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ORIGINAL ARTICLE

# Solid-state fermentation with *Serratia marcescens* Xd-1 enhanced production of prodigiosin by using bagasse as an inertia matrix

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**Abstract** Prodigiosin has attracted great interest for several decades for its proapoptotic anticancer and other activities. However, the low productivity of prodigiosin under submerged fermentation (SmF) limits its commercial application. In this study, *S. marcescens* Xd-1 was newly isolated from a mouldy tofu sample and was used to produce prodigiosin by solid-state fermentation (SSF) using bagasse as an inertia matrix. The result showed that *S. marcescens* Xd-1 was adapted to SSF for prodigiosin production and could bear a higher concentration of glycerol. Process parameters were systematically examined to improve the conversion of glycerol to prodigiosin by a response surface methodology. The maximal yield of prodigiosin (40.86 g $kg^{-1}$ dry solid) was achieved with glycerol 1.17 g $g^{-1}$ bagasse, soy peptone 0.33 g $g^{-1}$ bagasse, an initial moisture content of 83.5 %, and 1-mm particles of bagasse by using a response surface methodology (RSM). In addition, the extraction ratio of prodigiosin increased rapidly to 90 % in 30 min by using ultrasonic-assisted reflux extraction. Our results expand the culture method of *S. marcescens* for prodigiosin production and enhanced the conversion of glycerol to prodigiosin.

**Keywords** Prodigiosin · *Serratia marcescens* · Solid-state fermentation · Bagasse · Glycerol · Inertia matrix

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## Introduction

Prodigiosin is a multifaceted, secondary metabolite with a tripyrrole structure that is produced by *Serratia marcescens*, *Pseudomonas magnesiorubra*, *Hahella chejuensis,* and other eubacteria (D'Aoust and Gerber 1974; Harris et al. 2004; Huh et al. 2007; Lee et al. 2011). It has attracted great interest for several decades for its proapoptotic anticancer activity (Pérez-Tomás et al. 2003; Regourd et al. 2007). Many researches have shown it has apoptotic activity against more than 60 cancer cell lines with a concentration of 2.1 μM, including lung, colon, kidney, hematopoietic, and breast cancers, and has low cytotoxicity in noncancerous cells (Montaner et al. 2000; Williamson et al. 2007; Perez-Tomas et al. 2010). Prodigiosin has also shown effective antimalarial, antifungal, immunosuppressive activity, and algicidal activity (Pandey et al. 2003, 2007; Patil et al. 2011; Park et al. 2012; Singh and Shekhawat 2012).

The carbon source has played a crucial role in prodigiosin production. As shown in Table 1, many studies have been performed to determine the optimal conditions for prodigiosin production by submerged fermentation (SmF). *S. marcescens* is the major source of prodigiosin production (Fürstner 2003; Montaner et al. 2000; Pryce and Terry 2000). There are many kinds of carbon sources utilized for prodigiosin production, such as glycerol, brown sugar, mannitol, peanut seed, and so on. In this study, glycerol was used as the carbon source under solid-state fermentation (SSF) condition. Glycerol is the principal byproduct of biodiesel production, which accounts for about 10 % by weight (Chi et al. 2007). It is a promising renewable carbon source that has been used in several types of fermentations by both prokaryotic and eukaryotic strains. Currently, the most promising metabolites are 1,3-propanediol, 2,3-butanediol, ethanol, butanol, citric acid, microbial lipids, and $H_2$ (Silva et al. 2009; Markov et al. 2011; Zhang et al. 2007; Chatzifragkou et al. 2011).

