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Effect of Precultural and Nutritional Parameters on Compactin Production by Solid-State Fermentation

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In the present study, production of compactin by *Penicillium brevicompactum* WA 2315 was studied. In the first step, various precultural parameters were studied by substituting one factor at a time. Subsequently, the effect of maltodextrin DE 18 on compactin production was studied. The optimized parameters gave maximum compactin production of 850 μg/gds as compared with 678 μg/gds before optimization. Statistical study was performed to further improve the production and develop a robust model. An improved yield of 950 μg/gds was obtained using the conditions proposed by the experimental model. The present study emphasizes the importance of precultural and nutritional parameters on the production of compactin, and further confirms the usefulness of solid-state fermentation for the production of industrially important secondary metabolites. It also confirms that complex nitrogen sources such as oil cakes can be used for the production of compactin.

Keywords: Compactin, *Penicillium brevicompactum* WA 2315, solid-state fermentation, fractional factorial design, Box-Behnken design

Fungi produce secondary metabolites such as growth regulators, and antifungal, antibacterial, and hypocholesteremic agents. In 1976, compactin was isolated from *Penicillium brevicompactum* using submerged fermentation. Compactin and its hydroxyl derivative, pravastatin [11], act as competitive inhibitors of the enzyme 3-hydroxy-3-methylglutaryl CoA, resulting in inhibition of cholesterol biosynthesis, and are useful against atherosclerosis. Compactin is also known as mevastatin, and belongs to the polyketide group of metabolites [4]. Commercially, compactin is produced by *Penicillium citrinum* [6, 7, 9, 11], *P. cyclopium* [1], and *A. terreus* [16]. Attempts had also been made to improve the yield of compactin by mutagenesis of fungal cultures [6, 9]. Production of compactin by submerged fermentation of mutated *P. citrinum* NCIM 768 had been reported earlier by Chakravarti and Sahai [3] using Placket-Burman and central composite designs.

The industrial processes usually involve large numbers of factors. The full factorial design with many factors results in an experimental design with a large number of experiments. This tends to be uneconomic and resource intensive. Therefore, a reduction in the number of experiments to a practical level is needed. The basic purpose of fractional factorial designs is to use a limited number of experiments to study the significant factors in a given experimental setting. These designs are a subset of full factorial designs and can be used for the screening of important factors for development of a robust process [21]. The Box-Behnken designs are similar to central composite designs, but do not consider the extreme points [2]. The solid-state fermentation (SSF) process offers major advantage in the form of use of cost-effective agro-industrial residues as substrate, and uses simple instrumentation and less processing of fermented material during downstream processing. The SSF process is being increasingly explored for production of industrially important microbial enzymes and secondary metabolites [17, 19, 20, 22].

This paper evaluates the interaction of precultural and nutritional parameters on compactin production by SSF using a statistical approach and tries to build a robust model for compactin production under SSF conditions. In the first step, precultural parameters were studied by one factor at a time. Subsequently, in the second step, fractional factorial

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design was used to identify the main factors having significant effect on the production of compactin, and later the process was further improved by performing the Box-Behnken design and identifying the level of significant factors for improved compactin yield.

MATERIALS AND METHODS

Materials

Glycerol and magnesium sulfate were purchased from Hi-Media Limited, Mumbai, India. Potassium dihydrogen phosphate and acetonitrile HPLC grade were purchased from S.D. Fine Chemicals Limited, Mumbai, India. Di-ammonium hydrogen phosphate (DiAHP) was purchased from Merck Ltd, Mumbai, India. Maltodextrin was provided by Casco Inc., Canada. Wheat bran (WB), soybean meal (SM), and groundnut oil cake (GOC) for SSF were purchased from the local market of Trivandrum City, India.

Microorganism Maintenance and Seed Preparation

P. brevicompactum WA 2315 was obtained from the culture collection of the Technical University of Budapest, Hungary. The culture was maintained on potato dextrose agar slants at 25°C for 12 days; the slants were subcultured every month. One ml of the spore suspension (10^8/ml) prepared from such slants was used to inoculate 50 ml of sterile seed medium in 250-ml flasks at 25±2°C, 180 rpm, for 2 days in an incubator shaker. Seed medium contained (g/l) glucose 20.0, glycerol 30.0, peptone 8.0, $MgSO_4$ 1.0, $NaNO_3$ 2.0, and soybean meal 20.0, and the pH was adjusted to 6.5±0.2.

