Statistical optimisation of process variables and large-scale production of *Metarhizium rileyi* (Ascomycetes: Hypocreales) microsclerotia in submerged fermentation

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**ABSTRACT**

Microsclerotia (MS) formation was successfully induced in *Metarhizium rileyi* (Ascomycetes: Hypocreales) in liquid culture. To optimise the process variables of liquid fermentation, we first used a two-level fraction design to confirm the variables, including inoculum density, initial pH, shaker speed, and temperature, affecting M. rileyi MS production. Three variables were found to be important. Subsequently, a 2\(^3\) full factorial central composite design (CCD) and response surface methodology were applied to ascertain the optimal level of each variable. A second-order polynomial was determined and shaker speed and inoculum density were found to be the primary variables affecting MS yields. Finally, we realised and optimised M. rileyi MS submerged fermentation based on previous findings. A maximum MS yields (3.84 × 10\(^4\) MS/mL) were recorded in submerged fermentation at an initial pH of 5.5, growth temperature of 26°C, inoculum density of 10%, higher aeration rate (150 rpm in the initial 3 days and 200 rpm in the subsequent 3 days), and higher agitation rate of 800 L/h sterile air.

**Introduction**

Microsclerotia (MS), with a diameter of 50–600 \(\mu\)m, are pseudoparenchymatous aggregations of hyphae, comprising only a few cells (Yin et al. 2012). They are produced as overwintering structures by many phytopathogenic fungi (Colotelo 1974; López-Escudero et al. 2006). Fungi can survive for long periods in adverse environments by producing MS. For biological applications, MS has been successfully induced in entomopathogenic fungi such as *Beauveria bassiana* (Ascomycetes: Hypocreales), *Beauveria brongniartii*, *Metarhizium anisopliae* (Ascomycetes: Hypocreales), *Metarhizium rileyi*, and *Purpureocillium lilacinum* in liquid culture (Jackson & Jaronski 2009; Wang et al. 2011; Yin et al. 2012; Yang et al. 2014; Song et al. 2016b). The stability of dried MS preparations and their potential to produce infective conidia when conditions are suitable for growth make MS a promising fungal propagule for use as a mycoinsecticide to manage insect pests (Jackson et al. 2010; Behle et al. 2013; Song et al. 2014a), as antagonists of plant pathogenic fungi (Kobori et al. 2015), as bioherbicides for the management macrophytes (Shearer 2007), or bionematicides against root knot nematodes (Song et al. 2016b). Therefore, for the commercial production of fungi that are not cost-effective by solid substrate fermentation, the conidia, the effective propagule against pests, can be replaced with the MS.

The *Nomuraea rileyi* as a well-known entomopathogenic fungus, re-classified as *M. rileyi* according to multigene phylogenetics (Kepler et al. 2014), thrives in obligate hosts, particularly, lepidopterous pests (Chen et al. 2012). The current large-scale solid fermentation methods for the production of *M. rileyi* conidia, the present effective component, are not cost-effective and require fastidious sporulation conditions along with stimulatory light, which limit large-scale fermentation and commercialisation of *M. rileyi* (Faria & Wraight 2007).

Our laboratory had successfully accomplished *M. rileyi* MS production in liquid amended medium (AM) culture (Yin et al. 2012; Song et al. 2013), and further research aimed at the scale-up fermentation for *M. rileyi* MS production is underway. The quality as well as quantity of MS propagules in liquid culture should be examined with equal attention, because both the...
parameters can be affected by medium composition and culture conditions. Recently, our group optimised the medium composition to improve MS yields (Song et al. 2014a). As the sources of medium are widespread, large-scale fermentation for M. rileyi MS production could be easily achieved. Our primary experiments suggested that shaker speed, inoculum density, pH, and temperature are the major culture variables that affect M. rileyi MS production. For the development of efficient fermentation, the culture conditions and liquid culture fermentation process should be optimised owing to their impact on the M. rileyi MS yields. Optimisation studies could also help in understanding the interactions among the parameters as well as the mechanism of MS development (Song et al. 2013, 2016a; Jiang et al. 2014; Liu et al. 2014; Zhou et al. 2015).

