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RESEARCH PAPER

Optimization of a Liquid Medium for Beauvericin Production in *Fusarium redolens* Dzf2 Mycelial Culture

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Abstract Beauvericin (BEA) is a proven and potent antibiotic compound useful for bio-control and a potential antifungal and anticancer agent for human. This study was to evaluate and optimize the nutrient medium for BEA production in mycelial liquid culture of a high BEA-producing fungus *Fusarium redolens* Dzf2 isolated from a medicinal plant. Among various organic and inorganic carbon and nitrogen sources, glucose and peptone were found the most favorable for the *F. redolens* Dzf2 mycelial growth and BEA production. Through a Plackett-Burman screening test on a basal medium, glucose, peptone, and medium pH were identified as the significant factors for mycelial growth and BEA production. These factors were optimized through central composite design of experiments and response surface methodology, as 49.0 g/L glucose, 13.0 g/L peptone and pH 6.6, yielding 198 mg/L BEA (versus 156 mg/L in the basal medium). The BEA yield was further increased to 234 mg/L by feeding 10 g/L glucose to the culture during exponential phase. The results show that *F. redolens* Dzf2 mycelial fermentation is a feasible and promising process for production of BEA.

Keywords: *Fusarium redolens*, mycelial culture, liquid medium, beauvericin, statistical optimization

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1. Introduction

Beauvericin (BEA) is a cyclic hexadepsipeptide mycotoxin which was originally isolated from *Beauveria bassiana* [1] and later from *Paecilomyces fumosoroseus* [2] and *Fusarium lateritium* [3]. BEA has strong antibacterial, antifungal, and insecticidal activities [1,3,4], and has also shown significant cytotoxic activity to various human cancer cell lines [5]. The notable antimicrobial and cytotoxic activities of beauvericin have attracted research interest in its application as a potential antibiotic and anticancer agent for human health care [6-8].

Beauvericin is most widely produced by many entomopathogenic *Fusarium* fungal species [9,10]. In addition to BEA, enniatins, which belong to another class of cyclic-peptides having entomopathogenic activities, are also produced by many of these *Fusarium* species. Nevertheless, BEA is the most common and abundant mycotoxin, and plays a major role in the insecticidal activities of these *Fusarium* species [11]. Since BEA is a fungal metabolite, fungal fermentation may be a feasible and efficient means for large-scale production of BEA. Up to date, however, there have been few or no reported studies on fungal fermentation processes for BEA production. Lee *et al.* [12] has recently reported a study on statistical optimization of liquid culture media for maximal BEA production by a *F. oxysporum* strain isolated from soil in Korea.

F. redolens Dzf2 is an endophytic strain of fungus isolated from the rhizome of *Dioscorea zingiberensis* C. H. Wright (Dioscoreaceae), a medicinal plant mainly distributed in China. Our previous study [13] has shown that the mycelial extract of *F. redolens* Dzf2 had strong antimicro-

bial activities, and BEA was the most abundant and active antimicrobial compound in the extract. The BEA content of *F. redolens* Dzf2 mycelia cultivated in potato dextrose broth was about 5 mg/g, which was even higher than the highest found previously in many *Fusarium* species, *e.g.* 3.2 mg/g in nearly 50 species by Logrieco *et al*. [9] and 0.72 mg/g in 15 species by Fotso *et al.* [10]. Therefore, *F. redolens* Dzf2 may be a more efficient and promising producer of BEA through fermentation technology.

As an early and essential step in the development of an efficient fermentation process for BEA production, this study was to examine and optimize the major nutrients and physicochemical conditions for *F. redolens* Dzf2 mycelial culture through statistical experiment design and data analysis. A screening test based on Plackett-Burman design was first conducted on numerous nutrient components and culture factors to identify the significant factors affecting the mycelial growth and BEA production. The significant factors were then optimized through central composite design (CCD) of experiments and response surface methodology (RSM). In addition, fed-batch culture was exercised for further enhancement of the BEA production.

