Regulation of different polyketide biosynthesis by green light in an endophytic fungus of mangrove leaf

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Abstract Light is an important environmental signal for many organisms. The light response reports of fungi usually focus on blue light and red light. Although the green light sensor has also been found in several fungi, the knowledge of the green light response in fungi is very limited. *Halorosellinia* sp. (No. 1403) is a light-sensitive endophytic fungus of mangrove leaf. In this study, we explored the specific effects of monochromatic blue light, red light, and green light on polyketides biosynthesis in *Halorosellinia* sp. (No. 1403), respectively. The major polyketides produced in *Halorosellinia* sp. (No. 1403) are octaketides (1403C and 1403R) and heptaketide (griseofulvin). All monochromatic light enhanced octaketide biosynthesis and inhibited heptaketide biosynthesis to some extent compared with the dark condition. Most prominently, the total production of octaketides was increased by 76%, and the production of heptaketide was decreased by 73% under green light in bioreactor. Therefore, green light can not only influence the secondary metabolism in fungi, but also it can influence different biosynthetic pathways in different ways. We speculate that the significant effect of green light on mangrove leaf endophytic fungus *Halorosellinia* sp. (No. 1403) may be a kind of environmental adaptation to plant photosynthesis.

Keywords Green light · Octaketide biosynthesis · Heptaketide biosynthesis · Environmental adaptation · Mangrove leaf endophytic fungus

Introduction

Light is an important environmental signal for many organisms. During decades of studies, light response has been found in many fungal species (Rodriguez-Romero et al. 2010) and a substantial number of metabolic pathways have been found to be triggered in fungi after illumination, including carbohydrate metabolism, nitrogen and sulfur metabolism, nucleotide and nucleoside metabolism, amino acid metabolism, fatty acid metabolism, and secondary metabolism (Tisch and Schmoll 2010). Several light-sensing systems have been found in fungi. Therein, the blue light-sensing system has been studied deeply in *Neurospora crassa* (Dunlap and Loros 2004), and the interplay between blue light-sensing system and red light-sensing system has been reported in *Aspergillus nidulans* (Purschwitz et al. 2008). Besides, the green light sensor NOP-1 has been found in several fungi (Bieszke et al. 1999, 2007; Estrada and Avalos 2009; Estrada et al. 2009), while the knowledge of the green light response in fungi is still very limited so far.

In our previous study, compound white light has been found to improve an anticancer polyketide 1403C production in *Halorosellinia* sp. (No. 1403), a light-sensitive endophytic fungus of mangrove leaf (Zhang et al. 2016). The anthraquinone derivative 1403C (also known as SZ-685C) shows remarkable cytotoxic activity against multiple cancer cell lines (Zhu et al. 2012; Chen et al. 2013; Wang et al. 2013, 2015); therefore, light shows an attractive effect in this case. The anthraquinone 1403C is

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a kind of octaketide. In addition, there are some other polyketides produced by *Halorosellinia* sp. (No. 1403), such as 1403R (Xia et al. 2007) and griseofulvin (Xia et al. 2011). As shown in Fig. 1a, 1403C and 1403R share a common octaketide biosynthetic pathway (Niu et al. 2012), and it has been proven that 1403R will convert into 1403C completely when culture broth pH is above

6.0 (Zhou et al. 2014; Zhang et al. 2016). However, heptaketide griseofulvin has a different biosynthetic pathway (see Fig. 1b, Cacho et al. 2013), so it is a competitive metabolite.

Compound white light is mainly composed of blue light, green light, and red light, respectively, and all of them may be sensed by fungi. In this study, we try to find out which

Fig. 1 Main polyketide biosynthesis pathways in *Halorosellinia* sp. **a** 1403C and 1403R, **b** griseofulvin

kind of monochromatic light exactly improves 1403C production, and what are the specific effects of different monochromatic light on these two main polyketide biosynthetic pathways in this light-sensitive fungus. So that we can have a brief insight of light-sensing systems in this mangrove leaf endophytic fungus.

Materials and methods

Strain

Halorosellinia sp. (No. 1403) (CCTCCM 201018) was a leaf endophytic fungus of *Kandelia candel* (L.) Druce collected in Hongkong Mai Po wetland, and was provided by professor Zhi-gang She in Sun Yat-sen University.