The application of statistical experimental design techniques in fermentation process development can result in improved MS yields, reduced process variability, and reduced development time and overall costs. Response surface methodology (RSM), a collection of mathematical and statistical techniques for building empirical models, can employ quantitative data obtained from properly designed experiments to solve multivariate equations simultaneously and carry out only a limited and fixed number of experiments. More importantly, this method can be used in the presence of complex interactions. RSM has been successfully employed for optimizing the biomass of many microorganisms, such as Bacillus thuringiensis, M. anisopliae, and Pichia anomala (Liu & Tzeng 1998; Vohra & Satyanarayana 2002; Prakash et al. 2008; Li et al. 2009; Shi et al. 2009).

The present study was aimed at optimisation of the variables of culture conditions and development of an optimal process of submerged fermentation for M. rileyi MS production. We first used a two-level fractional factorial design (FFD) to confirm the important variables that significantly affect M. rileyi MS yields. Then, central composite design (CCD) and RSM methods were applied to optimise the major variables, including shaker speed, inoculum density, and temperature. The results showed that shaker speed and inoculum density were the primary variables that significantly affected M. rileyi MS yields. Finally, we developed and optimised the process of submerged fermentation for M. rileyi MS production in a 30-L bioreactor based on the confirmed primary variables.

Materials and methods

Strain and inoculum preparation

The M. rileyi strain, CQNr01, was obtained from the Chongqing Engineering Research Center for Fungal Insecticides, Chongqing, China. The strain had been used to study the optimisation of culture medium for MS production (Yin et al. 2012; Song et al. 2014a) and study the molecular mechanism of MS development in our earlier research (Song et al. 2013, 2016a; Jiang et al. 2014; Liu et al. 2014; Zhou et al. 2015). The strain was cultured on Sabouraud’s maltose agar fortified with 1% yeast extract (SMAY) under continuous light at 25°C. After 14 days, the conidia were harvested and suspended in sterile water with 0.1% Tween 80 at a concentration of $1 \times 10^8$ conidia/mL. For subsequent optimisation of the culture conditions, the conidial suspension was inoculated into 250-mL sterile Erlenmeyer flasks containing 100 mL of liquid AM. Prior to autoclaving, the pH of the liquid AM was adjusted to 6.0 with HCl or NaOH. After 6 days following inoculation, the MS matured and samples were collected to measure the MS yields.

For the preparation of blastospore inoculum for 30-L bioreactor studies, the conidial suspension was inoculated into five 1-L baffled Erlenmeyer flasks containing 400 mL of liquid SDA medium (glucose, 4%; peptone, 0.25%; and yeast extract, 0.5%) and incubated at 25°C and 250 rpm on a rotary shaker incubator for 48 h.

Two-level FFD

A total of four variables were studied for MS production, namely, inoculum density, initial pH, shaker speed, and temperature. To determine the accuracy of the results of statistical experiments, we used $2^{4-1}$ FFD leading to eight sets of experiments, performed in duplicate, to identify the important variables affecting MS yields. Each variable was represented at two levels, high and low, denoted by (1) and (−1) (Table 1).

CCD

After identifying the important variables, $2^3$ factorial CCD was adopted to optimise the levels of the variables. The full CCD with six star points ($\alpha = 1.682$) and three replications of the centre point, leading to 20 sets of experiments, was used to optimise the
production of *M. rileyi* MS. The MS yields were considered as the response, and the levels of each variable are given in Table 2. The results of CCD are usually used to fit a second-order polynomial equation because they represent the behaviour of such systems more appropriately than the first-order designs (Liu & Tzeng 1998; Souza et al. 1999; Prakash et al. 2008; Shi et al. 2009).

For a three-variable system, the model equation is as follows:

\[
Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11}A^2 + \beta_{22}B^2 + \beta_{33}C^2 + \beta_{12}AB + \beta_{23}BC + \beta_{13}AC
\]  

where *Y* is the predicted response (MS yields); *β*0 is the intercept; *β*1, *β*2, and *β*3 are the linear effects; *β*11, *β*22, and *β*33 are the squared coefficients; and *β*12, *β*23, and *β*13 are the interaction coefficients.