Fermentation

Five g substrate WB+GOC (1:1) was taken in a conical flask and supplement was added to it with an initial moisture content of 58%. Supplement contained glycerol 16.0% w/v, magnesium sulfate 0.75% w/v, glucose 11.0% w/v, DiAHP 2.3% w/v, KH_2PO_4 2.0% w/v, and maltose 5.0% w/v and the pH was adjusted to 7.5±0.2 [19]. All media components were sterilized at 121°C for 15 min. Fermentation was carried out at 25±2°C for 7 days. Two ml of seed culture was added per 5 g of substrate. All the experiments were done in triplicates.

Effect of Culture Age

To see the effect of culture age on the production, slants were incubated for different time periods of 4, 8, 12, and 16 days and the effect of slant age on the production of compactin was studied. The spore density was kept constant at 10^8 spores/ml. Slants with 12 days of incubation was used as controls.

Effect of Spore Count

To study the effect of initial spore density used for the seed inoculum preparation on compactin production, 1-ml spore suspensions with a spore count of 10^6, 10^7, 10^8, and 10^9 spores/ml were inoculated in the seed media. After 48 h of incubation, 2 ml of seed was added to the production media and its effect on the production of compactin was studied. The spore suspension with 10^8 spores/ml was used as the control.

Effect of Seed Age

To study the effect of incubation period of seed culture on compactin production, seed medium was incubated for 24 h, 48 h, 72 h, and 96 h at 25±2°C. Two ml of the pre-grown seed culture was inoculated into the production media and evaluated for production of compactin. Seed culture with 48 h growth was used as the control.

Effect of Inoculum Volume

To study the effect of inoculum size on compactin production, different volumes of seed culture (0.5, 1.0, 2.0, and 3.0 ml) were inoculated in the production media. The spore density was kept constant at 10^8 spores/ml. Two ml inoculum volume was used as the control.

Effect of Maltodextrin as Carbon Source

Glucose and maltose from the supplement were replaced with maltodextrin DE 18 at different concentrations (2.0–14.0% w/v). The concentration of maltodextrin DE 18 so optimized was used for further studies.

Fractional Factorial Design

Factorial designs are a type of design of experiments (DOE) that allow researchers to study the main effects among various factors used in a process and their effect on overall process output. Fractional factorial designs are a subset of full factorial designs. The high and low levels selected for this study represented the extremes of normal operating ranges. The DOE software package (Design-Expert software from Stat- Ease, Inc.; http://www.statease.com) was used to calculate all the effects that could be estimated from the data. The factors studied were inoculum volume (A), inoculum age (B), maltodextrin (C), glycerol (D), DiAHP (E), $MgSO_4$ (F), KH_2PO_4 (G), and pH (H). The experimental design used in the study is detailed in Table 1.

Box-Behnken Design

The effects of inoculum volume, inoculum age, glycerol, and KH_2PO_4 were further studied using response surface methodology (RSM). A Box-Behnken design consisting of four variables at three different levels with total 29 runs was used for study. The design included five replicates of the central point. The three levels of each variable were coded as −1, 0, and +1, which corresponded to the lower, middle, and higher values, respectively. The individual parameters studied were inoculum volume (A), inoculum age (B), glycerol (C), and KH_2PO_4 (D). The software Design-Expert (Version 6.0.6, Stat-Ease Inc., Mas, U.S.A.) was used for experimental design, data analysis, and quadratic model building. The response surface graphs were obtained using the software to understand the effect of variables individually and in combination, and to determine their optimum levels. The experimental setup of RSM is shown in Table 3.

Analytical Determination

Compactin from the fermented substrate was estimated by HPLC using the procedure as described by Konya *et al.* [11]. The fermented substrate was initially extracted with 50 ml of ethyl alcohol by shaking on an orbital shaker at 25±2 and 180 rpm for 1 h. The extract was then centrifuged at 6,000 rpm using a Remi cooling centrifuge. The supernatant was injected onto an HPLC. A Jasko HPLC system fitted with a reverse-phase column Waters Spherisorb 5 ODS2 (4.6 mm×250 mm) was used. The mobile phase consisted acetonitrile:water (60:40) with a pH value adjusted to pH 3±0.2 by adding H_3PO_4. The flow rate was maintained at 0.8 ml/min and the detection was done at 237 nm. Compactin from Themis Medicare, Mumbai was used as the standard.

