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# Enhancing production of a 24-membered ring macrolide compound by a marine bacterium using response surface methodology*

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**Abstract:** A 24-membered ring macrolide compound, macrolactin A has potential applications in pharmaceuticals for its anti-infectious and antiviral activity. In this study, macrolactin A was produced by a marine bacterium, which was identified as *Bacillus subtilis* by 16S ribosomal RNA (rRNA) sequence analysis. Electrospray ionization mass spectrometry (ESI/MS) and nuclear magnetic resonance (NMR) spectroscopy analyses were used to characterize this compound. To improve the production, response surface methodology (RSM) involving Box-Behnken design (BBD) was employed. *Faeces bombycis*, the main by-product in sericulture, was used as a nitrogen source in fermentation. The interactions between three significant factors, *F. bombycis*, soluble starch, and $(NH_4)_2SO_4$ were investigated. A quadratic model was constructed to fit the production and the factors. Optimum medium composition was obtained by analysis of the model. When cultivated in the optimum medium, the production of macrolactin A was increased to 851 mg/L, 2.7 times as compared to the original. This study is also useful to find another way in utilizing *F. bombycis*.

**Key words:** 24-membered ring macrolide, Enhancing production, Response surface methodology, *Faeces bombycis*, Marine bacterium

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## 1 Introduction

Macrolactin A is a 24-membered polyene macrolide isolated from a deep sea bacterium (Gustafson *et al*., 1989). This compound exhibits potential applications in pharmaceutical for its anti-infectious and antiviral activity. A variant of macrolactin A shows inhibitory activity against the methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) (Romero-Tabarez *et al*., 2006). Macrolactin A also exhibits antiviral activity against Herpes Simplex I and II, and against human immunodeficiency virus (HIV) (Bärmann *et al*., 2000). Unfortunately, the application of macrolactin A is limited due to its low fermentation titers. Optimizing the medium composition is an effective method to improve its productivity. The conventional method to optimize the medium is the one-factor-at-a-time method, which is time-consuming and cannot account for interactions between different parameters. Response surface methodology (RSM) is a statistical method used to describe and predict the response of a system of multiple variables with fewer experiments, therefore time and cost effective (Deepak *et al*., 2008; Mutalik *et al*., 2008; Zhang *et al*., 2012).

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Sericulture is an important traditional industry in China. The production of dry mulberry cocoon in China was 740390 t in 2006 (Wei *et al*., 2009). *Faeces bombycis* is the main waste product in sericulture, and there are about 200000 t of dried *F. bombycis* produced in China per year (Zhang *et al*., 1999; Yang *et al*., 2002). It is therefore necessary to search for a reasonable way to utilize this waste. *F. bombycis* is mainly used as a fertilizer and as a constituent of animal feed or it is disposed as a waste. As *F. bombycis* is rich in proteins (about 15.4% of the total dry weight) and contains all essential amino acids (Yang *et al*., 2002), it can be used as a source of nitrogen in fermentation medium.

In this study, a marine bacteria *Bacillus subtilis* ZJUIBE-076 was isolated from a soil sample of the East Sea, Zhoushan, Zhejiang, China. Macrolactin A produced by this strain was characterized by electrospray ionization mass spectrometry (ESI/MS) and nuclear magnetic resonance (NMR) analyses. Then *F. bombycis* was used as a nitrogen source in fermentation. RSM had been made to optimize the medium.

## 2 Materials and methods

### 2.1 Strain, medium, and culture conditions

The marine bacterium was isolated from a soil sample of the East Sea, Zhoushan, Zhejiang, China and serially numbered as ZJUIBE-076.

Seed culture medium contained (g/L): glucose 15, yeast extract 10, $MgSO_4{\cdot}7H_2O$ 0.5, $FeSO_4$ 0.01, $KH_2PO_4$ 1, $CaCO_3$ 4 (pH 7.0). The original fermentation medium contained (g/L): soluble starch 56, soybean meal 11.6, $(NH_4)_2SO_4$ 18.4, $K_2HPO_4{\cdot}3H_2O$ 1.4, NaCl 2.8, $MgSO_4{\cdot}7H_2O$ 0.7, $CaCO_3$ 4 (pH 7.0). The concentration of each component was adjusted according to the experimental design. Different single nitrogen sources tested in the fermentation medium were: *F. bombycis*, yeast extract, peptone, soybean meal, beef extract, $NH_4Cl$, and $(NH_4)_2SO_4$. Different combined nitrogen sources were: *F. bombycis* combined with yeast extract, peptone, soybean meal, beef extract, $NH_4Cl$, and $(NH_4)_2SO_4$, respectively. The concentration of seven single nitrogen sources was 30.0 g/L. In six combined nitrogen sources, the content of *F. bombycis* was 11.6 g/L, and the other nitrogen sources was 18.4 g/L. Other compositions in medium were the same value as in original medium mentioned above. The original medium was referred as a control for comparison.