**Table 1. Different variables screened and their levels in the two-level FFD.**

| Variables                          | Symbols | Levels     |
|-----------------------------------|---------|------------|
| Shaker speed (rpm)                | *X*_1   | 150 200 250 |
| Initial pH                        | *X*_2   | 5.5 6.0 6.5 |
| Temperature (°C)                  | *X*_3   | 21 23 25   |
| Inoculum density (%)              | *X*_4   | 7 10 13    |

**Table 2. Range, code values, and levels of independent variables in CCD.**

| Variables | Name            | Levels of variables |
|-----------|-----------------|---------------------|
| Shaker speed (rpm) | *X*_1 | −α 0 +α |
| Inoculum density (%) | *X*_2 | 8 12 16 20 28 30 |
| Temperature (°C) | *X*_3 | 22.5 24 26 28 29.4 |

The aeration and agitation rates were found to affect the concentration of dissolved oxygen during submerged fermentation. Therefore, the agitation and aeration rates were varied, as shown in Figure 1. The effects of these variations on MS production were evaluated by analysing the MS yields and biomass.

**Assessment of MS production and statistical analysis**

All the experiments were repeated three times. To analyse the biomass, 100 mL of fresh culture broth was weighed and the suspensions were subsequently centrifuged (5000 rpm). The resulting precipitate was weighed and dried to constant weight in an oven at 95°C. The biomass was estimated by determining the dry weight. The MS yields were measured as described by Jackson and Jaronski (2009). One-way ANOVA was employed to analyse the variance, and the proportion of variance, explained by the polynomial models obtained, was given by the multiple coefficient of determination, *R*^2^. The statistical software SPSS 16.0 (SPSS, Chicago, IL, USA) and GraphPad Prism 5 were used for statistical analysis.

**Results**

**Identification of important variables by using FFD**

The corresponding FFD matrix was used for the screening of the four variables. Table 3 presents the experimental design and results of 2^4−1 FFD experiments. The resulting effects (Exi) of the variables on the response, associated *F* values, and significant levels are shown in Table 4. A *p* value <0.1 for the shaker speed indicated that this variable was significant for the MS yields, while the other variables were less significant. An increase in the levels of shaker speed, inoculum density, and temperature had positive effects on the MS yields. However, an increase in the level of initial pH had a negative effect on the MS yields. The shaker speed, inoculum density, and temperature were selected based on the *p* values, and CCD was used for further optimisation.

**Optimisation of the MS yields by using RSM**

The CCD and corresponding experimental data are presented in Table 5. By applying ANOVA to the
The experimental data, the results of CCD were fitted by a polynomial quadratic equation. Although temperature was excluded, the linear, quadratic, and interaction coefficients of the variables examined were found to be significant at $F_{2,19} = 5.611$, $p = 0.013 < 0.05$ and were retained in the reduced models. The equation that emerged was as follows:

$$
\text{MS production (×10^8 MS/mL)} = 6.02 ± 0.4a
$$

**Figure 1.** Trends in the hourly mean records of the dissolved oxygen level in the 30-L bioreactor in various trials. Trials 1–6 comprised treatments with various aeration and agitation rates (Trial 1: 100 rpm and 600 L/h; Trial 2: 100 rpm for the first 3 days and 150 rpm for the subsequent 3 days and 600 L/h; Trial 3: 150 rpm and 600 L/h; Trial 4: 150 rpm and 800 L/h; Trial 5: 150 rpm for the first 3 days and 200 rpm for the subsequent 3 days and 800 L/h; and Trial 6: 150 rpm for the first 3 days and 200 rpm for the subsequent 3 days and 1200 L/h). Standard error bars indicate variation in measurements.

**Table 3.** Two-level FFD matrix for the screening of important variables for MS production.

| Run no. | Shaker speed | Initial pH | Temperature | Inoculum density | MS production (×10^8 MS/mL) |
|---------|--------------|------------|-------------|------------------|-----------------------------|
| 1       | 1            | 1          | 1           | 1                | 6.02 ± 0.4a                 |
| 2       | −1           | −1         | 1           | 1                | 5.45 ± 0.52b                |
| 3       | −1           | 1          | −1          | 1                | 5.56 ± 0.32ab               |
| 4       | 1            | −1         | −1          | 1                | 5.63 ± 0.14ab               |
| 5       | −1           | 1          | 1           | −1               | 5.52 ± 0.26ab               |
| 6       | 1            | −1         | 1           | −1               | 5.84 ± 0.24ab               |
| 7       | 1            | 1          | −1          | −1               | 5.51 ± 0.48ab               |
| 8       | −1           | −1         | −1          | −1               | 5.02 ± 0.19b                |

All the experiments were repeated three times. Means followed by the different letters within a row are significantly different (Duncan’s multiple range tests).