Table 1. Design and response of the fractional factorial design.

Run no.	Inoculum volume (ml)	Inoculum age (h)	Maltodextrin (%)	Glycerol (%)	DiAHP (%)	$MgSO_4$ (%)	KH_2PO_4 (%)	pH	Yield of compactin (μg/gds)
1	1 (−1)	72 (+1)	8 (+1)	20 (+1)	3 (+1)	0.25 (−1)	1 (−1)	6.5 (−1)	644±9
2	2 (+1)	72 (+1)	8 (+1)	10 (−1)	1.5 (−1)	0.25 (−1)	2 (+1)	6.5 (−1)	681±56
3	2 (+1)	72 (+1)	5 (−1)	10 (−1)	3 (+1)	0.75 (+1)	1 (−1)	6.5 (−1)	619±34
4	2 (+1)	48 (−1)	8 (+1)	10 (−1)	3 (+1)	0.25 (−1)	1 (−1)	7.5 (+1)	491±18
5	2 (+1)	48 (−1)	5 (−1)	20 (+1)	3 (+1)	0.25 (−1)	2 (+1)	6.5 (−1)	558±22
6	2 (+1)	72 (+1)	5 (−1)	20 (+1)	1.5 (−1)	0.25 (−1)	1 (−1)	7.5 (+1)	587±20
7	1 (−1)	72 (+1)	8 (+1)	10 (−1)	1.5 (−1)	0.75 (+1)	1 (−1)	7.5 (+1)	577±30
8	2 (+1)	72 (+1)	8 (+1)	20 (+1)	3 (+1)	0.75 (+1)	2 (+1)	7.5 (+1)	901±18
9	1 (−1)	72 (+1)	5 (−1)	10 (−1)	3 (+1)	0.25 (−1)	2 (+1)	7.5 (+1)	642±33
10	1 (−1)	48 (−1)	5 (−1)	10 (−1)	1.5 (−1)	0.25 (−1)	1 (−1)	6.5 (−1)	487±22
11	2 (+1)	48 (−1)	5 (−1)	10 (−1)	1.5 (−1)	0.75 (+1)	2 (+1)	7.5 (+1)	401±4
12	1 (−1)	48 (−1)	5 (−1)	20 (+1)	3 (+1)	0.75 (+1)	1 (−1)	7.5 (+1)	688±15
13	1 (−1)	48 (−1)	8 (+1)	20 (+1)	1.5 (−1)	0.25 (−1)	2 (+1)	7.5 (+1)	646±23
14	2 (+1)	48 (−1)	8 (+1)	20 (+1)	1.5 (−1)	0.75 (+1)	1 (−1)	6.5 (−1)	616±25
15	1 (−1)	48 (−1)	8 (+1)	10 (−1)	3 (+1)	0.75 (+1)	2 (+1)	6.5 (−1)	507±16
16	1 (−1)	72 (+1)	5 (−1)	20 (+1)	1.5 (−1)	0.75 (+1)	2 (+1)	6.5 (−1)	853±47

Results are mean±SD of three determinations. Values in parenthesis are coded values.

Results and Discussion

Effect of Preculture Studies on Production of Compactin

Optimum production of 725 μg/gds was obtained from 12-day-old slants. A decrease in production was observed with the 16-day-old slant (Fig. 1). The spore count varied from 10^6–10^9 spores/ml. Fig. 2 indicates that 10^8 spores/ml supported the maximum production of 741 μg/gds, but addition of 10^9 spores/ml decreased the production to 668 μg/gds. Fig. 3 illustrates that seed culture with 72 h incubation time supported maximum production of 726 μg/gds, and increasing the incubation time to 96 h showed no significant effect on the production. Fig. 4 shows that 2 ml of seed culture gave maximum production of 692 μg/gds, after which a decrease in production was observed.