The media and fermentation conditions

The seed medium was prepared in artificial seawater (ASW I), which consisted of 10 g glucose l^{-1} , 2 g tryptone l^{-1} , and 1 g yeast extract l^{-1} . The fermentation medium was prepared in 40% modified artificial seawater (ASW II), which consisted of 12.36 g glucose l^{-1} , 1.05 g tryptone l^{-1} , 6.08 g beef extract l^{-1} , and 0.246 g $MnSO_4 \cdot H_2O$ l^{-1} . The formulas of ASW I and ASW II were described by Zhou et al. (2013).

Halorosellinia sp. (No. 1403) was first grown on seed plate at 28 °C for 5 days. Small pieces of mycelia agar were cutoff, and transferred into 100-ml seed medium in a 500-ml baffled Erlenmeyer flask. The first-stage seed was obtained by incubated at 28 °C and 170 rpm for 72 h. Then, 5-ml first-stage seed was inoculated into fresh seed medium and cultured for another 36 h to obtain the second stage seed.

Shake flask fermentation was carried out in a 250-ml Erlenmeyer flask containing 50 ml fermentation medium, the second stage seed culture was inoculated (containing mycelia equal to 0.22 g dry biomass l^{-1}), and cultured at 28 °C and 170 rpm for 144 h. Bioreactor fermentation was carried out in a 5-l bioreactor (Shanghai Guoqiang Bioengineering Equipment Co., Ltd., China) containing 3.3 l fermentation medium equipped with double-layer six-flat-blade Rushton disc turbine impeller. The inoculation amount and cultivation temperature were the same as shake flask fermentation. The rotating speed (250–400 rpm) and aeration (0.13–1.31 vvm) were controlled identically and adjusted synchronously to keep the dissolved oxygen tension (DOT) not lower than 30% before 48 h and between 30 and 40% after that. Besides, the broth pH was controlled not lower than 3.8 by feeding NaOH solution (0.5 mmol l^{-1}).

Light irradiation conditions

The white fluorescent light was produced by Philips Electronic N.V, Dutch. Compound white light is mainly composed of three wavelength ranges 430–440 nm, 540–550 nm, and 610–620 nm, which represent for blue light, green light, and red light, respectively (see Fig. 2a). Different monochromatic lights were obtained by corresponding optical filters. As shown in Fig. 2b–d, three kinds of monochromatic light could be obtained, which were blue light, green light, and red light, respectively.

For shake flask culture, the Erlenmeyer flasks were illuminated from the top, and the light intensity was controlled at 350 ± 20 lx. For bioreactor fermentation, a self-designed barrel-shaped stainless steel shade evenly equipped with eight lamps was used which could surround the glass body of bioreactor to generate light irradiation for bioreactor fermentation. The light intensity was controlled at 400 ± 20 lx on body inner wall of the bioreactor.

Analytical methods

The quantitative method of 1403C, 1403R, and griseofulvin was described by Yu et al. (2012). The off-line pH and residual glucose of culture were measured by an FE20 pH meter (Mettler-Toledo International Inc., Switzerland) and a biosensor instrument SBA-40E (Biology Institute of Shandong Academy of Science, China), respectively. The biomass was determined by dry cell weight (DCW). The on-line dissolved oxygen tension (DOT) and the CO_2 excretion were monitored by an electrode (Mettler-Toledo International Inc., Switzerland) and a CO_2 analyzer (Electrolab Biotech Ltd., Britain).

The light irradiation intensity was measured using a VC1010A digital illuminometer (Hangzhou Victor Electronic Device Co., Ltd., China). The spectra of light sources were determined by an ASD FieldSpec 3 spectrograph (Analytical Spectral Device Inc., America).

Results and discussion

The effects of different monochromatic light on the polyketides production in shake flask

To explore the effects of different monochromatic light on the polyketides production in *Halorosellinia* sp. (No. 1403), it was cultivated under blue light, green light, and red light; furthermore, dark condition was set as a control.