**Table 4.** Analysis of the two-level FFD for the screening of important variables.

| Variables       | Name      | F value | Exi  | p Value |
|-----------------|-----------|---------|------|---------|
| Shaker speed    | $X_1$    | 4.648   | +    | 0.075   |
| Initial pH      | $X_2$    | 0.617   | −    | 0.462   |
| Temperature     | $X_3$    | 2.062   | +    | 0.201   |
| Inoculum density| $X_4$    | 0.842   | +    | 0.394   |

**Table 5.** CCD plan matrix in coded value and the observed response.

| Run no. | $X_1$ | $X_2$ | $X_3$ | Observed response (×10^8 MS/mL) |
|---------|-------|-------|-------|---------------------------------|
| 1       | 1     | 1     | 1     | 6.12 ± 0.32a                    |
| 2       | 1     | 1     | −1    | 6.21 ± 0.15a                    |
| 3       | 1     | −1    | 1     | 4.95 ± 0.21cd                   |
| 4       | 1     | −1    | −1    | 4.97 ± 0.18cd                   |
| 5       | −1    | 1     | 1     | 5.38 ± 0.45bc                   |
| 6       | −1    | 1     | −1    | 5.24 ± 0.52bc                   |
| 7       | −1    | −1    | 1     | 4.91 ± 0.14cd                   |
| 8       | −1    | −1    | −1    | 4.89 ± 0.64cd                   |
| 9       | 1.682 | 0     | 0     | 6.03 ± 0.36a                    |
| 10      | −1.682| 0     | 0     | 4.79 ± 0.24cd                   |
| 11      | 0     | 1.682 | 0     | 6.12 ± 0.26a                    |
| 12      | 0     | −1.682| 0     | 5.57 ± 0.35ab                   |
| 13      | 0     | 0     | 1.682 | 4.48 ± 0.25d                    |
| 14      | 0     | 0     | −1.682| 4.51 ± 0.28d                    |
| 15      | 0     | 0     | 0     | 5.83 ± 0.32ab                   |
| 16      | 0     | 0     | 0     | 5.79 ± 0.26ab                   |
| 17      | 0     | 0     | 0     | 5.81 ± 0.38ab                   |
| 18      | 0     | 0     | 0     | 5.82 ± 0.25ab                   |
| 19      | 0     | 0     | 0     | 5.82 ± 0.14ab                   |
| 20      | 0     | 0     | 0     | 5.81 ± 0.18ab                   |

All the experiments were repeated three times. Means followed by the different letters within a row are significantly different (Duncan’s multiple range tests).
where \( Y \) is the MS yields and \( X_1 \) and \( X_2 \) are the coded levels of shaker speed and inoculum density, respectively.

The fit of the model was expressed by the \( R^2 \) value, which provided a measure of the extent to which the variability in the observed response values could be explained by the experimental variables and their interactions (Liu & Tzeng 1998; Vohra & Satyanarayana 2002). The corresponding ANOVA results are given in Table 6. However, the \( R^2 \) value was 0.398 for MS production, indicating that the model was inadequate.

It had been pointed out that when a large amount of data are included in the analysis, a model with a significant lack of fit could still be used (Taragano & Pilosof 1999). Therefore, in the present study, we performed many experiments to predict data quality by evaluating the difference between the predicted data and measured results. Based on the variables shaker speed and inoculum density (Table 2), the results showed that there was a significant difference between the predicted data (4.55 \( \times 10^4 \) MS/mL) and measured results (3.41 \( \pm 0.7 \times 10^4 \) MS/mL) for the treatment group subjected to minimal culture conditions (shaker speed: 200 rpm, inoculum density: 8%) \((F_{1, 4} = 785.68, p < 0.001)\). Similarly, there was also a significant difference between the predicted data (5.56 \( \times 10^4 \) MS/mL) and measured results (5.80 \( \pm 0.03 \times 10^4 \) MS/mL) for the treatment group subjected to moderate culture conditions (shaker speed: 250 rpm, inoculum density: 13%) \((F_{1, 4} = 192.00, p < 0.001)\). However, the predicted data (6.07 \( \times 10^4 \) MS/mL) and measured results (6.06 \( \pm 0.12 \times 10^4 \) MS/mL) for the treatment group subjected to maximal culture conditions (shaker speed: 300 rpm, inoculum density: 18%) showed no difference \((F_{1, 4} = 0.009, p > 0.05)\). Furthermore, no difference \((F_{1, 4} = 1.36, p > 0.05)\) between the obtained MS yields (5.98 \( \pm 0.06 \times 10^4 \) and 6.08 \( \pm 0.11 \times 10^4 \) MS/mL, respectively) following treatment with different inoculum densities (13% and 18%) under a shaker speed of 300 rpm was noted.