2. Materials and Methods

2.1. Chemicals

Most of the reagents for culture media and chemical analysis were from Sigma (St. Louis, MO, USA) and a few from other recognized commercial suppliers. These were of either reagent grade (for culture media) or analytical grade (for analysis).

2.2. Microorganism and culture conditions

F. redolens Dzf2 was isolated from the rhizome of *Dioscrea zingiberensis*, a Chinese medicinal plant, and identified by rDNA sequencing (GenBank accession number DQ446211) [13]. Stock culture of *F. redolens* Dzf2 mycelia was maintained on solid potato-dextrose-agar (PDA) medium at 25°C. Mycelial liquid culture was maintained in 250 mL Erlenmeyer flasks, each filled with 50 mL medium, placed on a rotary shaker controlled at 150 rpm and 25°C for 7 days or a specified culture period. The inoculums for the mycelial liquid culture was prepared by shaking incubation of mycelia from the solid stock culture in potato dextrose broth (PDB) for 2 days and diluted to 10^5 spores/mL, and 1 mL was added to each flask.

All experiments in this study were carried out in the shake-flask cultures in triplicate and the results were averaged. The basal medium (before optimization) used in this study consisted of 40 g/L glucose, 10 g/L peptone, 0.6 g/L NaCl, 0.6 g/L K_2HPO_4, 0.2 g/L $MgSO_4$, 0.05 g/L $FeSO_4$, and pH 7.0. It was formulated based on the media used for *F. oxysporum* cultures [14,15] and our preliminary tests, which was more favorable for *F. redolens* Dzf2 mycelial growth and BEA production than the PDB used in our previous study [13].

2.3. Determination of biomass, nutrient concentrations, and BEA content

F. redolens Dzf2 mycelial biomass was separated from the liquid medium by centrifugation and rinsed twice with distilled water, and then dried at 65°C to constant dry weight (dw). Biomass concentration (g/L) in the results was represented by the mycelial dry weight (dw) in per liter of culture medium. Glucose concentration in the liquid medium was analyzed by a YSI 2700 Biochemistry Analyzer (YSI Inc., Yellow Springs, Ohio, USA). Total nitrogen concentration (organic and inorganic) in the medium was analyzed by the standard Kjeldahl method (ASTM 3590 2002) using NH_4Cl as a reference, and expressed as total Kjeldahl nitrogen (TKN).

BEA was extracted from dry mycelial powder with methanol-ethyl acetate (50:50 v/v) in an ultrasonic bath (three times, 30 min each), and then centrifuged to attain a solid-free extract. BEA content in the extract solution was analyzed by HPLC using a C18 column, acetonitrile-H_2O (85:15 v/v) as the mobile phase and UV detection at 210 nm. The peak area was calibrated to BEA content with a chemical standard (Sigma). BEA present in the culture medium was negligible and not determined. The volumetric BEA yield (mg/L) shown in the results is the product of biomass concentration (g/L) and BEA content (mg/g).

2.4. Evaluation of nitrogen and carbon sources

Before the statistical optimization experiments, we first evaluated various nitrogen and carbon sources for mycelial growth and BEA production (Table 1). Three organic nitrogen sources, peptone (PEP), corn steep liquor (CSL), and yeast extract (YE) and 3 inorganic nitrogen sources were tested both separately and in various combinations, all at a fixed total nitrogen of 1.0 g/L (each at 0.5 g/L for two in combination). Seven different sugars (carbon sources) were tested at a fixed concentration of 40 g/L. All these were tested as the alternative nitrogen (to peptone) or carbon sources (to glucose) in the basal medium. One-way ANOVA was performed for experimental data comparison and Duncan's new multiple range test (MRT) for multiple comparison [16].

