. 2004. Occurrence of vesicular-arbuscular mycorrhizal (VAM) fungi and their effect on plant growth in endangered vegetations. *J. Microbiol. Biotechnol.* **14:** 885–890.

32. Song, H. H., J. H. Ahn, Y. H Lim, and C. Lee. 2006. Analysis of beauvericin and unusual enniatins co-produced by *Fusarium oxysporum* FB1501 (KFCC 11363P). *J. Microbiol. Biotechnol.* **16:** 1111–1119.

33. Tomoda, H., H. Nishida, X. X. Huang, R. Masuma, Y. K. Kim, and S. Omura. 1992. New cyclodepsipeptides, enniatins D, E, and F produced by *Fusarium* sp. FO-1305. *J. Antibiot.* **45:** 1207–1215.

34. Vohra, A. and T. Satyanarayana. 2002. Statistical optimization of the medium components by response surface methodology to enhance phytase production by *Pichia anomala*. *Process Biochem.* **37:** 999–1004.