**Table 1** Production of prodigiosin by different strains and carbon sources

| Strains | Fermentation | Yield | Carbon sources | References |
|---|---|---|---|---|
| *S. marcescens* UTM1 | 5-L Bioreactor | 8000 mg $L^{-1}$ | Brown sugar | Aruldass et al. 2014 |
| *S. marcescens* TKU011 | Shake flasks | 978 mg $L^{-1}$ | Squid pen powder | Wang et al. 2012 |
| *S. marcescens* UCP1549 | Shake flasks | 49500 mg $L^{-1}$ | Cassava wastewater | Araújo et al. 2010 |
| *S. marcescens* | Shake flasks | 2423.4 mg $L^{-1}$ | Glycine | Su et al. 2011 |
| *S. marcescens* S389 | Shake flasks | 2950 mg $L^{-1}$ | Ethanol | Song et al. 2000 |
| *S. marcescens* Nima | 5-L bioreactor | 125 mg $L^{-1}$ | Glycerol | Wang et al. 2004 |
| *S. marcescens* B6 | 5-L bioreactor | 583 mg $L^{-1}$ | Glycerol | Tao et al. 2005 |
| *S. marcescens* | Shake flasks | 37600 mg $L^{-1}$ | Peanut seed broth | Shahitha and Poornima 2012 |
| *S. marcescens* | Shake flasks | 4800 mg $L^{-1}$ | Sweet potato extract | Suryawanshi et al. 2014 |
| *S. marcescens* MO-1 | Shake flasks | 277.74 mg $L^{-1}$ | Mannitol | Kurbanoglu et al. 2015 |
| *S. marcescens* N10612 | Shake flasks | 1303 mg $L^{-1}$ | Sucrose | Zang et al. 2014 |
| *S. marcescens* $SR_1$ | Shake flasks | 765.1 mg $L^{-1}$ | Casein | Parani and Saha 2008 |
| *Z. rubidus* S1-1 | Shake flasks | 47.8 mg $L^{-1}$ | Glycerol | Jong et al. 2011 |
| *H. chejuenesis* KCTC 2396 | Shake flasks | 2600 mg $L^{-1}$ | Sucrose | Kim et al. 2008 |

However, there are great differences in the yields of prodigiosin by different strains, and furthermore, prodigiosin produced by *S. marcescens* is mostly bound to bacterial envelopes and still a small part is released into the broth (Purkayastha et al. 1960; Kobayashi and Ichikawa 1991). So, the separation and purification processes have some bottlenecks that prevent large-scale production of prodigiosin by SmF. For industrialization purposes, a high-output and cost-effective method for prodigiosin production is demanded.

O'Rear (O'Rear et al. 1992) found that prodigiosin-producing *S. marcescens* will adhere to hydrophobic surfaces, whereas anon-prodigiosin-producing species will not. Therefore, prodigiosin may be involved in the attachment of *S. marcescens* to hydrophobic surfaces in its natural habitat, which means that *S. marcescens* might be adapting to the environmental conditions in SSF. We, therefore, isolated a new strain of *S. marcescens* for the production of prodigiosin. To enhance the efficient conversion of glycerol to prodigiosin, we studied the culture conditions required to produce a higher yield of prodigiosin using bagasse as a carrier in SSF.

## Materials and methods

### Isolation of the strain

Bean products and soil samples were inoculated in a 250-mL flask containing 50 mL of Luria-Bertani broth (lysogeny broth; LB). The broth was cultivated at 200 rpm for 2 h at 28 °C; it was then diluted and spread onto an LB agar plate to obtain a single colony. Cultures from the single colony were periodically maintained on LB agar medium containing 10 g $L^{-1}$ peptone, 5 g $L^{-1}$ yeast extract, 10 g $L^{-1}$ NaCl, and 1.5 g $L^{-1}$ agar after growth on LB agar medium for 24 h at 28 °C. Prodigiosin is a kind of red intracellular pigment. It has a connection between the color of the colony and yield of prodigiosin. To screen for strains with high prodigiosin production, the red colony collected was inoculated into LB broth at 28 °C for 16 h as seed cultures. And then it was inoculated into an SmF medium containing 20 g $L^{-1}$ glycerol, 5 g $L^{-1}$ soybean peptone and cultured at 28 °C for 48 h. The yield of prodigiosin was then detected with high-performance liquid chromatography (HPLC). The standard sample of prodigiosin was purchased from Sigma-Aldrich Co.

### Effect of inertia matrices on prodigiosin production

In this experiment, bagasse, wheat straw powder, and wood chips were chosen as inertia matrices for investigation of their effects on prodigiosin productivity under SSF. *S. marcescens* Xd-1 was cultivated on LB liquid medium and was used for seed cultures for 16 h at 28 °C. The inertia matrices were pretreated as described by Pintado (Pintado et al. 1998) for use as an inert carrier for prodigiosin production in SSF. The particle size of inertia matrices were 1 mm. Initially, 2 g of pretreated inertia matrices was added to a 250-mL Erlenmeyer flask before other nutrient content was added. The initial contents of glycerol and soy peptone were 0.75 g $g^{-1}$ bagasse and 0.6 g $g^{-1}$ bagasse, respectively. The initial moisture content was 80 %. The contents of the flask were mixed and autoclaved for 20 min at 121 °C. After cooling, each flask was inoculated with 10 % (v/w) seed cultures. Fermentation was carried out at 28 °C for 48 h with mixing every 12 h.

### Effect of glycerol concentration on prodigiosin production

Effect of concentrations of glycerol ranging from 0.1 to 1.5 g $g^{-1}$ bagasse on prodigiosin production by SSF was investigated. The inoculum density was 10 % (v/w) seed cultures and was incubated at 28 °C for 48 h with mixing every 12 h.