2.5. Experiment design and data analysis

A screening test was constructed by the Plackett-Burman (P-B) design on all six medium components of the basal medium and the medium (pH), to examine their effects and

Table 1. Mycelial biomass and BEA content of *F. redolens* Dzf2 mycelial cultures with various nitrogen and carbon (sugar) sources in the basal medium (on day 7)

Nutrients	Biomass (g/L)	BEA content (mg/g)	Final medium (pH)
Nitrogen (all with 40 g/L glucose)			
NH_4Cl + CSL	11.2 ± 0.79^{b}	1.96 ± 0.32^{f}	2.29 ± 0.13^{h}
NH_4Cl + PEP	10.9 ± 0.43^{bc}	4.96 ± 0.73^{d}	2.29 ± 0.15^{h}
NH_4Cl + YE	10.3 ± 0.22^{c}	1.86 ± 0.12^{f}	2.33 ± 0.16^{h}
YE + CSL	15.5 ± 0.35^{a}	3.40 ± 0.49^{e}	7.86 ± 0.17^{a}
YE + PEP	11.3 ± 0.29^{b}	6.36 ± 0.34^{c}	7.12 ± 0.23^{b}
PEP + CSL	15.8 ± 0.47^{a}	5.83 ± 0.42^{c}	6.77 ± 0.58^{bc}
NH_4Cl	1.36 ± 0.33^{e}	4.86 ± 0.45^{d}	5.25 ± 0.14^{f}
KNO_3	0.39 ± 0.14^{f}	6.1 ± 0.87^{c}	6.63 ± 0.20^{bc}
Urea	0.92 ± 0.19^{ef}	3.16 ± 0.18^{e}	6.03 ± 0.05^{de}
CSL	8.10 ± 0.60^{d}	6.59 ± 0.49^{c}	5.60 ± 0.13^{ef}
YE	10.6 ± 0.07^{bc}	8.27 ± 0.32^{b}	6.35 ± 0.59^{cd}
PEP	15.2 ± 0.23^{a}	10.5 ± 0.66^{a}	4.46 ± 0.36^{g}
Sugar (all with 10 g/L PEP)			
Lactose	5.67 ± 0.10^{e}	7.75 ± 0.54^{e}	8.02 ± 0.48^{a}
Arabinose	12.9 ± 0.70^{d}	5.38 ± 0.10^{g}	4.34 ± 0.16^{cd}
Xylose	15.2 ± 0.51^{c}	6.19 ± 0.10^{f}	4.55 ± 0.23^{bcd}
Fructose	13.3 ± 0.74^{d}	6.21 ± 0.05^{f}	3.94 ± 0.03^{e}
Sucrose	15.9 ± 0.55^{bc}	9.86 ± 0.45^{b}	4.51 ± 0.18^{bcd}
Glucose	15.2 ± 0.44^{c}	10.7 ± 0.28^{a}	4.75 ± 0.04^{b}
Maltose	16.5 ± 0.22^{ab}	8.96 ± 0.23^{c}	4.67 ± 0.00^{bc}

All values represent means ± S.E.M; values marked with different letters "a,b,c…" indicate significant difference at $p < 0.05$ by Duncan's method for multiple comparison. Nutrient symbols: PEP-peptone, YE-yeast extract, CSL-corn steep liquor.

to identify the significant factors on BEA production. P-B design allows for the screening of n variables with $n + 1$ runs, each variable set at two levels, high (H) and low (L) (Table 2) [14]. Therefore, 12 runs were designed for the seven test variables plus four dummy variables. The significant variables or factors screened out by the above P-B test were optimized through central composite design (CCD) of experiments and response surface methodology (RSM). The experimental results were fitted to a predictive quadratic polynomial equation for the correlation of response to the test variables as given by (Box *et al.* [17]),

$$Y = \beta_0 + \sum_{i=1}^{i=3} \beta_i x_i + \sum_{i=1}^{i=3} \beta_{ii} x_i^2 + \sum_{i=1}^{i=3} \sum_{j=1}^{i=3} \beta_{i,j} x_i x_j \quad (1)$$

