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Effect of Salicylic Acid and Methyl Jasmonate on Growth and Pigment Production in *Monascus purpureus*

Jialan Zhang[1]*, Piao Liu[2], Yingbao Liu[2], Zhixin Zhen[2], Li Li[2] and Mengxiang Gao[2]*
[1]College of Animal Science, [2]College of Life Science, Yangtze University, Jingzhou, Hubei 434 025, China

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The influence of salicylic acid (SA) and methyl jasmonate (MeJa) on the biomass and pigments production in *Monascus purpureus* was investigated. With 100 µmol/L SA- and 10 µmol/L MeJa-treated cultures, the maximum biomass were 4.70 and 4.33 g/L and significantly higher than the control, respectively. Supplemented with 10 µmol/L SA or MeJa, extracellular yellow, orange and red pigments increased, respectively, compared to the control. Supplemented with 10 µmol/L SA or 30 µmol/L MeJa, intracellular yellow, orange and red pigment production increased mostly compared to the control. AP activity was significant induction with SA and MeJa supplementation and the most significant at the 8th day of induction. These results indicate that proper concentration SA or MeJa enhance the biomass and pigment production in *Monascus purpureus*. Salicylic acid improved biomass and pigment production significantly more than MeJa.

Keywords: AP activity, Biomass, Extracellular pigment, Health care, Intracellular pigment

Introduction

Monascus pigment (MP) is one of the main metabolites of *Monascus* with bright colour, strong heat resistance, and high safety. This pigment is a food grade natural pigment and has been used throughout history for millennia in some Asian countries.[1] In recent years, the biological properties of MP have been discovered.[2,3] Especially, it has a potential application prospect in health care products and even medicine.

The liquid fermentation of MP has been industrialized and is increasingly favored[4] for process automation, large-scale production, product application convenience and uniform colour. Salicylic acid (SA) could regulate some physiological processes as an elicitor.[5,6] Methyl jasmonate (MeJa) as a cellular regulator could determine the secondary metabolites yield for environmental change.[7,8]

In this work, the biomass and MP production in *Monascus purpureus* was evaluated by the addition of SA and MeJa to the culture media.

Materials and Methods

Strain, culture medium and culture duration

Monascus purpureus strain SKY219 was supplied by the College of Life Science, Yangtze University.

The potato liquid medium consisted of 200 g/L potato (peeled), 20 g/L glucose, 3 g/L potassium dihydrogen phosphate, 1.5 g/L magnesium sulfate, 1.5 g/L peptone. The reagents were purchased from Solarbio ST Co. Beijing, China.

The inducer added to the liquid medium were 0, 10, 50, 100, 250, 500, 750, 1000 µmol/L of SA and 0, 10, 30, 50, 70, 90, 110 µmol/L of MeJa. The 250 mL flasks with 100 mL medium were inoculated at 30°C, 150 rpm for 12 days. Each experiment was repeated five times. The mycelium and fermentation broth was collected separately to determinate biomass and pigment.

Determination of biomass and pigment

The biomass was determined by cell dry weight in fermentation broth by a method suggested by Wan *et al*.[9] The fermented broth was withdrawn for centrifugation to harvest mycelium and the supernatant broth. The mycelium was dried to measure the biomass. The intracellular pigments were extracted with anhydrous ethanol by sonication for 40 min, 120 W from a part of the dry mycelium. The absorbance value of the extracts was used to estimate intracellular yellow, orange and red pigments at 410, 465 and 500 nm by spectrophotometer (UV1601PC, Shimadzu, Japan), respectively. Additionally, The extracellular pigments were extracted with distilled water from the supernatant. The pigment yield was estimated and expressed in absorbance units multiplied by the dilution factor.

[1]Authors for Correspondence
E-mail: zjlgpy88@163.com; mxgao0398@163.com

Extracellular Enzyme Activities

The supernatant was used for enzyme analysis by centrifugation fermentation broth at 22 400 × g for 10 min. Acid proteinase (AP) activity was determined by the standard curve method suggested by Dai *et al.*[10] The unit of enzyme activity is also defined as him.