There are various factors governing the fungal growth and morphology during fermentation. The amount, type, and age of the inoculum are of prime importance in determining the morphology and the general pattern of fungal fermentation. The effect of inoculum volume and stability of spores on production of compactin by submerged fermentation was studied earlier (Y. P. Wang *et al.* 2001. U.S. patent 6323021).

Table 2. ANOVA of fractional factorial design to study the significance of factors.

Source	Sum of squares	Coefficient estimate	Mean square	F value	Prob>F
Model	245,147.5	618.62	17,510.53	1,429.43	0.0207
Inoculum volume (A)	2,256.25	−11.87	2,256.25	184.18	0.0468
Inoculum age (B)	77,006.25	69.37	77,006.25	6,286.22	0.0080
Maltodextrin (C)	3,249	14.25	3,249	265.22	0.0390
Glycerol (D)	73,984	68	73,984	6,039.51	0.0082
DiAHP (E)	2,550.25	12.625	2,550.25	208.18	0.0441
$MgSO_4$ (F)	11,342.25	26.625	11,342.25	925.89	0.0209
KH_2PO_4 (G)	14,400	30	14,400	1,175.51	0.0186
pH (H)	64	−2	64	5.22	0.2625
AB	6,972.25	20.87	6,972.25	569.16	0.0267
AC	42,025	51.25	42,025	3,430.6	0.0109
AD	1,369	−9.25	1,369	111.75	0.0600
AE	8,372.25	22.87	8,372.25	683.44	0.0243
AG	36	−1.5	36	2.93	0.3362
AH	1,521	−9.75	1,521	124.16	0.0570
Residual	12.25		12.25		
Corrected total	245,159.8				

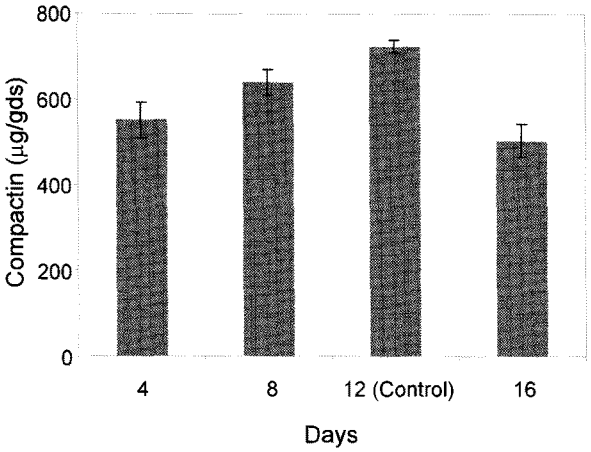

Fig. 1. Effect of slant age on compactin production by *P. brevicompactum* WA 2315.

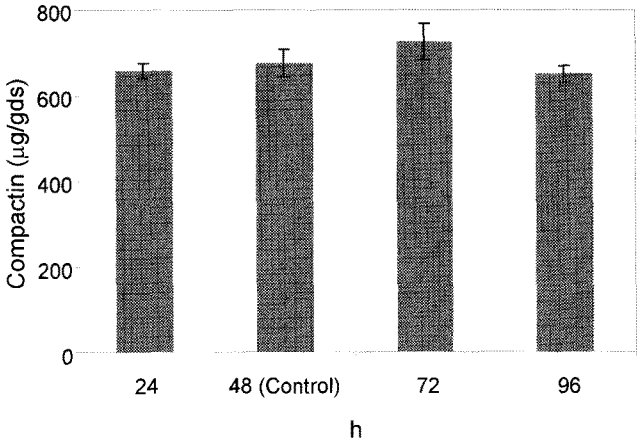

Fig. 3. Effect of incubation time of seed culture on compactin production by *P. brevicompactum* WA 2315.

However, in their study, it was also observed that although inoculum volumes between 4% and 10% were good, spore suspension stored at different time intervals was also stable and did not lead to loss in compactin yield. In the present study, contrary to the earlier finding, it was observed that increasing the age of slant resulted in a decrease in production. Moreover, increased inoculum volume and higher spore count had negative effects on compactin production, whereas the inoculum age at 72 h and 96 h had not much difference. Similar studies in citric acid fermentation under SSF conditions revealed that type, age, and size of inoculum affected the citric acid production under SSF conditions [5].