### Effect of nitrogen sources on prodigiosin production

Six nitrogen sources were used to enhance the conversion of glycerol to prodigiosin by *S. marcescens* Xd-1; four of these nitrogen sources were organic (casein hydrolysate, yeast extract, tryptone, and soy peptone) and two were inorganic ($(NH_4)_2SO_4$ and $NaNO_3$). The nitrogen sources were added into the initial solid medium at 0.6 g $g^{-1}$ bagasse. Then, the effects of different concentrations of soy peptone (0.25, 0.4, 0.5, 0.6, 0.75, and 1.0 g $g^{-1}$ bagasse) on the prodigiosin yield of *S. marcescens* Xd-1 in SSF were studied.

### Effect of initial moisture content on prodigiosin production

Six different initial moisture levels, i.e., 70 %, 75 %, 80 %, 83 %, 85 %, and 87 % (w/w substrate) were established in the inertia matrix to study the effects of the initial moisture content on prodigiosin production. The initial total moisture content of the substrate was calculated with the following formula:

$$\%\text{moisture content} = \frac{\text{water(g)} + \text{water in seed culture(g)}}{\text{substrate(g)} + \text{water(g)} + \text{water in seed culture(g)}} \times 100\%$$

### Effect of particle size on prodigiosin production

Bagasse with different particle sizes (0.1, 1, 2, 3, and 5 mm) was used to study the influence of particle size on the yield of prodigiosin in SSF of *S. marcescens* Xd-1.

### RSM analysis for prodigiosin production

The response surface methodology (RSM; Box–Benhnken design) procedure was employed to screen the cultural conditions and medium compositions for prodigiosin production. Glycerol, soybean peptone, and initial moisture content were optimized as the major factors. Three variables with designed levels (−1, 0, +1) and three replicates are shown in Table 2. The results were analyzed by using Design expert 8.0 program.

### Ultrasound-assisted reflux extraction of prodigiosin from *S. marcescens* Xd-1

In this experiment, the extraction ratio of prodigiosin by ultrasound-assisted reflux extraction was studied. Extraction was carried out at different concentrations of ethanol (70 %, 80 %, and 90 %) with an extraction temperature of 55 °C, a solid–liquid ratio of 1:5 (w/v), and ultrasonic power of 105 W.

### Analysis of prodigiosin

Solid cultures of *S. marcescens* Xd-1 (5 g) were extracted with 50 mL of ethanol by refluxing in a 45-°C water bath for 1 h. After filtration, the ethanol solution was maintained at 4 °C in preparation for the next experiment.

The concentration of prodigiosin was analyzed with HPLC. An Amethyst C18 column (4.6 × 150 mm i.d.; Sepax Co., Newark, DE, USA) was used for analysis. The mobile phase was composed of 1 % AcOH/$H_2O$-$CH_3CN$ (0 min, 25:75; 15 min, 90:10; 15.5 min, 25:75; 21 min, 25:75). The flow rate was 0.6 mL $min^{-1}$, the injection volume was 20 μL, and the UV detection wavelength was set at 535 nm. The operating temperature was maintained at 37 °C.

### Detection of pH

Solid cultures of *S. marcescens* Xd-1 (5 g) were put in 50 mL of distilled water for 15 min at room temperature. Then, the extraction liquid was gathered by filtration and pH was measured by pH meter.

### Statistical analysis

All of the experiments were carried out in triplicate. Analysis of variance (ANOVA) and regression analysis were conducted, and contour plots were drawn using the Design-Expert 8.0 software package. The results are expressed as the mean ± standard deviation (SD)

## Results and discussion

### Screen strains for prodigiosin production

As shown in Table 3, six strains with red colonies were selected for prodigiosin detection. The strain Xd-1 had the highest yield of prodigiosin, which reached 1.14 g $L^{-1}$. The 16S rDNA nucleotide sequence of strain Xd-1 was amplified with the following primers: 27 F: 5'-AGA GTT TGA TCC TGG CTC AG-3', 1492R: 5'-GGT TAC CTT GTT ACG ACT T-3'. The fragments from polymerase chain reactions were sequenced and identified as *S. marcescens* based on the 16S rDNA sequence. The prodigiosin product was purified from

**Table 2** Three variables and the levels in Box–Behnken design

| Variables | Coded levels | | |
|---|---|---|---|
| | −1 | 0 | +1 |
| $X_1$: Glycerol (g $g^{-1}$ bagasse) | 0.75 | 1.00 | 1.25 |
| $X_2$: Soybean peptone (g $g^{-1}$ bagasse) | 0.3 | 0.4 | 0.5 |
| $X_3$: Initial moisture content (%) | 80 | 83 | 85 |

the fermentation broth and identified with liquid chromatography–mass spectrometry and HPLC.