Where Y is the target (objective) response, β_0 a constant, β_i the linear coefficient, β_{ii} the quadratic coefficient, and $\beta_{i,j}$ the interaction coefficient. The response (objective function) considered in the optimization study is volumetric BEA yield. The equation uses coded variables of the actual variables as given by $x_i = (X_i - X_i^*)/\Delta X_i$, where x_i is the coded value of the ith test variable, X_i the actual value of the ith test variable, X_i^* the value of X_i at the center point of the investigated area, and ΔX_i the step size. The analysis of variance (ANOVA) for the experimental data and model coefficients were computed with Statistic 6.0 software (StatSoft, Inc., OK).

Table 2. Levels and values of variables tested in Plackett-Burman design (all component concentrations in g/L)

Level	PEP	Glc	pH	$FeSO_4$	$MgSO_4$	K_2HPO_4	NaCl
H	13	50	7	0.075	0.3	0.9	0.9
L	7	30	4	0.025	0.1	0.3	0.3

Glc = glucose.

2.6. Fed-batch culture

Fed-batch culture was exercised for further improving the BEA production based on the optimized medium composition and pH for the batch culture. For a preliminary evaluation in this study, we only tested one fed-batch scheme, the feeding of 10 g/L glucose to the culture during exponential growth (on day 2) of the mycelial culture.

3. Results and Discussion

3.1. Comparison of nitrogen and carbon sources

Table 1 shows the mycelial biomass, BEA content of mycelial biomass, and medium pH in *F. redolens* Dzf2 mycelial cultures supplemented with different nitrogen sources and sugars. The cultures supplemented with an organic nitrogen nutrient (PEP, CSL or YE) all achieved fairly high biomass concentrations of 8.1~15.5 g/L, while the cultures with only inorganic nitrogen (NH_4Cl, KNO_3, or urea) showed poor growth with very low biomass concentrations (< 1.4 g/L). Among all single and combined nitrogen sources, PEP as a single nitrogen source was most favorable for both biomass growth and BEA accumulation, giving rise to the highest volumetric BEA yield of 158 mg/L. The combined use of two nitrogen nutrients in a culture had no beneficial effect on mycelial growth or BEA accumulation in most cases except for NH_4Cl + CSL and YE + CSL compared to that with a single nitrogen nutrient in culture. The strong dependence of the *F. redolens* Dzf2 mycelial culture on a complex organic nitrogen nutrient is distinct from the *F. oxysporum* fungus reported by Lee *et al.* [12] which grew most favorably in a chemically defined medium but poorly in potato dextrose broth and malt extract broth. This *F. oxysporum* fungus could also grow to a high density in a complex medium of yeast and malt extract broth, but at a much slower rate (10 g dw/L in 8 ~ 9 days) than in the chemically defined medium (10 g dw/L in 2 days).

According to the biomass data (PEP versus NH_4Cl + PEP), the addition of NH_4Cl had a negative effect on mycelial growth in the culture supplemented with PEP. The cultures supplemented with NH_4Cl plus an organic nitrogen nutrient had a final medium pH of about 2.3, much lower than the initial medium pH 7 and the final medium pH of other cultures without NH_4Cl. The pH drop or medium acidification caused by NH_4Cl is most probably attributed to the uptake of NH_3 by the fungal cells and the concomitant release of H^+ into the culture medium. With its negative effects on mycelial growth and pH stability, NH_4Cl or ammonium is not a favorable medium component for the *F. redolens* Dzf2 mycelial culture.

Of the 7 different sugars tested as carbon sources for the *F. redolens* Dzf2 mycelial culture, glucose, sucrose, xylose, and maltose yielded the similar biomass concentrations, which are notably higher than those by the other three sugars. The BEA contents with glucose, sucrose, and maltose (8.96 ~ 10.66 mg/g) are notably higher than with the other sugars (5.38 ~ 7.75 mg/g). Overall, glucose was the most favorable carbon source for both mycelial growth and BEA production, giving rise to the highest volumetric BEA yield of 161 mg/L. The cultures supplied with different sugars mostly had the similar final medium pH between 4.0 and 4.7 except for that with lactose.