Effect of Maltodextrin as Carbon Source

The concentration of maltodextrin DE 18 was varied from 2.0–14.0% w/v. Fig. 5 indicates that 8.0% w/v of maltodextrin DE 18 gave a compactin yield of 850 µg/gds. However, further increase in maltodextrin DE 18 concentration resulted in a loss of production.

The maltodextrin addition had been studied earlier to improve the production of lovastatin [12]. It was observed that addition of maltodextrin in SMF for lovastatin production resulted in improved productivity. In this study, it was also observed that replacement of readily assimilable carbon sources such as glucose and maltose with maltodextrin increased the production of compactin. This may be possibly due to the ability of slow metabolizing carbon sources to meet the energy requirement during the extended stationary phases during which the production of secondary metabolites takes place.

The yield data from the precultural study suggested that although the one-factor-at-a-time approach was good for initial screening of factor, variation in yield due to non-robustness of the process could be observed, requiring a more robust approach. Hence, statistically designed experiments were used for further production improvements.

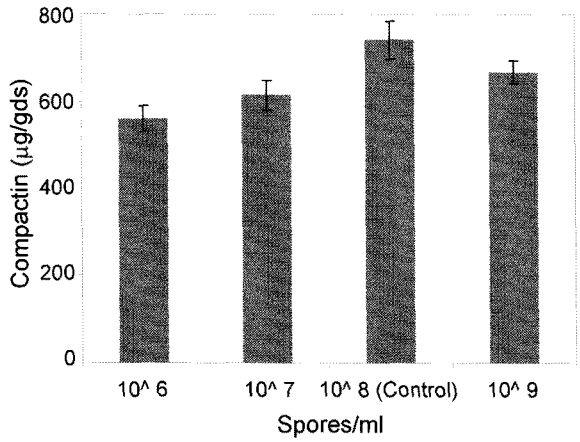

Fig. 2. Effect of spore count on compactin production by *P. brevicompactum* WA 2315.

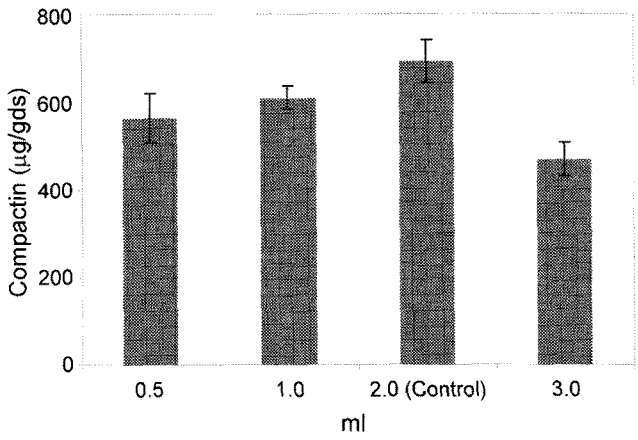

Fig. 4. Effect of inoculum size on compactin production by *P. brevicompactum* WA 2315.

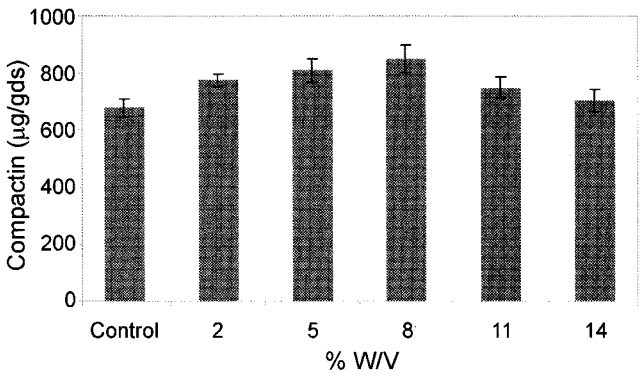

Fig. 5. Effect of maltodextrin DE 18 on compactin production by *P. brevicompactum* WA 2315.

Fractional Factorial Design

The ANOVA of the regression model indicated that inoculum volume, inoculum age, maltodextrin, glycerol, DiAHP, $MgSO_4$, and KH_2PO_4 had significant effects on compactin production. The effects were both positive and negative in nature, with the concentration of maltodextrin, glycerol, inoculum age, DiAHP, $MgSO_4$, and KH_2PO_4 being positive in nature. The effect of inoculum volume was negative in nature. This suggested that whereas the concentrations of DiAHP, $MgSO_4$, KH_2PO_4, glycerol, maltodextrin, and age of the inoculum needs to be increased, the inoculum volume needs to be decreased. The findings suggested that significant main effects were present and needed to be investigated further. The fractional factorial designs are useful for screening of various factors for an improved process and had been used recently for optimization of the fermentative process [13, 14]. In the present study, fractional factorial design was also used to identify the significant main effects.