Seed culture inoculated from a slant was cultivated at 30 °C in a 250-ml flask containing 25 ml seed medium on a reciprocal shaker for 24 h with 200 r/min. The seed was then transferred into the fermentation medium. Fermentations were carried out in 500 ml flasks containing 50 ml fermentation medium. The amount of inoculum was 8%. Culture flasks were incubated for 36 h at 30 °C with 200 r/min. The entire liquid medium was sterilized in the autoclave at 121 °C for 20 min. All experiments were performed in triplicate.

### 2.2 *Faeces bombycis*

*F. bombycis* was obtained from the Zhejiang Academy of Agricultural Sciences (Hangzhou, China). Samples were vacuum-dried at 60 °C to a stable moisture content of less than 10% (0.1 g/ml), then finely ground to powder.

### 2.3 Identification of the marine bacterium

Genomic DNA was extracted from the strain in logarithm growth phase as a template for polymerase chain reaction (PCR), and the 16S ribosomal RNA (rRNA) gene was amplified by PCR. To determine the 16S rRNA sequence, the genomic DNA was isolated by the cetyltrimethyl ammonium bromide (CTAB)/NaCl method. The DNA manipulations for the cloning, transformation, plasmid isolation, ligation, and electrophoresis were carried out according to the method described previously (Sambrook and Maniatis, 1989). PCR was performed to amplify a partial 16S rRNA fragment of the strain using the universal general primers (forward primer P1: 5′-AGAGTTTGATCCTGGCTCAG-3′; reverse primer P2: 5′-AAGGAGGTGATCCAGCCGCA-3′). The amplified PCR product was then purified from agarose gels and ligated into a pGEM-T vector. 16S rRNA of the bacterium was compared with others obtained from GenBank. Alignments and similarity comparison referred to Clustal W method (Thompson *et al*., 1994).

### 2.4 Isolation and identification of macrolactin A

The cell-free culture supernatant was adjusted to

pH 2.0 with 6 mol/L HCl, and then was purified by adsorption column ($\Phi$ 2 cm×97 cm) packed with Amberlite XAD-8 macroporous resin (Roman-Hass). The active fraction was eluted with 95% ethanol. The ethanol soluble compound was obtained by evaporating the ethanol, and then subjected to lichropre Si60 (Merck Chemicals) silica gel column chromatography ($\Phi$ 2.5 cm×20 cm). The eluates of peaks were further purified by preparative high performance liquid chromatography (HPLC).

Then purified compound was identified by ESI/MS and NMR analyses. ESI/MS was carried out on a Finnigan™ LCQ™ Advantage instrument of Thermo Finnigan (Thermo Electron Corporation, San Jose, CA, USA). The electrospray source was conducted at a spray voltage of 5 kV, a capillary temperature of 320 °C, and a capillary voltage of 32 V. For NMR analysis, the samples were dissolved in chloroform-$d_3$, and then were detected with an AVANCE 400 spectrometer (Bruker, Karlsruhe, Germany).

### 2.5 Analytical methods

The production of macrolactin A was measured by HPLC according to the following procedures. Two milliliter of the fermentation broth was extracted with 2 ml of methanol for 30 min. The samples were obtained by centrifugation at 4000 r/min for 20 min to discard the precipitates. Then the samples were run on Agilent ZORBAX SB-C18 (5 μm, 4.6 mm×250 mm). The elution was detected at 280 nm. Mobile phases were: (a) methanol; and (b) water. The compound was eluted with a linear gradient of solvent (a) increasing from 5% to 35% (0–30 min), then from 35% to 45% (31–60 min) at a flow rate of 1 ml/min. Temperature of the column was maintained at 25 °C.