Results and Discussion

Effect of SA and MeJa Concentration on Biomass

The biomass was more than the control in response to 10–250 µmol/L SA or 10–50 µmol/L MeJa (Fig. 1). The maximum biomass were 4.70 and 4.33 g/L (extremely bigger than the control, $p<0.01$) adding with 100 µmol/L SA and 10 µmol/L MeJa, while the minimum biomass was 500 µmol/L SA and 70 µmol/L MeJa, respectively. The findings suggested that the biomass was affected by SA or MeJa. The optimal concentration of SA or MeJa was found to significantly increase the biomass. Salicylic acid or MeJa at lower and higher concentrations had not affected or decreased biomass. Sivanadhan *et al.*[11] also found that biomass production was not significant and was completely inhibited by 150–250 µmol/L SA and MeJa treated cultures of *Withania somnifera*. Moreover, α-terpinene-7-a1 in *Cuminum cyminum L.* increased with 1 mmol/L of SA and 0.1 mmol/L of MeJa-treated cultures, but other levels of SA and MeJa decreased α-terpinene-7-a1.[12] Danaee *et al.*[13] reported that high levels of MeJA (>10 mmol/L) restrains the callus growth extracorporeal callus induction of *Phyllanthus pulcher*. The fresh and dry weight of *Rehmannia glutinosa* roots was significantly reduced by MeJa and SA, especially at 150–200 µmol/L.[14]

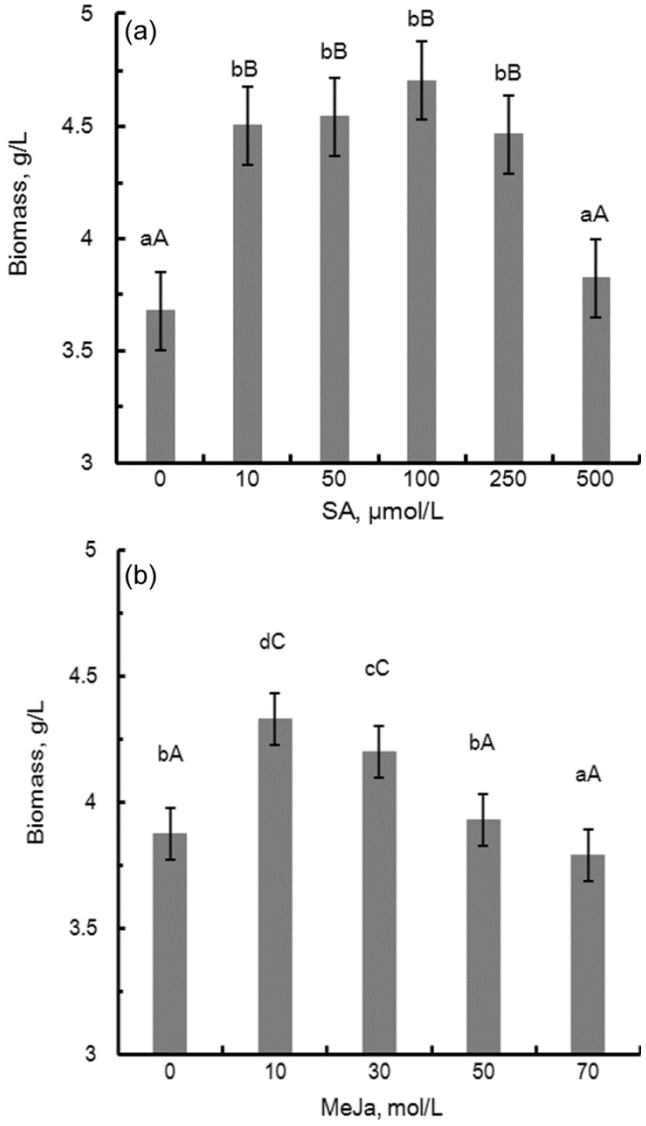

Fig. 1 — Effect of SA (a) and MeJa (b) concentrations on mycelia biomass in *Monascus purpureus*; Values are mean ± SD (n=5); Capital letters denote $p<0.01$; lowercase letters denote $p<0.05$