Box-Behnken Design

The experimental runs and results for the Box-Behnken design are shown in Table 3. There were a total of 29 runs for optimizing the four individual parameters in the current Box-Behnken design for the production of compactin. The data were analyzed by multiple regression analysis using the Design Expert software, and a polynomial equation

Table 3. Box-Behnken design and response of factors on compactin yield.

Run	Inoculum volume (ml)	Inoculum age (h)	Glycerol %	KH_2PO_4 %	Compactin (µg/gds)
1	0 (1.5)	1 (72)	-1 (8)	0 (1.4)	726±42
2	0 (1.5)	0 (60)	0 (14)	0 (1.4)	773±47
3	0 (1.5)	1 (72)	1 (20)	0 (1.4)	792±29
4	-1 (1)	0 (60)	0 (14)	-1 (0.8)	873±52
5	-1 (1)	0 (60)	1 (20)	0 (1.4)	849±41
6	1 (2)	-1 (48)	0 (14)	0 (1.4)	890±36
7	1 (2)	1 (72)	0 (14)	0 (1.4)	749±38
8	0 (1.5)	0 (60)	-1 (8)	-1 (0.8)	715±55
9	0 (1.5)	1 (72)	0 (14)	-1 (0.8)	769±32
10	0 (1.5)	0 (60)	0 (14)	0 (1.4)	773±47
11	0 (1.5)	0 (60)	0 (14)	0 (1.4)	773±47
12	0 (1.5)	1 (72)	0 (14)	1 (2.0)	768±52
13	1 (2)	0 (60)	-1 (8)	0 (1.4)	673±34
14	0 (1.5)	-1 (48)	0 (14)	1 (2.0)	819±42
15	0 (1.5)	0 (60)	0 (14)	0 (1.4)	773±47
16	-1 (1)	-1 (48)	0 (14)	0 (1.4)	861±36
17	0 (1.5)	0 (60)	1 (20)	-1 (0.8)	865±58
18	0 (1.5)	-1 (48)	-1 (8)	0 (1.4)	821±51
19	-1 (1)	0 (60)	-1 (8)	0 (1.4)	691±33
20	0 (1.5)	-1 (48)	1 (20)	0 (1.4)	860±45
21	0 (1.5)	0 (60)	-1 (8)	1 (2.0)	721±38
22	1 (2)	0 (60)	1 (20)	0 (1.4)	819±32
23	0 (1.5)	0 (60)	0 (14)	0 (1.4)	773±47
24	-1 (1)	0 (60)	0 (14)	1 (2.0)	764±28
25	0 (1.5)	-1 (48)	0 (14)	-1 (0.8)	867±53
26	1 (2)	0 (60)	0 (14)	1 (2.0)	822±41
27	-1 (1)	1 (72)	0 (14)	0 (1.4)	907±45
28	1 (2)	0 (60)	0 (14)	-1 (0.8)	729±51
29	0 (1.5)	0 (60)	1 (20)	1 (2.0)	884±47

Results are mean±SD of three determinations. Values in parenthesis are actual values.

was derived to represent compactin yield as a function of the independent variables tested. The experimental data were statistically analyzed using the analysis of variance (ANOVA) and the results are presented in Table 4.