### 2.6 Experimental design and data analysis

#### 2.6.1 RSM experiment

Using "Plackett-Burman (PB) design" the effects of *F. bombycis*, soluble starch, $(NH_4)_2SO_4$, $K_2HPO_4{\cdot}3H_2O$, NaCl, $MgSO_4{\cdot}7H_2O$, initial pH, and $CaCO_3$ on macrolactin A production were studied. Based on previous PB experiments, three variables, *F. bombycis*, soluble starch, and $(NH_4)_2SO_4$, were proven to be significant to the production of macrolactin A and were selected for further optimization by RSM using Box-Behnken design (BBD) (Wei *et al*., 2011). In BBD, all three variables were tested simultaneously using three different levels. Fifteen experiments were carried out containing three replications at the central point. Macrolactin A production (mg/L) corresponding to combined effects of three variables was studied in their specified ranges, *F. bombycis* (0.6–4.4 g/L), soluble starch (20–32 g/L), and $(NH_4)_2SO_4$ (7.5–11.5 g/L) as shown in Table 1. The coded levels of three variables used for RSM experiment are shown in Table 2.

For statistical calculations the factors are coded according to the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X},\ i = 1, 2, 3, \quad (1)$$

where $x_i$ is the coded value, $X_i$ is the real value, $X_0$ is the midpoint of $X_i$, and $\Delta X$ is the step change value.

The mathematical relationship of response and variables is formulated by the following equation:

$$Y = a_0 + \sum a_i x_i + a_{ii} x_i^2 + \sum a_{ij} x_i x_j,\ i, j = 1, 2, 3, \quad (2)$$

**Table 1 Values of variables at different levels in BBD**

| Coded level | $X_1$ (g/L) | $X_2$ (g/L) | $X_3$ (g/L) |
|---|---|---|---|
| −1 | 0.6 | 20 | 7.5 |
| 0 | 2.5 | 26 | 9.5 |
| 1 | 4.4 | 32 | 11.5 |

$X_1$, $X_2$, $X_3$: concentrations of *F. bombycis*, soluble starch, $(NH_4)_2SO_4$, respectively

**Table 2 Experimental and predicted results of BBD**

| Run | $x_1$ | $x_2$ | $x_3$ | Macrolactin A (mg/L) | |
|---|---|---|---|---|---|
| | | | | Actual | Predicted |
| 1 | −1 | −1 | 0 | 80 | 118 |
| 2 | +1 | −1 | 0 | 423 | 439 |
| 3 | −1 | +1 | 0 | 201 | 185 |
| 4 | +1 | +1 | 0 | 700 | 662 |
| 5 | −1 | 0 | −1 | 206 | 201 |
| 6 | +1 | 0 | −1 | 604 | 620 |
| 7 | −1 | 0 | +1 | 110 | 94 |
| 8 | +1 | 0 | +1 | 468 | 473 |
| 9 | 0 | −1 | −1 | 725 | 693 |
| 10 | 0 | +1 | −1 | 777 | 799 |
| 11 | 0 | −1 | +1 | 548 | 527 |
| 12 | 0 | +1 | +1 | 679 | 711 |
| 13 | 0 | 0 | 0 | 811 | 804 |
| 14 | 0 | 0 | 0 | 824 | 804 |
| 15 | 0 | 0 | 0 | 777 | 804 |

$x_1$, $x_2$, $x_3$: coded values of concentrations of *F. bombycis*, soluble starch, $(NH_4)_2SO_4$, respectively

where $Y$ is the predicted response, $a_0$ is the intercept, $x_i$ and $x_j$ are the coded independent variables, $a_i$ is the linear coefficient, $a_{ii}$ is the quadratic coefficient, and $a_{ij}$ is the interaction coefficient.

2.6.2 Software and data analysis

Minitab (Version 15) was applied for experimental designs and analyses of the RSM results. Data analysis of the equation was performed to evaluate analysis of variance (ANOVA).

## 3 Results and discussion

### 3.1 Isolation and identification of *Bacillus* sp. ZJUIBE-076

About 80 strains were isolated from a deep-sea soil sample. Among these strains, one bacterium showed the highest inhibitory activity against several pathogenic bacteria. The amplified fragment (1513 bp) of 16S rRNA gene sequence of this strain was determined. When compared to the reference sequence of other bacteria using BLAST, the 16S rRNA sequence of this strain showed 100% similarity with corresponding sequence of the type strain of *B. subtilis* AU53 (GenBank accession number EU257436). Therefore, this strain was identified as *B. subtilis*. Phylogenetic affiliations of ZJUIBE-076 with related *B. subtilis* are shown in Fig. 1.