## Effect of inertia matrices on conversion of glycerol to prodigiosin

As shown in Fig. 1, bagasse was the best inertia matrix for fermentation (with a prodigiosin yield of 20.13 g $kg^{-1}$ dry solid), followed by wood powder. The prodigiosin yield was lowest (only 10.6 g $kg^{-1}$ dry solid) when wheat straw powder was used as the inertia matrix. Because wheat straw powder had a closer fiber structure, it had a lower glycerol-absorbing capacity than that of bagasse. The distribution of glycerol was uneven, and most of it had gathered in the bottom of the substrate; therefore, the biosynthesis of prodigiosin was restricted.

## Effect of glycerol concentration on prodigiosin production

The glycerol concentration has an important effect on growth and product synthesis during fermentation processes. In SSF, the yield of prodigiosin was enhanced as the concentration of glycerol was increased from 0.1 to 1.5 g $g^{-1}$ bagasse, beyond which a decrease in the prodigiosin yield was observed (Fig. 2a). The maximum yield of prodigiosin (24.14 g $kg^{-1}$ dry solid) was recorded in 1.0 g $g^{-1}$ bagasse glycerol.

As shown in Table 1, as compared to other carbon sources, the yield of prodigiosin was lower by using glycerol in SmF. One reason could be a lower concentration of glycerol in the fermentation broth and higher glycerol content could trigger catabolite repression. Tao JL et al. studied a two-step feeding

**Table 3** Yield of prodigiosin from selected strains

| Strains | Color of colony | Prodigiosin (g $L^{-1}$) | Sample |
|---|---|---|---|
| Xd-1 | Garnet | 1.14 ± 0.05 | Mouldy tofu |
| Xd-4 | Purplish red | 0.92 ± 0.03 | Soil from tofu factory |
| Xd-9 | Red | 0.64 ± 0.05 | Soil from orange orchard |
| Xd-15 | Purplish red | 0.85 ± 0.04 | Odor soybean curd |
| Xd-17 | Pink | 0.33 ± 0.01 | Mouldy tofu |
| Xd-22 | Red | 0.71 ± 0.04 | Dried bean curd |

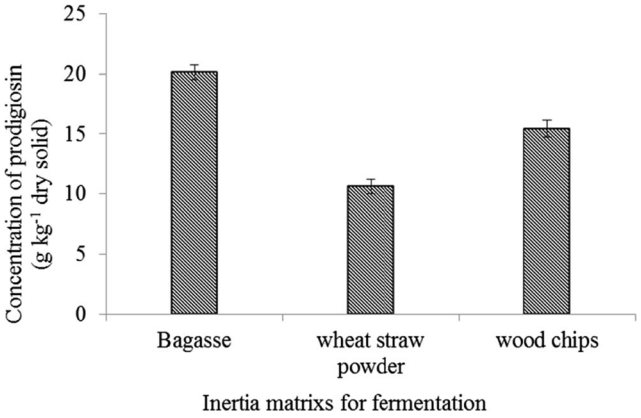


**Fig. 1** Effect of medium components on prodigiosin production. Three kinds of loose inertia matrices were used for solid-state fermentation. Bagasse was the best inertia matrix for prodigiosin production with a yield of 20.13 g $kg^{-1}$ dry solid

strategy in a 5-L bioreactor in which production of prodigiosin was 583 mg $L^{-1}$ and the glycerol concentration in the medium being was always about 5 g $L^{-1}$, about 0.5 % (w/w; Tao et al. 2005). While, *S. marcescens* Xd-1 could bear a high concentration of glycerol [1.0 g $g^{-1}$ bagasse glycerol, about 7 % (w/w)] under SSF conditions. Zhang BB et al. showed that the phenomenon of glycerol concentration gradient existed in SSF, which could well-explain the resistance effect to high concentration of glycerol in SSF (Zhang et al. 2015). Based on these results, the cultivation of *S. marcescens* Xd-1 using the agricultural byproduct bagasse as an inert carrier was suitable for efficient conversion of high concentrations of glycerol into prodigiosin. Further study of mechanisms by which SSF could bear higher concentrations of glycerol is required.\