Effect of SA and MeJa Induction Time on Mycelia Biomass

The biomass yield increased at first then decreased with culture time both SA (100 µmol/L) and MeJa (10 µmol/L) supplemented, as well as that of the control (Fig. 2). The bigger of biomass were followed by SA or MeJa added from day 6 to 12 comparing to the control. The promotion effects of SA or MeJa added were most significant at the 8th day of induction. The peak of biomass was 4.09 and 4.02 g/L. This is 1.11 and 1.13 fold higher than that of the control,

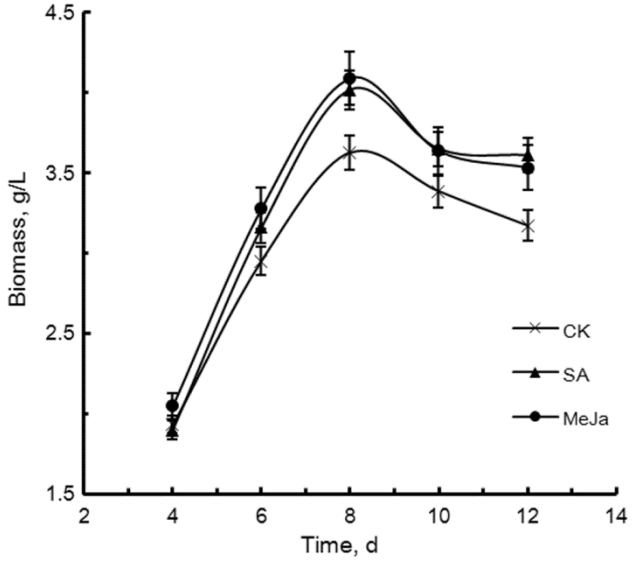

Fig. 2 — Effect of induction time on mycelia biomass in *Monascus purpureus* following SA (100 µmol/L) or MeJa (10 µmol/L) supplementation; Values are mean ± SD (n=5)

respectively. However, there was no significant difference in biomass between SA and MeJa supplementation at the same stage.

Effect of SA and MeJa Concentration on Extracellular and Intracellular Pigment

The influence of SA or MeJa on extracellular and intracellular yellow, orange and red pigments is shown in Fig. 3. The difference in concentrations of SA and MeJa were separately added to the liquid medium with various exposure times for 12 days of culture. The same trends were shown between the three pigments' production and SA or MeJa concentrations (Fig. 3).

It is shown in Fig. 3(a) that the three extracellular pigments produced were significantly increased at 10 µmol/L SA and were 1.48, 1.27 and 1.24 fold higher than the control of yellow, orange and red pigments, respectively. There were no different effects of SA concentrations on pigment production at 100 µmol/L. Salicylic acid could inhibit the pigments' production at 50, 250 and 500 µmol/L.

In Fig. 3(b) it can be observed that the three extracellular pigments produced were significantly increased at 10 and 30 µmol/L MeJa and the production was larger at the higher concentrations. The yellow, orange and red pigments produced were 1.48, 1.48, 1.78 fold higher than that of the control, respectively. MeJa did not influence pigments yield at 50 µmol/L.

Among the different concentrations of SA or MeJa tested, 10 µmol/L of SA (Fig. 3(c)) and 30 µmol/L of MeJa (Fig. 3(d)) with 12 day exposure time increased the production of intracellular yellow pigment (SA: 181.5 U/g; 2.31-fold; MeJa: 142.3 U/g; 1.46-fold),