### 3.2 Identification of the compound

ESI/MS and NMR analyses were performed on the purified compound obtained by silica gel column chromatography and preparative HPLC. The compound is colorless oil with the ESI/MS *m/z* 425 $[M+Na]^+$ (Fig. 2). The NMR analysis of the compound and the results are showed in Fig. 3. The NMR data obtained are as follows: $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 7.25 (1H, dd, $J$=15.2, 12.3 Hz, H-4), 6.56 (1H, m, H-9), 6.53 (1H, m, H-3), 6.17 (1H, dd, $J$=15.2, 10.4 Hz, H-17), 6.11 (1H, m, H-10), 6.07 (1H, m, H-5), 6.02 (1H, dd, $J$=13.9, 10.4 Hz, H-18), 5.76 (1H, dd, $J$=15.2, 5.4 Hz, H-8), 5.65 (1H, dd, $J$=13.9, 7.0 Hz, H-19), 5.60 (2H, m, H-2, 16), 5.50 (1H, m, H-11), 5.02 (1H, m, H-23), 4.52 (1H, m, H-15), 4.35 (1H, m, H-7), 3.98 (1H, m, H-13), 2.50 (1H, m, H-12a), 2.45 (2H, m, H-6), 2.41 (1H, m, H-12b), 2.15 (1H, m, H-20a), 2.09 (1H, m, H-20b), 1.74 (2H, m, H-14), 1.65 (1H, m, H-22a), 1.57 (1H, m, H-22b), 1.49 (2H, m, H-21), 1.27 (3H, d, $J$=6.3 Hz, $H_3$-24); $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 166.3 (s, C-1), 142.7 (d, C-3), 139.4 (d, C-5), 136.0 (d, C-8), 135.0 (d, C-19), 132.8 (d, C-16), 130.7 (d, C-17), 130.3 (d, C-10), 129.9 (d, C-18), 129.8 (d, C-4), 127.5 (d, C-11),