## Influence of nitrogen sources on prodigiosin production

The results revealed that *S. marcescens* Xd-1 produced the maximum level of prodigiosin (24.46 g $kg^{-1}$ dry solid) in the presence of soy peptone (Fig. 2b). Pigmentation was delayed in media supplied with inorganic nitrogen sources ($(NH_4)_2SO_4$ and $NaNO_3$), and the growth of *S. marcescens* Xd-1 was poorer. Yeast extract and tryptone could be used to promote the proliferation of *S. marcescens* Xd-1, but the biosynthesis of prodigiosin was lower than with soy peptone. The types of nitrogen sources are very important for prodigiosin production of *S.marcescens* Xd-1. Research has shown that *S. marcescens* has a very weak ability to produce prodigiosin when inorganic nitrogen sources were used in the medium (Hejazi and Falkiner 1997; Rokem and Weitzman 1987; Hardjito et al. 2002). These results suggest that the choice of nitrogen source is species-specific for different fermentation systems and plays a crucial role in cell growth, production, and secretion of prodigiosin (Bennett and Bentley 2000; Kurbanoglu et al. 2015).

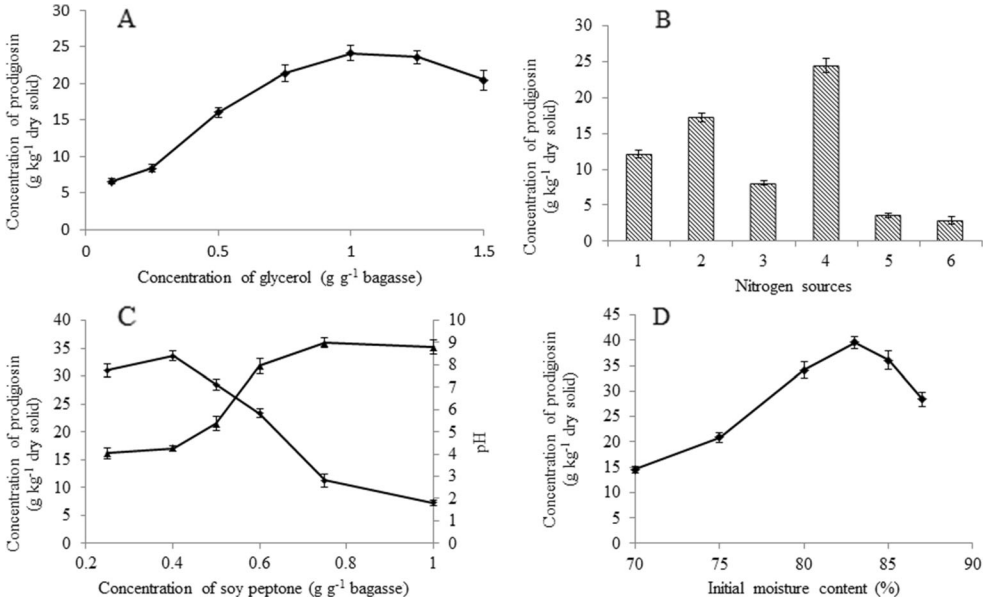


**Fig. 2** Effects of fermentation conditions on prodigiosin production. (A) The glycerol concentration has an important effect on prodigiosin product. *S. marcescens* Xd-1 could bear a high concentration of glycerol (1 g $g^{-1}$ bagasse) in solid-state fermentation. (B) Six nitrogen sources were used to enhance the conversion of glycerol to prodigiosin by *S. marcescens* Xd-1. 1 Casein hydrolysate; 2 yeast extract; 3 tryptone; 4 soy peptone; 5 $(NH_4)_2SO_4$; 6 $NaNO_3$. (C) Effect of soy peptone contents on prodigiosin production and final medium pH. (D) Effect of initial moisture content on prodigiosin production. The yield of prodigiosin markedly increased as the initial moisture content rose from 70 % to 83 %

### Influence of concentration of soy peptone on prodigiosin production

As shown in Fig. 2c, the maximum yield of prodigiosin (33.63 g $kg^{-1}$ dry solid) was obtained with the addition of 0.4 g $g^{-1}$ bagasse of soy peptone to the medium. A further increase in soy peptone content resulted in a clear decrease in the prodigiosin yield. These results might be directly attributable to the proportion variance of the carbon and nitrogen sources. Higher concentrations of soy peptone contributed to increased growth of *S. marcescens* Xd-1; however, further increases in soy peptone proved disadvantageous for the synthesis of prodigiosin by diverting more carbon into cell growth.