Based on these experimental results, peptone was chosen as the nitrogen source and glucose as the carbon source for the mycelial culture in the following optimization experiments.

3.2. Screening of significant factors using plackett-burman design

Table 3 shows the ANOVA data for the experimental results from the 12 run P-B screening test. Of the seven factors tested, six (five nutrient components and medium pH) had positive effects, and one (NaCl) had a negative effect on volumetric BEA yield. Three factors, peptone, and pH, had a significant effect at a confidence level $p < 0.05$. Therefore, these three were taken as the significant variables in the following optimization experiments.

3.3. Optimization of the factors with CCD and RSM

For the optimization of three factors (peptone, glucose, and pH) with central composite design (CCD), a 2^3 factorial design is required which contains six star points ($\alpha = \pm 1.68$) and two replicates at the center points with a total of 16 runs. The coded and actual values of these significant factors in the CCD design were shown in Table 4, the other medium components of the basal medium were fixed at the center points of P-B design, *i.e.* 0.05 g/L $FeSO_4$, 0.2 g/L $MgSO_4$, 0.6 g/L K_2HPO_4, and 0.6 g/L NaCl.

The CCD experimental data of volumetric BEA yield (Y, mg/L) at various conditions were fitted to the second-order polynomial model (Eq. 1), yielding the following equation,

$$Y = -609.81 + 22.69X_1 - 2.17X_1^2 + 9.25X_2 - 0.11X_2^2 + 130.67X_3 - 12.67X_3^2 + 0.24X_1X_2 + 3.37X_1X_3 - 0.17X_2X_3 \quad (2)$$

Where X_1, X_2, and X_3 are the actual values of peptone, glucose, and pH, respectively. All coefficients computed from the statistic software have been rounded to two decimal places for simplicity and consistency. The optimal variable values computed with Eq. (2) are: 13.0 g/L, peptone; 49.0 g/L, glucose; and pH 6.6, with which the maximum BEA yield is predicted as 194.2 mg/L.

ANOVA of the CCD experimental results (Table 5) yielded R-square value of 0.9598, F value of 12.61 and p

Table 3. ANOVA for BEA yield response from the P-B screening test ($R^2 = 0.935$)

Factors	PEP	Glc	pH	$FeSO_4$	$MgSO_4$	K_2HPO_4	NaCl
Effect	44.79	36.61	48.43	3.465	5.221	0.384	−1.869
p value	0.011	0.021	0.008	0.745	0.628	0.971	0.86

Glc = glucose.

Table 4. Coded values (x) and actual values of variables tested in CCD

x_i	X_1 (PEP, g/L)	X_2 (Glc, g/L)	X_3 (pH)
+1.68	15.05	56.82	8.02
+1	13	50	7
0	10	40	5.5
−1	7	30	4
−1.68	4.95	23.18	2.98

Glc = glucose.

Table 5. ANOVA for BEA yield response from the CCD experiments ($R^2 = 0.9498$)

Sources	Sum of squares	Degree of freedom	Mean square	F-value	$p > F$
Model	30743	9	3416	12.61	0.002968
PEP	7369	1	7369	27.21	0.001984
PEP^2	3172	1	3172	11.71	0.01410
GLC	3076	1	3076	11.36	0.01505
GLC^2	1160	1	1160	4.284	0.08391
pH	10862	1	10862	40.10	0.000725
pH^2	5925	1	5925	21.88	0.003406
PEP*GLC	527.5	1	527.5	1.948	0.2123
PEP*pH	2178	1	2178	8.043	0.02972
GLC*pH	70.44	1	70.44	0.2601	0.6283
Residual	1625	6	270.9		
Lack of fit	1612	5	322.4	24.70	0.1516
Corrected total	32368	15			

> F value of 0.002968, respectively, which all indicate the high significance and reliability of the polynomial model for correlating the experimental results. The F value (24.70) and $p > F$ value for lack of fit (0.1516) indicates that model lack of fit is insignificant.