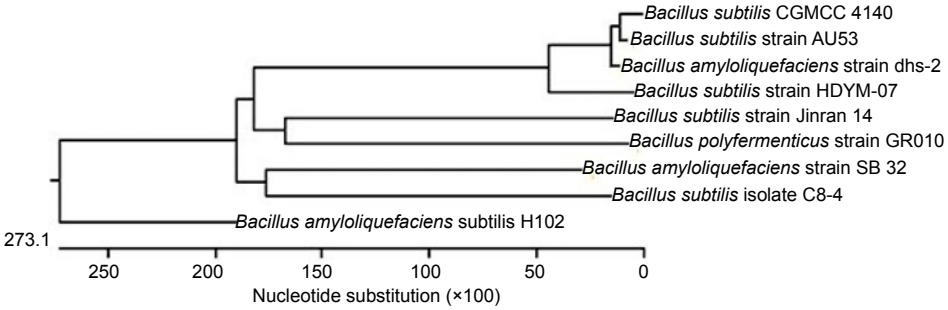


**Fig. 1 Phylogenetic tree of *Bacillus* sp. ZJUIBE-076 based on 16S rRNA sequence**

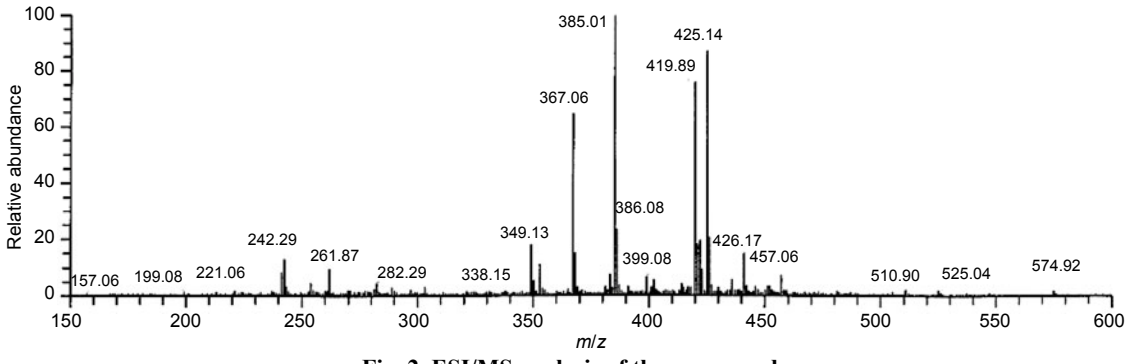


**Fig. 2 ESI/MS analysis of the compound**

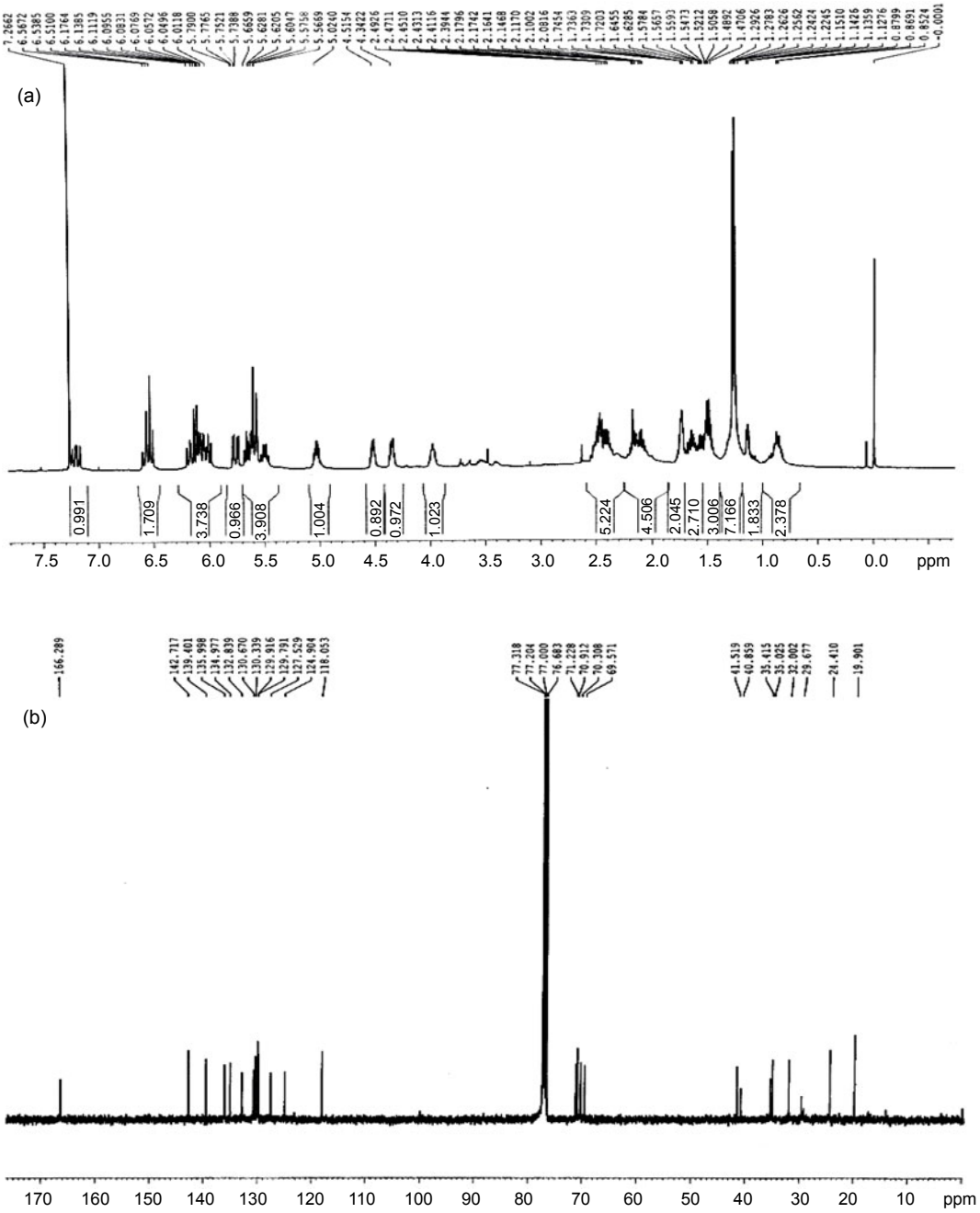


**Fig. 3 $^{1}$H NMR spectrum (a) and $^{13}$C NMR spectrum (b) of the compound**

124.9 (d, C-9), 118.1 (d, C-2), 71.2 (d, C-7), 70.9 (d, C-23), 70.3 (d, C-15), 69.6 (d, C-13), 41.5 (t, C-6), 40.9 (t, C-14), 35.4 (t, C-12), 35.0 (t, C-22), 32.0 (t, C-20), 24.4 (t, C-21), 19.9 (q, C-24).