The effect of pH has been reported to play an important role in prodigiosin production due to its involvement in carbon source uptake and essential amino acid synthesis (Mohammed et al. 2012). Interestingly, the pH of the solid medium increased as the soy peptone concentration increased. The pH was about 4.1 when the concentration of soy peptone was less than 0.4 g $g^{-1}$ bagasse, and the substrate was dark red. As the soy peptone concentration exceeded 0.6 g $g^{-1}$ bagasse, the pH of the solid medium increased rapidly, reaching 8.0 or even more, but the substrate was purplish red or light red. Williams et al. showed that lower environmental pH values inhibited prodigiosin synthesis in SmF (Williamson et al. 2005). However, the results contrasted to those in SSF. The differences in the biomass were tested by the colony-counting method. The results showed that the biomass content of the solid medium increased significantly with the increase in soy peptone ($1.5 \times 10^7$ to $7.2 \times 10^8$ colony-forming units per gram of bagasse as the concentration of soy peptone increased from 0.25 to 1.0 g $g^{-1}$ bagasse). Results for the effect of peptone on biomass and prodigiosin production are in agreement with the observation of Wen TS et al. (Su et al. 2011).

### Influence of initial moisture content on prodigiosin production

The maximum yield of prodigiosin (39.54 g $kg^{-1}$ dry solid) was attained when the initial moisture content was 83 % (Fig. 2d). The yield of prodigiosin markedly increased as the initial moisture content rose from 70 % to 83 %. However, a further increase in the initial moisture content led to a notable decrease in prodigiosin production. The initial moisture content could play an important role in SSF, especially for bacterial fermentation. The low initial moisture content reduced the water activity of the substrate too far for cell growth and metabolite production. In contrast, higher initial moisture content resulted in a decrease in the porosity of the bagasse, thereby decreasing the diffusion of gas exchange (Corona et al. 2005; Singhania et al. 2009).

### Influence of particle size on prodigiosin production

As shown in Table 4, the maximum prodigiosin yield (40.11 g $kg^{-1}$ dry solid) was obtained for the bagasse with a 1-mm particle size. An increase or decrease in the particle size led to a reduction in the prodigiosin yield. In the SSF process, the availability of surface area plays a critical role in microbial

**Table 4** Effects of particle size on prodigiosin yield in SSF by *S. marcescens* Xd-1

| Particle size (mm) | Prodigiosin (g $kg^{-1}$ dry solid) |
|---|---|
| 0.1 | 29.29 ± 1.07 |
| 1 | 40.11 ± 1.63 |
| 2 | 38.51 ± 0.94 |
| 3 | 34.57 ± 1.28 |
| 5 | 27.26 ± 0.73 |

attachment, mass transfer of various nutrients and inertia matrices, and subsequent microbial growth and metabolite production. The availability of surface area, in turn, depends on the particle size of the substrate or support matrix (Prakasham et al. 2006). In general, smaller substrate particles provide a larger surface area for microbial attachment and metabolite production. However, substrate particles that are too small may lead to substrate agglomeration in most cases, which may interfere with microbial respiration and mass transfer, especially for air and heat transfer. Oxygen is necessary in the production of prodigiosin and *S. marcescens* cannot synthesis prodigiosin in anaerobic conditions (Heinemann et al. 1970). In contrast, larger particles provide better respiration efficiency (due to their increased interparticle space) but provide limited surface for microbial attachment. Thus, an appropriate particle size is required for a particular process in SSF (Ellaiah et al. 2004; Sangeetha et al. 2004).

## The response surface analysis for prodigiosin production

As shown in Table 5, further optimization of culture conditions was carried out by the Box–Benhnken design (BBD). Fifteen trails were performed to determine the culture conditions for prodigiosin production in solid state fermentation by *S. marcescens* Xd-1. To investigate the simultaneous influences of the variables, regression analysis was performed to fit the response function to the experimental data (Table 6). The fit value of the model, or $R^2$ (determination coefficient), was calculated as 0.9537, indicating that 95.37 % of the variance in the response of prodigiosin production could be explained by the second-order polynomial prediction equation given below. The ANOVA results prove this model to be appropriate. $Y = 40.24 + 2.10X_1 - 1.80X_2 - 0.33X_3 - 1.29X_1X_2 + 2.66X_1X_3 + 0.19X_2X_3 - 2.69X_1^2 - 1.85X_2^2 - 2.82X_3^2$ where Y is the prodigiosin yield (predicted response), and $X_1$, $X_2$, and $X_3$

**Table 5** Results of code levels for three variables in the Box–Benhnken design and observed prodigiosin yield in fifteen trails

| Runs | $X_1$ | $X_2$ | $X_3$ | Prodigiosin (g $kg^{-1}$ dry solid) |
|---|---|---|---|---|
| 1 | −1 | 0 | −1 | 36.79 |
| 2 | 0 | −1 | −1 | 37.88 |
| 3 | −1 | 0 | 1 | 30.1 |
| 4 | 1 | 1 | 0 | 35.85 |
| 5 | −1 | 1 | 0 | 32.69 |
| 6 | −1 | −1 | 0 | 33.03 |
| 7 | 0 | 0 | 0 | 40.68 |
| 8 | 0 | 0 | 0 | 39.49 |
| 9 | 0 | 1 | −1 | 33.21 |
| 10 | 0 | 1 | 1 | 33.63 |
| 11 | 1 | 0 | −1 | 34.11 |
| 12 | 1 | 0 | 1 | 38.06 |
| 13 | 1 | −1 | 0 | 41.36 |
| 14 | 0 | −1 | 1 | 37.54 |
| 15 | 0 | 0 | 0 | 40.54 |