3.4. Response contour plots

Fig. 1 presents the two-dimensional response surface contours plotted with data predicted from Eq. (2), each with two variables changing over the test range and the third variable fixed at the level corresponding to $x_i = 0$. All contour lines are convex with the maximum response (BEA level) falling within the design boundary. The contour lines on the glucose-peptone plot (Fig. 1A) and the pH-peptone plot (Fig. 1B) are all oriented diagonally, implying a significant interaction between two factor effects on the response (BEA yield), the contour lines on the pH-glucose plot (Fig. 1C) are nearly horizontal, implying a weak interaction between the two factor effects on BEA yield. Moreover, in the pH-peptone plot Fig. 1B, the BEA yield decreases with the decrease of medium pH from 5.0 to 2.5. This pH effect on BEA yield may also be accountable for the negative effect of NH_4Cl in the culture supplemented with peptone shown earlier in Table 1 (causing sharp pH drop from 4.46 for PEP ~ 2.29 for NH_4Cl + PEP).

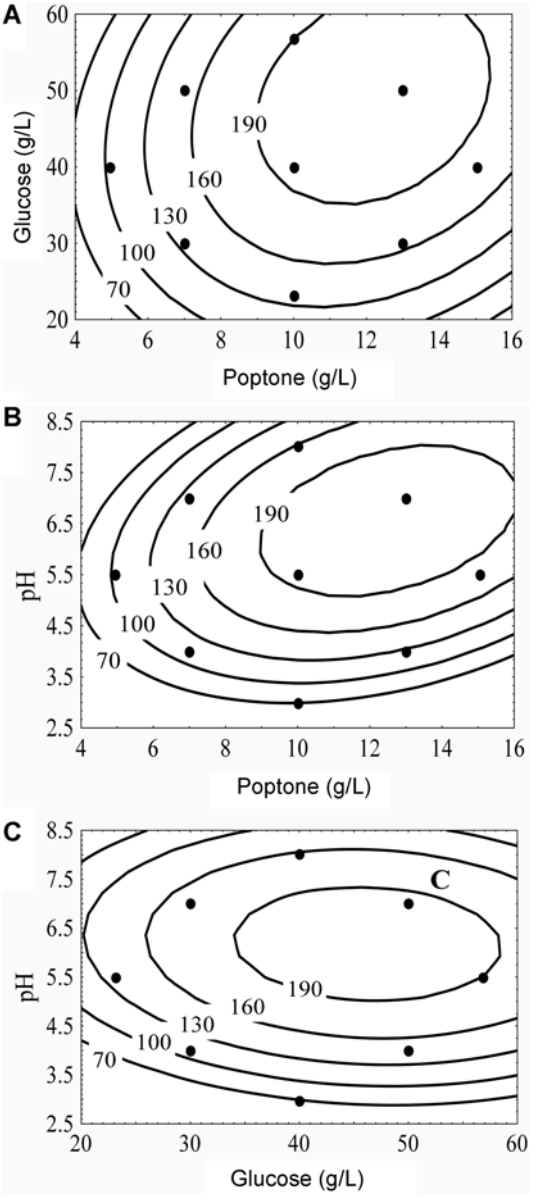

Fig. 1. Contour plots of volumetric BEA yield versus the test variables: (A) glucose and peptone, (B) pH and glucose, and (C) pH and peptone (values on the contour surface representing BEA yield in mg/L).