Its $^{13}$C NMR spectrum reveal one ester carbonyl carbon at $\delta$ 166.3 (s), one methyl resonance at $\delta$ 19.9 (q), six methylene carbons at $\delta$ 41.5 (t), 40.9 (t), 35.4 (t), 35.0 (t), 32.0 (t), 24.4 (t), four oxygen-bearing methines at $\delta$ 71.2 (d), 70.9 (d), 70.3 (d), 69.6 (d) in addition to twelve $sp^2$ methines. Examination of the $^{1}$H NMR spectrum of the compound reveals the presence of one aliphatic doublet methyl at $\delta$ 1.27 (3H, d, $J$=6.3 Hz), four oxymeyhine protons at $\delta$ 5.02 (1H, m), 4.52 (1H, m), 4.35 (1H, m), 3.98 (1H, m), twelve olefinic protons at $\delta$ 5.50–7.25 were observed. The above spectral data of the compound are identical to

those of macrolactin A (Gustafson *et al*., 1989). The structure of this compound is showed in Fig. 4.

### 3.3 Effects of different nitrogen sources on the production

It is believed that *F. bombycis* could be an ideal nitrogen source for containing plenty of proteins (Yang *et al*., 2002); therefore, we compared *F. bombycis* with other nitrogen sources to study the effects on macrolactin A production. Our results indicate that productivity is strongly influenced by nitrogen sources (Fig. 5). When seven nitrogen sources (*F. bombycis*, yeast extract, peptone, soybean meal, $NH_4Cl$, $(NH_4)_2SO_4$, and beef extract) were used alone, *F. bombycis* was the best.

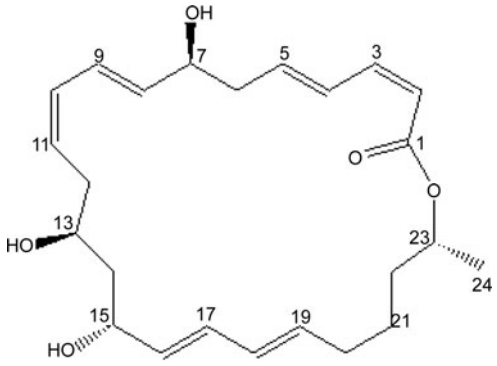


**Fig. 4 Chemical structure of macrolactin A produced by *Bacillus* sp. ZJUIBE-076**

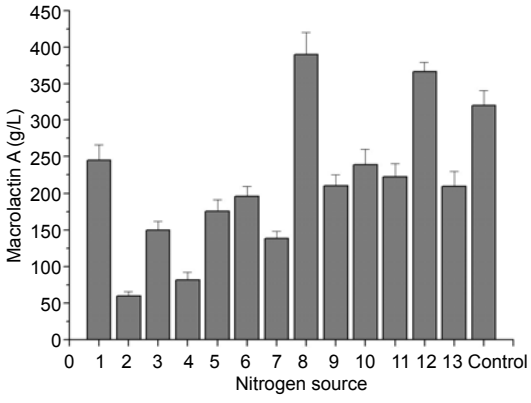


**Fig. 5 Effects of different nitrogen sources on production of macrolactin A**

1: *F. bombycis*; 2: $NH_4Cl$; 3: peptone; 4: $(NH_4)_2SO_4$; 5: yeast extract; 6: soybean meal; 7: beef extract; 8: *F. bombycis*+$(NH_4)_2SO_4$; 9: *F. bombycis*+peptone; 10: *F. bombycis*+soybean meal; 11: *F. bombycis*+yeast extract; 12: *F. bombycis*+$NH_4Cl$; 13: *F. bombycis*+beef extract. Control: the original medium soybean meal+$(NH_4)_2SO_4$

Fig. 5 also shows *F. bombycis* combined with $(NH_4)_2SO_4$ or $NH_4Cl$ is better than the nitrogen sources used alone. *F. bombycis* can be seen as a slowly utilized organic nitrogen source for a high content of proteins. Inorganic nitrogen sources, such as ammonium salts, are rapidly utilized, which makes them optimal for growth but is usually repressive for secondary metabolite biosynthesis (Demain, 2006). So, the combination of slowly (*F. bombycis*) and rapidly (ammonium salts) utilized nitrogen sources is optimal for macrolactin A production.

