

Establishment hairy root culture system to produce secondary metabolites of *Salvia miltiorrhiza*

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Abstract

Salvia miltiorrhiza Bunge (Labiatae) is an important traditional Chinese medicinal plant for treating the cardiovascular related diseases. Factors affecting genetic transformed hairy root of *S. miltiorrhiza* for production of secondary metabolites were investigated in this study.

Materials & Methods

Plant material-Hairy roots induced from *in vitro* *Salvia miltiorrhiza* leaf explant were used in this study. Unless specified, root tips with 1 cm in length were cultured into B5¹ basal salt medium containing with 3% sucrose and additional 1% agar for solid medium. All cultures were kept in dark at 25°C in liquid culture shaking at 100 rpm

Results

1. Effect of medium salt formula and strength on secondary metabolite production of *S. miltiorrhiza* hairy root culture-Three kind secondary metabolites were measured using HPLC, the result showed that hairy root grown on B5¹ medium contained the highest of tanshinone II A, tanshinone I and cyptotanshinone, followed by root grown on Woody Plant Medium (WPM)², half strength of Murashige and Skoog (MS³) medium and full strength MS medium (Fig. 1). No secondary metabolite was detected on N6⁴ medium because of no growth of hairy root in this medium.

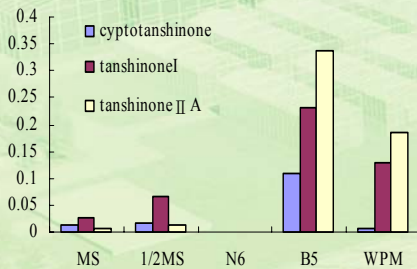


Figure 1. Effect of medium salt formula on production of secondary metabolite of *Salvia miltiorrhiza* hairy root.

2. Effect of sucrose concentration on secondary metabolite production of *S. miltiorrhiza* hairy root- Contents of tanshinones were found increased along with sucrose concentration. It was found that 3% sucrose concentration was necessary for production of tanshinone. Sucrose concentration of 6% would increase tanshinone II A accumulation after 6 weeks of culture (Fig. 2).

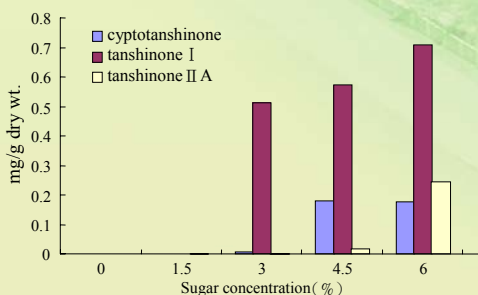


Figure 2. Effect of sucrose concentration on secondary metabolite production of *Salvia miltiorrhiza* hairy root.

3. Effect of liquid and solid medium on secondary metabolite production of *S. miltiorrhiza* hairy root- Tanshinone contents of roots grown in the solid culture was found 3 times higher than that of in the liquid culture (Fig. 3)

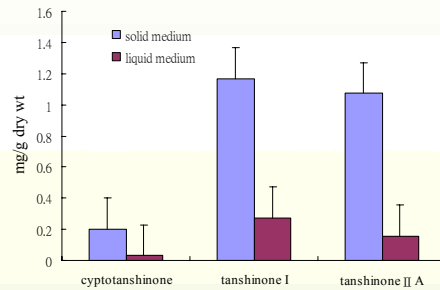


Figure 3. Effect of liquid and solid medium on production of cyptotanshinone, tanshinone I and tanshinone II A for hairy root of *Salvia miltiorrhiza*.

4. Effect of illumination culture condition on secondary metabolite production of *S. miltiorrhiza* hairy root-The results showed that hairy root grown under lightness condition had all three tanshinones production suppressed especially for the cyptotanshinone and tanshinone I (Tab. 1).

Table 1. Effect of light and dark growth condition on hairy root of *Salvia miltiorrhiza* production of cyptotanshinone, tanshinone I and tanshinone II A.

Illumination culture treatment	Cyptotanshinone (mg/g dry wt.)	tanshinone I (mg/g dry wt.)	tanshinone II A (mg/g dry wt.)
Light	0.000	0.000	0.002
Dark	2.523	1.321	0.005

5. Time course study on tanshinone production of hairy root lines HR-1, HR-2 and HR-3- The hairy roots were harvested every two weeks up to 12 weeks of culture. All three lines showed continuously increasing in tanshinones before 10 weeks however tanshinones content of line HR-1 decreased afterwards (Fig. 4). The tanshinone I, tanshinone II A and cyptotanshinone contents in different hairy root lines was varied which indicated the genetic unique of every and each transformed hairy root lines (Fig. 4).

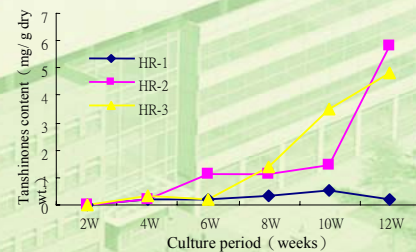


Figure 4. Time course of hairy root lines on production of tanshinones.

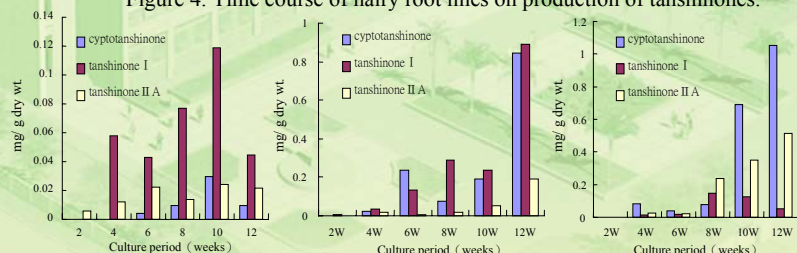


Figure 5. Time course of hairy root line HR-1, HR-2 and HR-3 on production of tanshinones.

Conclusion

The maximum tanshinone contents were achieved after 10 to 12 weeks of culture for different hairy root lines grown on B5 solid medium containing sucrose higher than 4.5% in dark condition. Time course of total yield and individual tanshinone content varied in different hairy root lines.