The ANOVA of the quadratic regression model indicated the model to be highly significant, as the F-value for the model was 5.96. There was only a 0.10% chance that a "model F-value" this large could occur as a result of noise. The model p value (0.001) also confirmed that the model was highly significant. The coefficient estimate and the corresponding Prob>F-values suggested that the inoculum volume, inoculum age, and glycerol had significant effects on compactin production. The analysis also showed that there were significant interactions between inoculum volume with inoculum age, and inoculum volume with KH_2PO_4. The model quadratic equation for compactin yield is provided below:

$$\text{Compactin } (\mu g/gds) = 773 - 21.91A - 33.91B + 60.16C - 3.33D + 15.45A^2 + 40.70B^2 - 10.91C^2 + 11.58D^2 - 46.75AB - 3AC + 50.5AD + 6.75BC + 11.75BD + 3.25CD$$

The A, B, C, and D refer to inoculum volume, inoculum age, glycerol, and KH_2PO_4, respectively. From the F-value statistic (Table 4), it was concluded that a change in glycerol concentration caused the major variation in compactin production. The R^2 statistic (Table 4) indicated that the model as fitted explained 85% of the variability in compactin production. A very high degree of precision and a good deal of reliability of the experimental values were indicated by a very low value of the coefficient of variation (CV=4.28). A validation run resulted in a yield of 950 μg/gds as compared with the model-predicted yield of 931 μg/gds. Results presented in Fig. 6 show the interaction between the inoculum volume and inoculum age. At lower inoculum age, the production of compactin increased with increase in inoculum volume, whereas at higher inoculum age the production of compactin decreased with an increase in inoculum volume.

The validation of model-predicted compactin yield with experimentally obtained yield suggested that the model-predicted response zone was the best region for compactin production under a defined experimental space. The interaction between the inoculum volume with inoculum age suggested that at lower inoculum age, the production of compactin increases with increase in inoculum volume, whereas at higher inoculum age the production of compactin decreases with an increase in inoculum volume. This may be due to competition for limiting nutrients as a higher volume of grown inoculum is introduced. Similarly, the interaction between inoculum volume and KH_2PO_4 was significant in SSF for production of compactin, suggesting a higher requirement of salts at a higher initial inoculum volume. This observation supports the interaction between age of inoculum with amount of inoculum, as the salts may become a limiting substrate for compactin production. Similarly, an increased nutrient requirement was observed with an increase in initial inoculum size while working with lactic acid fermentation using *Lactobacilli* [10]. There had been various reports on utilization of the Box-Behnken design for optimization of fermentative production [8, 15, 18].

Table 4. ANOVA of Box-Behnken design to study the significance of factors and their interaction effects.

Source	Sum of squares	Coefficient estimate	Mean square	F value	Prob>F
Model	96,974.99	773	6,926.78	5.95	0.001
Inoculum volume (A)	5,764.08	-21.91	5,764.08	4.95	0.0429
Inoculum age (B)	13,804.08	-33.91	13,804.08	11.87	0.0039
Glycerol (C)	43,440.33	60.16	43,440.33	37.36	<0.0001
KH_2PO_4 (D)	133.33	-3.33	133.33	0.11	0.7399
A^2	1,550.01	15.45	1,550.01	1.33	0.2675
B^2	10,749.2	40.70	10,749.20	9.24	0.0088
C^2	773.01	-10.91	773.01	0.66	0.4285
D^2	870.31	11.58	870.31	0.74	0.4015
AB	8,742.25	-46.75	8,742.25	7.52	0.0159
AC	36	-3	36	0.03	0.8628
AD	10,201	50.5	10,201	8.77	0.0103
BC	182.25	6.75	182.25	0.15	0.6981
BD	552.25	11.75	552.25	0.47	0.5019
CD	42.25	3.25	42.25	0.03	0.8515
Residual	16,274.25	14	1,162.44		
Lack of fit	16,274.25	10	1,627.42		
Pure error	0	4	0		
Corrected total	113,249.2	28			