### 3.4 Optimization by RSM experiment

In this study, three significant factors (*F. bombycis*, soluble starch, and ammonium sulfate) were investigated to evaluate their effects on production. Levels of three variables are shown in Table 1. The center point of corresponding factor was set to be *F. bombycis* 2.5 g/L, soluble starch 26.0 g/L, and $(NH_4)_2SO_4$ 9.5 g/L, respectively. Experimental design matrix is presented in Table 2. Data were analyzed using Minitab 15 software. Second-order model for Eq. (2) was established after analysis of the data:

$$Y = 804 + 199.8x_1 + 72.6x_2 - 63.4x_3 + 39x_1x_2 - 10x_1x_3 + 19.8x_2x_3 - 394.1x_1^2 - 58.9x_2^2 - 62.9x_3^2. \quad (3)$$

Statistical analysis was used to identify the effect of each factor on macrolactin A production (Table 3). A $p$-value (<0.05) indicates the model is significant. The coefficient of determination ($R^2$) of the model is 0.9918, indicating that 99.18% of the variability could be explained by this model (Eq. (3)). Therefore, the model is reliable for macrolactin A production in this study. Table 2 also shows the observed and predicted production is a very good fit. Table 3 indicates *F. bombycis* ($x_1$), soluble starch ($x_2$), and $(NH_4)_2SO_4$ ($x_3$) have significant effects on the production ($p$<0.05), as well as the quadratic terms of each factors ($p$<0.05). In contrast, the interactions between *F. bombycis* and soluble starch, *F. bombycis* and $(NH_4)_2SO_4$, soluble starch and $(NH_4)_2SO_4$, have insignificant effects on the production ($p$>0.1).

Statistical ANOVA of the model was performed (Table 4). ANOVA shows that the model is highly significant with $F$ value of 67.23 ($p$=0.0001). The model also shows statistically insignificant lack of fit ($p$=0.210), which means the model is accurate for predicting macrolactin A production.

**Table 3 Regression coefficients and significance in RSM model***

| Term | Coefficient | SE coefficient | *t* | *p* |
|---|---|---|---|---|
| Constant | 804.00 | 23.25 | 34.574 | 0.0001 |
| $x_1$ | 199.75 | 14.24 | 14.027 | 0.000 |
| $x_2$ | 72.63 | 14.24 | 5.100 | 0.004 |
| $x_3$ | −63.38 | 14.24 | −4.450 | 0.007 |
| $x_1^2$ | −394.12 | 20.96 | −18.802 | 0.000 |
| $x_2^2$ | −58.88 | 20.96 | −2.809 | 0.038 |
| $x_3^2$ | −62.87 | 20.96 | −3.000 | 0.030 |
| $x_1x_2$ | 39.00 | 20.14 | 1.937 | 0.111 |
| $x_1x_3$ | −10.00 | 20.14 | −0.497 | 0.641 |
| $x_2x_3$ | 19.75 | 20.14 | 0.981 | 0.372 |

*$R^2$ = 99.18%; SE: standard error

**Table 4 ANOVA for the regression model**

| Source | Sum of squares | *df* | Mean square | *F* | *p* |
|---|---|---|---|---|---|
| Model | 981620 | 9 | 109069 | 67.23 | 0.0001 |
| Lack of fit | 6934 | 3 | 2311 | 3.92 | 0.210 |
| Pure error | 1178 | 2 | 589 | | |
| Cor total | 989732 | 14 | | | |

*df*: degree freedom

The 2D contour is a simple representation of the regression model. Fig. 6 shows 2D contour plots for medium optimization on macrolactin A production. Each figure presents the effects of two factors on the production, when the third factor is set at zero level. Fig. 6a indicates that an increase in *F. bombycis* led to increased production when its concentration is below 2.5 g/L. This result is also shown in Fig. 6c. *F. bombycis* can be seen as a complex nitrogen source for a high content of proteins. It was reported that complex nitrogen sources could sustain high antibiotic production, because they were supposed to be linked to the slow release of nitrogenous components during the course of fermentation (Gao *et al*., 2009). Figs. 6a and 6b show that increasing soluble starch led to increased production. It was also proven in some literature that the preferred carbon source for the production of secondary metabolites was starch (Syed *et al*., 2009). Ammonium salts are usually repressive for secondary metabolites fermentation (Demain, 2006). As shown in Figs. 6b and 6c, $(NH_4)_2SO_4$ has a significant negative effect on the production. How ever, as a readily utilized nitrogen source,