3.5. Validation experiments and culture time courses

To verify the optimization results derived from Eq. (2), we repeated the mycelial culture experiments in the optimal medium with 49.0 g/L glucose, 13.0 g/L peptone, and pH 6.6, and attained a BEA yield of 197.8 mg/L, which was in close agreement with the model-predicted response of 194.2 mg/L. Therefore, the repeat experiments verified the optimization results and the accuracy of model equation. Fig. 2 shows the batch culture time courses of cell growth, BEA production, glucose, and nitrogen consumption, and pH change in the optimal medium. The mycelial culture exhibited a lag phase in the initial 24 h (day 1), a rapid (or exponential) growth period between day 1 and 3, a slow growth between day 3 and 5, and a stationary period in the rest 2 ~ 3 days of culture. The volumetric BEA yield showed a rapid and almost a linear from day 1 to 6.

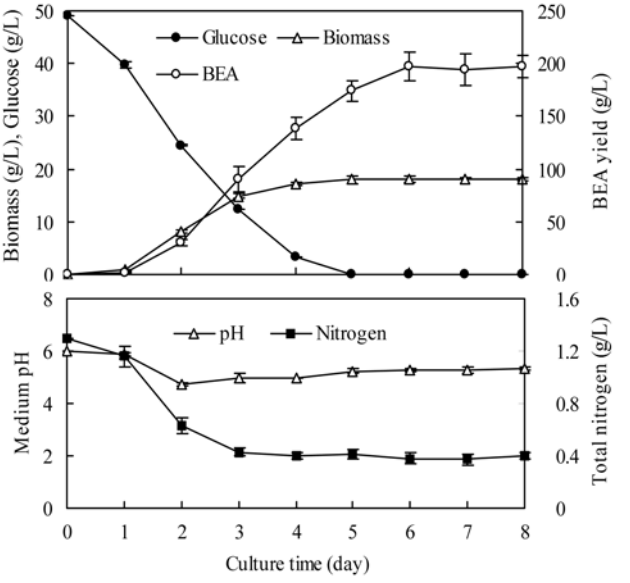

Fig. 2. Batch culture time courses of *F. redolens* Dzf2 in the optimal medium for BEA production (49.0 g/L glucose, 13.0 g/L peptone, and pH 6).

Glucose in the culture was consumed rapidly between day 1 and 4 and depleted on day 5. The total nitrogen (TKN) in culture medium showed a rapid decrease between day 1 and 3, and a slight decrease between day 3 and 4, and a stable and lower level in the remaining culture period. The rate of nitrogen consumption (*i.e.* decrease in total nitrogen) showed a close correlation with the mycelial growth (increase in biomass concentration). The medium pH showed a notable drop during the first two days but a slightly upward trend in the remaining culture period.

As BEA production occurred both in the rapid mycelial growth period (a small portion) and stationary growth period (a major portion), BEA is a mixed or partially growth-associated product. The mixed-growth association pattern of BEA production in *F. redolens* Dzf2 is similar to the accumulation of BEA and related enniatin antibiotics in other fungi such as *Paecilomyces fumosoroseus* [18,19] and *Fusarium sambucinum* [20]. It has been suggested that the enzymes for enniatin biosynthesis are constitutive and expressed in all growth phases of the fungal cultures. The cessation of BEA production during the late days (after day 6) of *F. redolens* Dzf2 culture is probably attributed to substrate deficiency for growth and biosynthesis.

Lee *et al.* [12] evaluated several complex and chemically defined culture media for mycelial growth and BEA production of a *F. oxysporum* species isolated from soil in Korea, finding that a chemically defined medium was most favorable. Through statistical optimization of the carbon and nitrogen sources and C/N ratio in the defined medium, they achieved a maximal BEA yield of 0.42 g/L with 108 mM (19.4 g/L) glucose and 25 mM (2.1 g/L) $NaNO_3$. This BEA yield is much higher than that (≈ 0.2 g/L) produced by our *F. redolens* Dzf2 culture in the optimized medium. To further enhance the BEA production by *F. redolens* Dzf2 in liquid fermentation, we may need to develop new medium formulations and to explore effective means such as those for strain improvement to stimulate the BEA biosynthesis (specific yield) of the fungal cells.