**MS fermentation in 30-L bioreactor**

To optimise the fermentation process for *M. rileyi* MS production, six trials were independently carried out (Figure 1). Under the controlled conditions, the whole fermentation process was completed within 6 days. Overall, varied concentrations of dissolved oxygen were observed among the six trials during fermentation. After 24 h of fermentation with low aeration rate and low agitation rate, the concentration of dissolved oxygen decreased slowly at different rates (Trials 1–3) (Figure 1). However, after 56 h of fermentation with higher aeration rate and higher agitation rate, the decrease in the concentration of dissolved oxygen was delayed and slow (Trials 4–6) (Figure 1). Subsequently, the concentration of dissolved oxygen rapidly declined at different rates. Considering the development of MS, these results indicated that the growth of polar hyphae composed of the MS and the forming MS consume abundant dissolved oxygen (Song et al. 2013, 2016a; Jiang et al. 2014; Liu et al. 2014; Zhou et al. 2015).

The indices for MS production in the 30-L bioreactor are shown in Figures 2 and 3. The MS harvested from 20-L culture medium of Trials 1–6 were, respectively, weighed and the MS yields were calculated (Figure 3). The *M. rileyi* MS yields varied with the aeration and agitation rates tested \((F_{5, 12} = 46.15, p < 0.001)\): Trial 1, 1.52 \( \pm 0.31 \times 10^4 \) MS/mL; Trial 2, 2.15 \( \pm 0.22 \times 10^4 \) MS/mL; Trial 3, 2.21 \( \pm 0.13 \times 10^5 \) MS/mL; Trial 4, 3.14 \( \pm 0.17 \times 10^4 \) MS/mL; Trial 5, 3.65 \( \pm 0.19 \times 10^4 \) MS/mL; and Trial 6, 3.52 \( \pm 0.25 \times 10^4 \) MS/mL (Figure 2(a)). Similarly, the biomass varied with the aeration and agitation rates examined \((F_{5, 12} = 203.08, p < 0.001)\): Trial 1, 13.41 \( \pm 0.61 \) g/L; Trial 2, 15.62 \( \pm 0.41 \) g/L; Trial 3, 17.33 \( \pm 0.64 \) g/L; Trial 4, 21.25 \( \pm 0.81 \) g/L; Trial 5, 24.36 \( \pm 0.42 \) g/L; and Trial 6, 23.35 \( \pm 0.22 \) g/L (Figure 2(b)). Furthermore, in Trials 4–6, especially in Trial 6, among the harvested MS, a larger number of matured MS with secondary mycelia growth were observed.

**Discussion**

In our preliminary experiments, we found that the biomass accumulation and MS yields were not
positively correlated. High biomass accumulation with mass hyphal growth could be achieved with low MS yields (Yin et al. 2012). Hence, in the present study, we used the MS yields as an indicator of culture productivity in optimizing the process variables. The effect of initial pH of the liquid AM on M. rileyi MS production was studied with the two-level FFD (Tables 1 and 3). The results showed that an increase in the levels of initial pH had negative effects on MS production (Table 4). Likewise, in Sclerotinia sclerotiorum, neutral or alkaline pH suppressed MS development, while acidic pH conditions favoured MS development (Rollins & Dickman 2001; Chen et al. 2004). In the present study, further investigation of the effect of increasing levels of initial pH showed that an increase in the pH was not favourable for M. rileyi MS development (data not shown). Therefore, the pH of the medium was adjusted to 6.0 and was not altered in the following experiments. Our fermentation trials revealed that the pH of the culture medium regularly changed during M. rileyi MS formation. Further studies on the effects of changing pH on M. rileyi MS differentiation are currently being carried out in our laboratory.