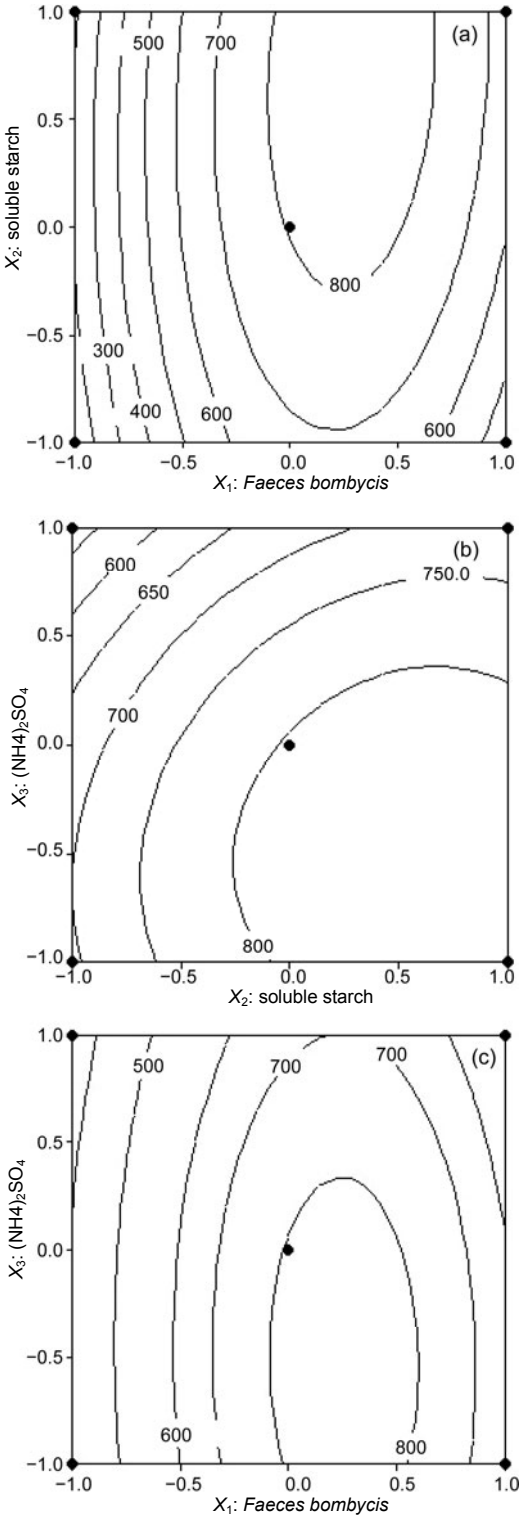


**Fig. 6 Contour plots for production of macrolactin A by changing components of *F. bombycis* and soluble starch (a), soluble starch and $(NH_4)_2SO_4$ (b), *F. bombycis* and $(NH_4)_2SO_4$ (c)**

Each figure presents the effects of two factors on the production, while the third factor is set at zero level

$(NH_4)_2SO_4$ is in favor of growth. So, the combined nitrogen sources of *F. bombycis* and $(NH_4)_2SO_4$ are optimal in this work, which also has been demonstrated in Fig. 5.

Based on calculation of the model (Eq. (3)), the optimum medium is obtained: *F. bombycis* 3.1 g/L, soluble starch 29.8 g/L, and $(NH_4)_2SO_4$ 8.6 g/L. The other component concentrations are the same values as in original medium: $K_2HPO_4{\cdot}3H_2O$ 1.4 g/L, NaCl 2.8 g/L, $MgSO_4{\cdot}7H_2O$ 0.7 g/L, $CaCO_3$ 4.0 g/L (pH 7.0). The predicted production in optimum medium is 870 mg/L. To verify the predicted results, validation experiments were performed in triplicate. The average experimental titer of macrolactin A is (851±39) mg/L, suggesting that experimental and predicted values are in good agreement.

## 4 Conclusions

An anti-bacteria compound was isolated from a marine microorganism and was identified as a macrolactin-type compound. Three significant factors in culture medium, *F. bombycis*, soluble starch, and $(NH_4)_2SO_4$, which influenced the production, were optimized by RSM. A polynomial regression model was established to describe the experimental results. The optimum medium is obtained by analysis of the mode, which is *F. bombycis* 3.1 g/L, soluble starch 29.8 g/L, $(NH_4)_2SO_4$ 8.6 g/L, $K_2HPO_4{\cdot}3H_2O$ 1.4 g/L, NaCl 2.8 g/L, $MgSO_4{\cdot}7H_2O$ 0.7 g/L, $CaCO_3$ 4.0 g/L (pH 7.0). When *Bacillus* sp. ZJUIBE-076 is cultivated in this medium, the production of macrolactin A is increased by about 170% from 320 to 851 mg/L as compared to the original. The results prove that *F. bombycis* could be used as a source of nitrogen in fermentation. Since *F. bombycis* is abundant in China, this study provides an effective and beneficial way for the application of this resource.

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## Compliance with ethics guidelines

Hua CHEN, Mian-bin WU, Zheng-jie CHEN, Ming-lu WANG, Jian-ping LIN, and Li-rong YANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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