The formation of 1403R is a reversible process (see Fig. 1a), and pH is an important factor affecting the balance. Former experiments showed that 1403R would not be converted into 1403C under pH 4.0 or 5.0, but when pH

Fig. 2 Spectrum of electric light source (1-430–440 nm, 2-540–550 nm, 3-610–620 nm). **a** White light, **b** blue light, **c** green light, and **d** red light

Fig. 3 Typical HPLC traces of the metabolites of *Halorosellinia* sp. (No. 1403) at different time points in shake flask

was controlled at 7.0, 1403R would not occur at all (Zhou et al. 2014). In a word, 1403R can be formed under low pH, and will be converted into 1403C when pH rising up to 6.0. In this study, as shown in Fig. 3, highest 1403R production occurs at 48 h together with low pH. Afterwards, 1403R is converted into 1403C along with pH rising, and the conversion process is completed at 120 h (Zhang et al. 2017). Hence, highest 1403C productions are achieved at that moment. Highest 1403C production, griseofulvin

production, and DCW under different irradiation conditions are shown in Table 1.

Interestingly, all monochromatic light enhanced octaketide 1403C production but inhibited heptaketide griseofulvin production. Therefore, *Halorosellinia* sp. (No. 1403) seemed to sense all these monochromatic light, and light showed different effects on different biosynthetic pathways. Light was benefit for the biosynthesis competition of octaketide against heptaketide. Green light showed

Table 1 Effects of different irradiation conditions on 1403C production, griseofulvin production, and DCW of *Halorosellinia* sp. (No. 1403) cultured in shake flask at 120 h

Irradiation condition	1403C (g l ⁻¹)	Griseofulvin (mg l ⁻¹)	DCW (g l ⁻¹)
Blue	2.49 ± 0.091 ^a	14.09 ± 0.79 ^a	5.25 ± 0.21
Green	2.70 ± 0.074 ^a	21.98 ± 1.36 ^a	4.90 ± 0.16
Red	2.22 ± 0.051	45.24 ± 2.29 ^a	5.30 ± 0.19
Dark	2.17 ± 0.055	54.78 ± 2.69	5.20 ± 0.21

^aSignificant at the 5% level ($P < 0.05$) comparing with the dark control

the most significant effect, the highest 1403C production was achieved under green light, which was 24% higher than that under dark condition, and the griseofulvin production was depressed by 60% under this condition ($P < 0.05$). On the contrary, red light showed the weakest effect, it could not improve the 1403C production significantly, and only inhibited griseofulvin production by 17% ($P < 0.05$). Blue light was less effective in promoting 1403C production compared with green light, but it could inhibit griseofulvin production most significantly.

Accordingly, three light-sensing systems may all exist in *Halorosellinia* sp. (No. 1403), and the rarely studied green light-sensing system may be the most effective one. *Halorosellinia* sp. (No. 1403) is an endophytic fungus of mangrove leaf, so we speculate that its effective green light-sensing system may be a kind of environmental adaptation. When photosynthesis is carried out in the leaves, red light and blue light will be absorbed by chlorophyll and carotenoid, and green light is the most abundant light source in its habitat. Hence, its metabolism is significantly influenced by green light.

The effects of different monochromatic light on the polyketides production in 5-l bioreactor

Fermentation in bioreactor usually resulted in lower 1403C production and higher griseofulvin production compared with that in shake flask, which was a bottleneck for 1403C mass production. Fortunately, the effect of illumination was also more significant in bioreactor (Zhang et al. 2016). Consequently, we investigated the fermentation process under different illumination condition more comprehensively in bioreactor.

The production of both octaketide (1403C and 1403R) and heptaketide (griseofulvin), as well as other process parameters in the fermentation of *Halorosellinia* sp. (No. 1403) in 5-l bioreactor under different illumination conditions are shown in Fig. 4. Consistent with the case in shake flask, higher octaketide production was obtained under illumination, and dark condition resulted in higher heptaketide production (Fig. 4a–e).

As shown in Fig. 4d, the highest total production of 1403C and 1403R under all conditions were achieved at 60 h. The highest total production was also achieved under green light, which was 4.85 mmol l⁻¹, and 76% higher than that under dark condition ($P < 0.05$). Moreover, the griseofulvin production under green light was 34 mg l⁻¹, which was 73% lower than that under dark condition ($P < 0.05$). Therefore, green light showed a very momentous effect on polyketide biosynthesis in *Halorosellinia* sp. (No. 1403) in bioreactor. Like the situation in shake flask, blue light was less effective in promoting octaketide production than green light, but a bit more effective in inhibiting griseofulvin production. The effect of red light was still the weakest, but it also showed significant difference compared with dark condition.