3.6. Fed-batch culture

The batch culture time courses (Fig. 2) show that a major portion of BEA was produced during the stationary phase. Extension of this period by feeding of limiting nutrients or fed-batch operation may prolong the BEA production and increase the yield. Based on this consideration, a fed-batch culture was performed with the optimal medium for BEA production (49 g/L glucose initially), and feeding of 10 g/L of glucose to the culture on day 2 (during the exponential growth phase). Fig. 3 shows the culture time courses with the glucose feeding, which are mostly similar to those in the batch culture in Fig. 2, and glucose was also exhausted on day 5 as in the batch culture. Over a period of 7 days, this fed-batch culture reached a higher (12%) biomass concentration and a higher (18%) BEA yield than those in the batch culture with the same medium but no feeding (Table 5). The significant improvement of BEA production with a simple feeding scheme demonstrated the effectiveness of fed-batch operation. In contrast, the use of a higher initial glucose (59 g/L) instead of feeding on the later day resulted in a BEA yield even lower than in the batch culture at a lower initial glucose (49 g/L). This suggests that high glucose concentration has an adverse effect on the BEA biosynthesis of the fungus.

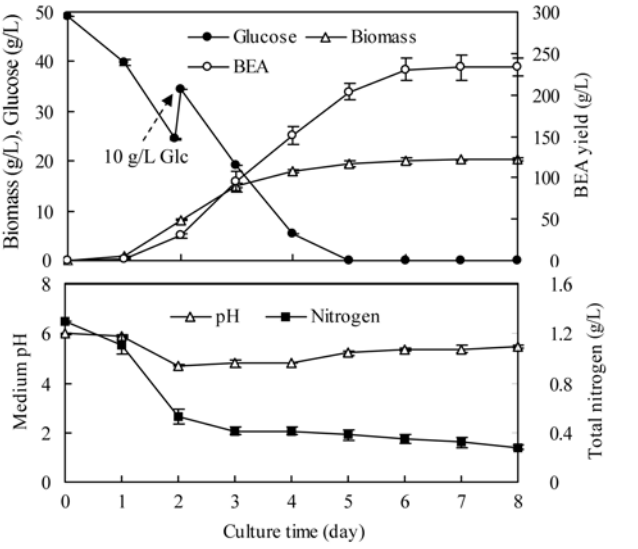

Fig. 3. Fed-batch culture time courses of *F. redolens* Dzf2 in the optimal medium (49.0 g/L initial glucose, 10 g/L, glucose fed on day 2).

Table 6. Effects of initial glucose concentration and feeding on mycelial biomass and BEA production (based on the optimal medium; over 7-day culture period)

Initial glucose and feeding	Biomass (g/L)	BEA (mg/L)
49 g/L; no feeding	18.1 ± 0.3	197.8 ± 10.5
49 g/L; 10 g/L on day 2	20.3 ± 0.5	233.5 ± 7.3
59 g/L; no feeding	18.7 ± 0.3	175.9 ± 11.7

4. Conclusion

An optimal medium composition has been derived for BEA production in *F. redolens* Dzf2 mycelial liquid cultures through statistic experiment design and data analysis, giving rise to a higher BEA yield of 198 mg/L. Peptone was the most favorable nitrogen and glucose the most favorable carbon source for the mycelial growth and BEA production. The BEA production by *F. redolens* Dzf2 exhibited a mixed-growth association, occurring partially

during mycelial growth and mostly during the stationary phase in a batch culture. Fed-batch operation has been shown an effective strategy for further enhancement of the BEA production. Our present study demonstrates the feasibility and promising potential of *F. redolens* Dzf2 mycelial fermentation for efficient production of BEA and related bioactive compounds.

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