**Table 6** Significance of regression equation for prodigiosin production from the Box-Behnken design

| Source | Sum of squares | df | Mean square | F value | *p* value prob. > F | |
|---|---|---|---|---|---|---|
| Model | 156.55 | 9 | 17.39 | 11.46 | 0.0076 | Significant |
| $X_1$ | 35.15 | 1 | 35.15 | 23.15 | 0.0048 | |
| $X_2$ | 26.03 | 1 | 26.03 | 17.14 | 0.0090 | |
| $X_3$ | 0.88 | 1 | 0.88 | 0.58 | 0.4798 | |
| $X_1 X_2$ | 6.68 | 1 | 6.68 | 4.40 | 0.0900 | |
| $X_1 X_3$ | 28.30 | 1 | 28.30 | 18.64 | 0.0076 | |
| $X_2 X_3$ | 0.14 | 1 | 0.14 | 0.095 | 0.7702 | |
| $X_1^2$ | 25.97 | 1 | 25.97 | 17.10 | 0.0090 | |
| $X_2^2$ | 12.67 | 1 | 12.67 | 8.34 | 0.0343 | |
| $X_3^2$ | 29.35 | 1 | 29.35 | 19.33 | 0.0070 | |
| Residual | 7.59 | 5 | 1.52 | | | |
| Lack of fit | 6.75 | 3 | 2.25 | 5.32 | 0.1624 | Not significant |
| Pure error | 0.85 | 2 | 0.42 | | | |
| Cor total | 164.14 | 14 | | | | |

**Fig. 3** Solid-state fermentation of *S. marcescens* Xd-1 using bagasse. The garnet color of the SSF product of *S. marcescens* Xd-1 was distributed uniformly on the bagasse

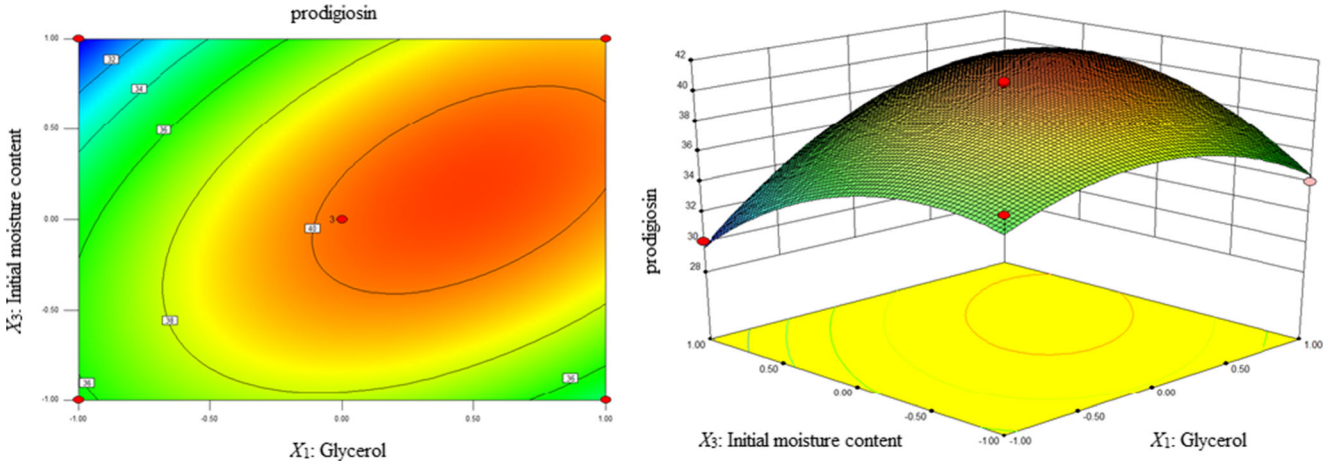


**Fig. 4** Effect of interaction of glycerol and initial moisture content on prodigiosin production. The corresponding contour plot of soy bean meal and inoculum density was obtained from the calculated response surface plot, and it was found to be significant ($p < 0.05$)

are the coded values of the test variables glycerol, soybean meal, and initial moisture content, respectively.