Although green light still showed best effect in bioreactor, while in fact green light showed better effect on the 1403C production potential—total production of 1403C and 1403R (76% higher than dark condition, Fig. 4d) than 1403C production itself (52% higher than dark condition, Fig. 4a). After 60 h, residual glucose was very low in bioreactor (Fig. 4f); therefore, the highest total production of 1403C and 1403R was achieved at 60 h. However, the conversion process of 1403R into 1403C continued, and the highest 1403C production was achieved later. Since 1403C was the most desired product in our study, so the conversion of 1403R into 1403C was very important. Under green light, pH dropped down and raised up faster than other conditions, so 1403R was formed and then converted earlier; as a result, highest 1403C production occurred 12 h earlier than other conditions. On the other hand, the degradation of metabolites (Fig. 4d) and mycelia (Fig. 4h) in the late stage (after 60 h) under green light was very severe. In particular, 1403C and 1403R production declined synchronously after 72 h, which meant that not all 1403R had converted into 1403C. Previously, we have proved that compound white light could promote 1403C production rate compared with dark condition before 60 h in 5-l bioreactor fermentation, but dark condition could prevent synchronous degradation of metabolites and mycelia after 60 h and ensured that 1403R could entirely

Fig. 4 Time profiles of process parameters of *Halorosellinia* sp. (No. 1403) cultured in 5-l bioreactor under blue light, green light, red light, and dark conditions. The light intensity was 400 ± 20 lx on body inner wall of bioreactor. **a** 1403C, **b** 1403R, **c** griseofulvin, **d** 1403C + 1403R (total production of 1403C and 1403R), **e** $Y_{p/x}$ (total production of 1403C and 1403R/DCW), **f** residual glucose, **g** broth pH, **h** DCW, **i** DOT (dissolved oxygen tension), and **j** CO_2 in exhaust gas

convert into 1403C. Thus, a light–dark shift strategy could achieve a 24% production enhancement compared with constant irradiation condition (Zhang et al. 2016). We believe that this effective light–dark shift strategy will also work under green light, and we estimate that the situation shift under green light would probably occurred between 48 and 60 h, so the cultural time may also be decreased compared with compound white light.

Compared with dark condition and red light, more glucose and oxygen were consumed (Fig. 4f, i), and more CO₂ occurred in exhaust gas (Fig. 4j) under green and blue light in the early stages (0–48 h). It implied that glucose metabolism was activated under green and blue light, so that more organic acid could be synthesized, resulted in lower pH in the first 36 h (Fig. 4g). Active glucose metabolism could provide sufficient substrates for cell growth and secondary metabolism. Highest octaketide production rate was achieved under green light (Fig. 4d), and highest growth rate was achieved under blue light (Fig. 4h). Consequently, $Y_{p/x}$ under green light was 28% higher than that under blue light (Fig. 4e).

Glucose will be synthesized through photosynthesis; therefore, green light and increasing glucose content may be signals for endophytic fungus to enhance glucose metabolism. In this study, adequate supply of glucose was ensured by an optimized medium (Zhou et al. 2013), so green light activated glucose metabolism, and more secondary metabolites were then produced. Furthermore, we can try fed-batch fermentation in conjunction with green light irradiation in the future to improve 1403C production further, while the effect of blue light was a bit different. Blue light induction system was thoroughly studied in *N. crassa*, and blue light was reported to improve carotenoids production by increasing the gene transcription level of carotenoids synthase (Schmidhauser et al. 1990, 1994). Similar phenomenon could also be observed in *Phycomyces blakesleeanus* (Blasco et al. 2001), *Mucor circinelloides* (Velayos et al. 2000), and *Blakeslea trispora* (Quiles-Rosillo et al. 2005). On the other hand, recently, intermittent illumination of green light-emitting diodes was found to increase mycelial biomass production of *Lentinula edodes* (Glukhova et al. 2014). However, in our study, blue light showed a better effect on mycelial growth, and green light promoted octaketide production more effectively. Therefore, the mechanism of the green light and blue light response in *Halorosellinia* sp. (No. 1403) needs to be further studied to let us have a better understanding of the phenomenon in fermentation process.

Conclusion

In a whole, illumination conditions could improve octaketide production in *Halorosellinia* sp. (No. 1403), and inhibited heptaketide production compared with dark

condition. Green light showed best effect on the desired octaketide 1403C production enhancement, which was probably derived from environmental adaptation. Thus, environmental factor should be taken into consideration in the attempt to improve the metabolites production of environmental microorganisms.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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