The three-dimensional response surfaces were plotted by using a statistically significant model to understand the interaction of the three important variables and determine the optimum level of each variable required for effective MS production. Although temperature had been shown to significantly influence MS production in other plant pathogenic fungi (Jackson et al. 2011), it was an excluded variable in the RSM design to achieve optimum M. rileyi MS production because the optimised temperature range may not be suitable for M. rileyi growth. At 24–28°C, significantly higher biomass accumulation

![Figure 2. Production of M. rileyi MS and biomass in various trials. (a) The MS yields obtained in various trials. (b) The biomass obtained in various trials. Trials 1–6 comprised treatments with various aeration and agitation rates (Trial 1: 100 rpm and 600 L/h; Trial 2: 100 rpm for the first 3 days and 150 rpm for the subsequent 3 days and 600 L/h; Trial 3: 150 rpm and 600 L/h; Trial 4: 150 rpm and 800 L/h; Trial 5: 150 rpm for the first 3 days and 200 rpm for the subsequent 3 days and 800 L/h; and Trial 6: 150 rpm for the first 3 days and 200 rpm for the subsequent 3 days and 1200 L/h). Standard error bars indicate variation in measurements. Means followed by different letters are significantly different (Duncan’s multiple range tests).](image1)

![Figure 3. Submerged fermentation of M. rileyi MS. (a) The picture of fermentation yields of M. rileyi MS. (b) The germination of dried MS after incubation on agar for 36 h at 25°C (scare bar is 100 μm).](image2)
and MS production were observed, whereas at temperatures above/below this range, the growth of *M. rileyi* was greatly reduced (data not shown). Therefore, 26°C was set as the growth temperature for fermentation in the bioreactor.

Although the model was inadequate, it also required a significant lack of fit when a large amount of data were included in the analysis (Levin et al. 2008). Nevertheless, the *p* value for the regression was <0.05 (Table 6), suggesting that at least one of the terms in the regression equation was significantly correlated to the response variable (Tarocco et al. 2005). The predicted conditions for MS yields against those measured exhibited imperfect matching with a confidence interval of 95%. The difference in the predicted data and measured experimental results confirmed that the model may not have included all the appropriate functions of independent variables or the experimental range may be too large for a quadratic model (Tarocco et al. 2005). We individually verified the two variables shaker speed and inoculum density in the equation and found that they independently had a significant effect on MS production (*p* = 0.026 and 0.036, respectively). However, the independent variable (temperature) had no significant effect on MS production (*p* = 0.49). The interactive terms (AB, AC, BC) had no significant effect on MS production (*p* = 0.50). Although the inoculum density could affect MS production, the MS yields were not significantly different when the inoculum densities exceeded a concentration of 13%. Nevertheless, the upper limit of inoculum concentration was lower during bioreactor fermentation (data not shown). Therefore, the inoculum density was set at 10% in the following fermentation experiments. Furthermore, we found that the shaker speed played a major role in *M. rileyi* MS production by influencing the dissolved oxygen levels during fermentation. The reactive oxygen species, the by-products of oxygen metabolism, mediate MS formation (Georgiou et al. 2006; Song et al. 2013; Jiang et al. 2014; Liu et al. 2014; Zhou et al. 2015). Therefore, for the optimisation of the submerged fermentation for *M. rileyi* MS production, dissolved oxygen level is an important monitor variable. However, the present study revealed that aeration and agitation rates were the major variables that affected the dissolved oxygen levels during fermentation, which, in turn, affected the MS yields (Figures 1 and 2). The low aeration and agitation rates resulted in low biomass accumulation and MS concentration. Therefore, for the optimum fermentation process for *M. rileyi* MS production, higher aeration and agitation rates should be employed. Based on the optimised process of fermentation of MS, we realise the stable submerged fermentation for *M. rileyi* MS production in 300-L bioreactor (data not shown).

The RSM was performed to optimise the process variables for *M. rileyi* MS production. One of the process variables, namely, shaker speed, obtained by the CCD was determined to significantly affect the MS yields. The optimal conditions for MS production were an initial pH of 5.5, growth temperature of 26°C, inoculum density of 10%, higher aeration rate (150 rpm in the initial 3 days and 200 rpm in the subsequent 3 days), and higher agitation rate of 800 L/h sterile air. To our knowledge, these findings on *M. rileyi* MS fermentation...
are novel, and the short duration of production cycle and lower cost of *M. rileyi* MS fermentation could promote its commercialisation.

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**Disclosure statement**

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