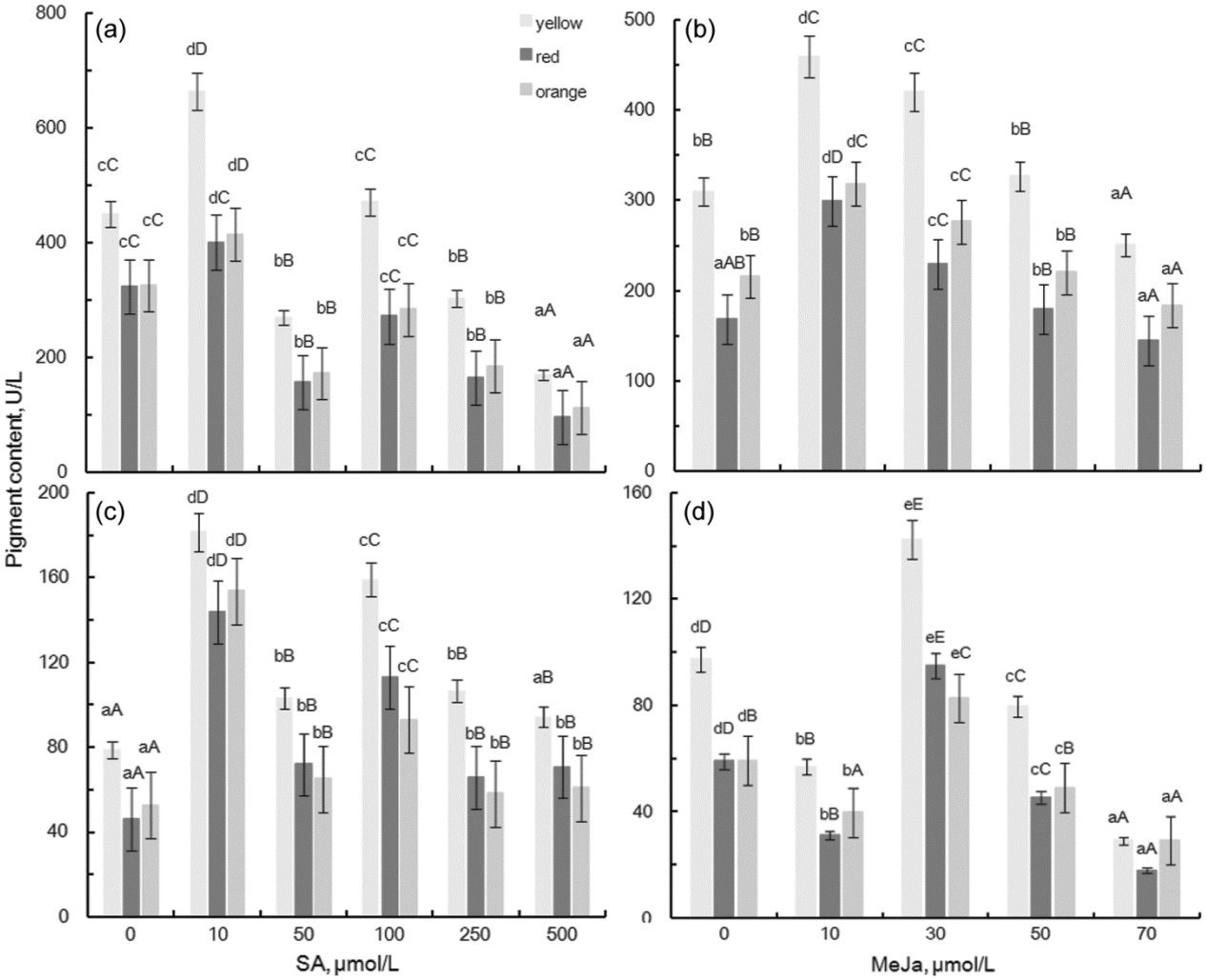

Fig. 3 — Effect of concentration on extracellular pigments content (SA (a) and MeJa (b)) and intracellular pigments content (SA (c) and MeJa (d)) in *Monascus purpureus;* Values are mean ± SD (n=5); Capital letters denote $p<0.01$; lowercase letters denote $p<0.05$