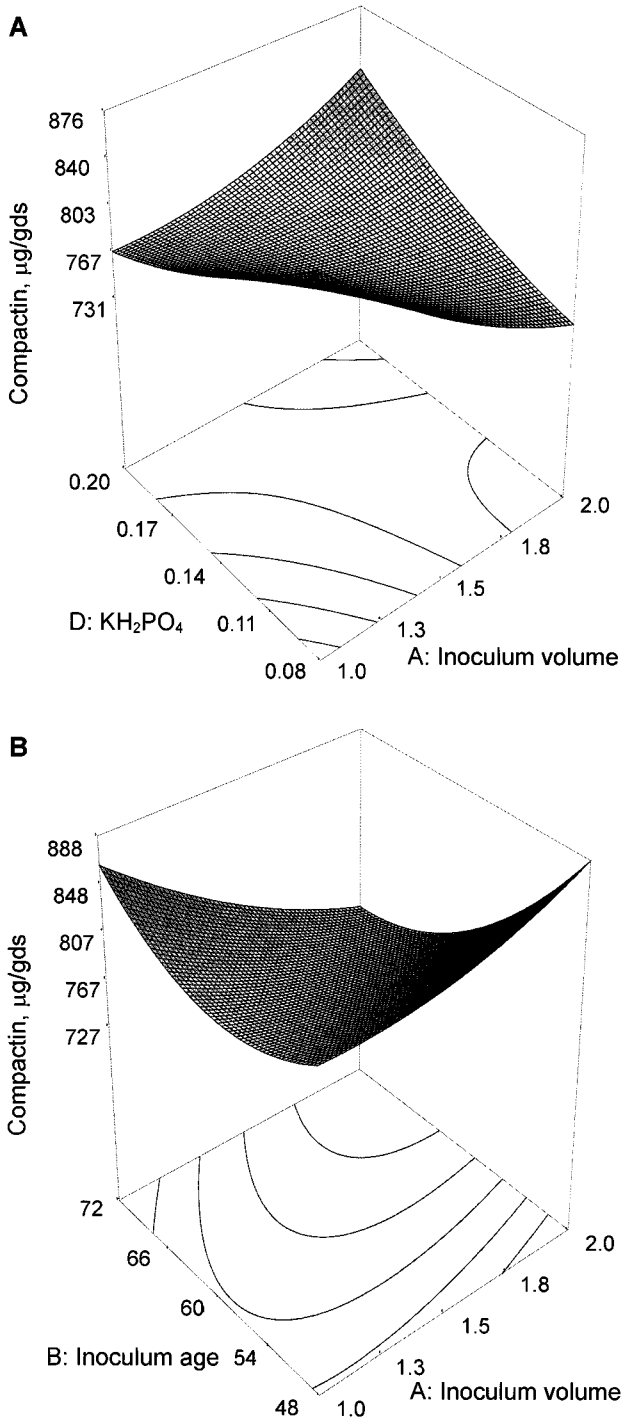

Fig. 6. Interaction graphs between inoculum volume and KH_2PO_4 (**A**) and inoculum volume and inoculum age (**B**).

In the present study, wheat bran + GOC was used as a complex carbon and nitrogen source apart from acting as a support matrix. However, earlier study for lovastatin production had suggested that a complex nitrogen source such as soybean meal gave a very poor yield of lovastatin. The detrimental effect of nitrogen on lovastatin production was also observed in inorganic nitrogen supplementation experiments. Furthermore, the results of experiments using fruit wastes as substrates showed poor lovastatin production [22]. However, in our earlier study, we had demonstrated that supplementation of selected inorganic nitrogen sources and yeast extract resulted in improved production of compactin [19], which was in contravention to earlier report for lovastatin production [22]. The present study confirms that agro-industrial residues such as wheat bran and groundnut oil cakes provide an excellent support matrix and are also useful as a carbon and nitrogen source.

The present study also suggests that precultural characteristics of *P. brevicompactum* WA 2315 affect the compactin production significantly. Replacement of glucose and maltose with slow metabolizing maltodextrin DE 18 also gave better production. Compactin production starting from 678 µg/gds was improved to 950 µg/gds with an improvement of 1.4 times using the combination of single factor and statistically designed experiments. This also proposes a robust model that can be used for compactin production with high degree of control and reproducibility within the defined experimental conditions. It was possible to determine the effect of nutritional and precultural parameters on the production of compactin by *Penicillium brevicompactum* WA 2315 and significant interactions among the parameters were observed. The earlier study for compactin production under SSF conditions [19] had already demonstrated the usefulness of statistical methods for process improvements, and the present study further supports that a combination of one-factor-at-a-time approach along with statistical designs can be used to develop robust operating conditions for compactin production under SSF. The SSF process can be further improved to make it a viable alternative for present commercial processes.

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Nomenclature

µg	microgram
gds	gram dry substrate
SSF	solid-state fermentation
CoA	coenzyme A
DiAHP	di-ammonium hydrogen phosphate
g/l	gram per liter
GOC	groundnut oil cake
WB	wheat bran

SM	soybean meal
mg/l	milligram per liter

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