By solving the model regression equation, the maximum production of prodigiosin and the optimum values of the variables can be acquired. The maximum prodigiosin production of 41.57 g $kg^{-1}$ dry solid was attained under the condition of 1.17 g $g^{-1}$ bagasse glycerol, 0.33 g $g^{-1}$ bagasse soy peptone, and an initial moisture content of 83.5 %. As shown in Fig. 3, the garnet color of the SSF product of *S. marcescens* Xd-1 was distributed uniformly on the bagasse.

To confirm these results, verified experiments under optimized conditions were carried out. The maximum production of prodigiosin produced experimentally was 40.68 g $kg^{-1}$ dry solid, which was in close agreement with the predicted yield. According to the $p$ value of each model term, the constants $X_1$, $X_2$, $X_1X_3$, $X_1^2$, $X_2^2$, $X_3^2$ were found to be significant ($p < 0.05$). The corresponding contour plot of glycerol and initial moisture content was obtained from the calculated response surface plot, and is shown in Fig. 4.

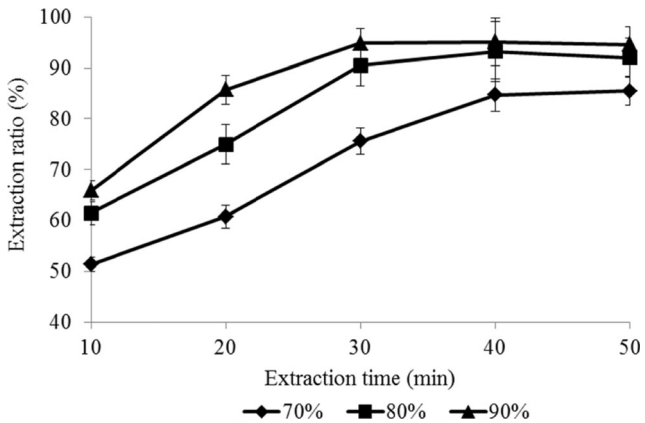


**Fig. 5** Ultrasonic-assisted reflux extraction of prodigiosin from S. marcescens Xd-1. The extraction ratio of prodigiosin increased rapidly as the concentration of ethanol increased from 70 % to 90 %. It's easy to extract prodigiosin from solid-state fermentation production of *S. marcescens* Xd-1

## Extraction of prodigiosin from solid-state fermentation production of *S. marcescens* Xd-1

As shown in Fig. 5, the extraction ratio of prodigiosin increased rapidly as the concentration of ethanol increased from 70 % to 90 %. The highest extraction ratio was 95.1 % when ethanol reached 90 %. A higher concentration of ethanol can be used to shorten the extraction time. During the initial 30 min, the extraction rate of prodigiosin was considerably increased, but rose little thereafter. Consequently, the optimum extraction time was 30 min in 90 % ethanol.

Downstream engineering in many fermentation processes is responsible for up to 60–70 % of the overall cost (Keller et al. 2001). Prodigiosin is mainly an intracellular product, and a small amount was secreted outside the cell. In SmF, some research on the recovery and separation of prodigiosin from a liquid has included ultrafiltration, organic solvent extraction, and macroporous polymeric resin adsorption processes (Kim et al. 1999; Wang et al. 2004; Juang et al. 2012; Juang and Yeh 2014). However, it is difficult to recover prodigiosin from both cells and broth. In SSF, it's simple and efficient to extract and recover prodigiosin with ethanol. In addition, there was almost no waste during the extraction process. Because the bagasse is an inertia matrix, it retained its fluffy appearance and adsorption property after the prodigiosin was extracted by ultrasound, so it could be reused.

## Conclusion

Prodigiosin is a multifaceted, secondary metabolite with a tripyrrole structure that has attracted great interest because of its apoptotic activity against a group of cancer cell lines and its

low cytotoxicity in noncancerous cells. The yield of prodigiosin is inhibited in SmF, and the separation and purification processes still have some bottle necks that prevent large-scale production. In this paper, *S. marcescens* Xd-1 was adapted to SSF for prodigiosin production by using bagasse as an inert carrier and could bear a higher concentration of glycerol. The maximal yield of prodigiosin (40.86 g $kg^{-1}$ dry solid) was achieved with 1.17 g $g^{-1}$ bagasse, glycerol, 0.33 g $g^{-1}$ bagasse soy peptone, an initial moisture content of 83.5 %, and 1-mm particles of bagasse by using a response surface methodology. In addition, the extraction of prodigiosin from SSF was more convenient than that from SmF. These results expand the culture method of *S. marcescen*s for prodigiosin production and demonstrate a superior means of producing useful activity metabolites using an agricultural byproduct as an inertia matrix under SSF.

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