orange pigment (SA: 153.7 U/g; 2.92-fold; MeJa: 82.8 U/g; 1.40-fold), and red pigment (SA: 143.7 U/g; 3.13-fold; MeJa: 95.0 U/g; 1.61-fold) compared to the control. And the intracellular yellow, orange and red pigment production was higher than the control at 10 and 100 µmol/L of SA, respectively. However, SA at 50 µmol/L showed no significant effect on the three intracellular pigments (Fig. 3(c)). Methyl Jasmonate at 30 µmol/L and 70 µmol/L showed a significantly promotion and inhibition of the three intracellular pigments, respectively (Fig. 3(d)). But the biomass was greater than that of the control at 100 µmol/L SA and 10 µmol/L MeJa (Figs 1(a) and 1(b)). The bacoside-A content could be increase by SA and MeJa (50 µmol/L) in shoot cultures of *Bacopa monnieri (L.)*.[15] The capsaicin yield in suspension cultures of Naga King Chili (*Capsicum chinense Jacq.*) could be increased by exposing cells to 1 mmol/L SA.[16] The withanolides yield could be increased by SA (100 µmol/L) in multiple shoots of *W. somnifera*.[11]

Effect of SA and MeJa Supplementation on AP Activity

During cultivation, AP activity increased at first then decreased with culture time supplement with SA (100 µmol/L) or MeJa (10 µmol/L), same as the control culture (Fig. 4). These data indicated that AP activity was promoted by SA supplementation from day 6 to 10 and MeJa supplementation from day 4 to 12. The levels of AP activity were the most significant at the 8th day of induction, compared to the control. The peak of AP activity was 29.87 and 28.08 U/mL SA and MeJa supplementation and 1.19 and 1.12 fold higher than the control, respectively. However, AP activity showed higher levels in MeJa supplementation than with SA supplementation at the 4th and 12th days of culture. There were significant induction effects of SA and MeJa supplementation on AP activity (Fig. 4). For fungi, AP is one of the key enzymes for growth and defense[10] and plays important roles in the developmental regulation, mycelium physiological maturity and fruiting body formation.[17] At the same time, proteinase is an inducible enzyme. Protease activity was increased by MeJa induce and thus promoted the ergosterol production in *Hericium erinaceus*.[10] Using MeJA could increase dry bean resistance against *S. sclerotiorum* by increasing the transcripts levels of pathogenesis-related protein.[18] Additionally, the responses to stress of plant was regulated by SA via signaling pathways.[5] However, until now, it was not fully understood the exact mechanism that SA and MeJa regulates secondary metabolite synthesis. The effect on fungi is less significant than that of plants. More works are needed to improve methods and research mechanisms and to get the satisfactory results.

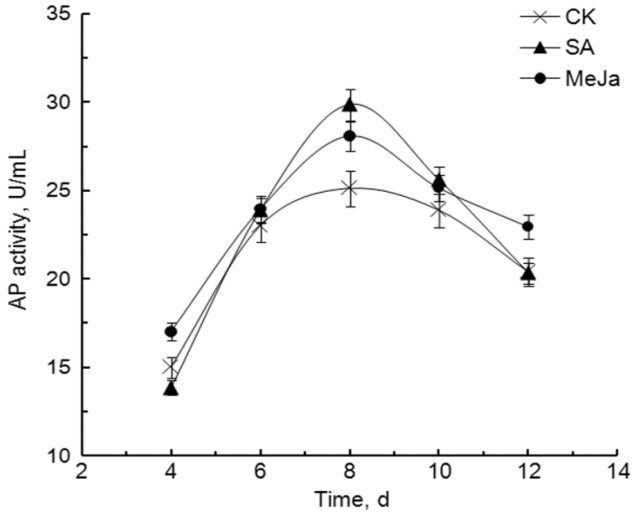

Fig. 4 — Effect of induction time on acid protease (AP) activity in *Monascus purpureus* liquid culture following SA (100 µmol/L) or MeJa (10 µmol/L) supplementation; Values are mean ± SD (n=5)

Conclusions

Proper concentration SA or MeJa induced biomass and pigment biosynthesis in *M. purpureus*. SA improved biomass and pigment production significantly more than MeJa. Therefore, SA is a better inducer of biomass and pigment in *M. purpureus*.

Acknowledgments

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