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BRIEF COMMUNICATION

Syringin production by *Saussurea medusa* cell cultures in a novel bioreactor

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Abstract

The culture of *Saussurea medusa* cell were cultured in an internal loop airlift bioreactor with sifter draft tube (ILABSDT) was investigated. Under the optimal culture conditions, which were inoculation size 1.5 g(d.m.) dm^{-3}, aeration rate 0.3 dm^3(air) dm^{-3}(medium) min^{-1}, and 14 mesh sifter holes, the maximum biomass, syringin content and syringin production reached 11.7 g(d.m.) dm^{-3}, 17.7 mg g^{-1} and 206.6 mg dm^{-3}, respectively. Among cell cultures in shake flask, bubble column bioreactor and ILABSDT, ILABSDT had the highest syringin productivity and reached 12.41 mg dm^{-3} d^{-1}.

Additional key words: aeration rate, inoculation size, mesh, sifter draft tube.

Saussurea medusa, belonging to the *Compositae* family, is one of the most famous Chinese traditional and rare medicinal plants. Syringin extracted from the aerial part of *S. medusa* is effective in the treatment of psychogenic behavior disorder and has a hypnosis inducing action, hepato-protective activities, anti-hypersensitivity effect as well as anti-inflammatory function on auto-immune diseases (Yang *et al.* 2004). Due to over-exploitation of the wild plants for commercial purpose and difficulty of cultivation, *S. medusa* has been listed as a protected plant by Chinese government (Chen *et al.*1999). Therefore, the culture of *S. medusa* cells is considered as a promising alternative for high efficient production of syringin. For the cell culture in scale up, several types of bioreactor, such as stirred tank and periodically submerged airlift bioreactor have been used in *S. medusa* cell cultured (Xing *et al*. 2000, Yuan *et al*. 2004). High cell density and fluid viscosity significantly reduce transfer efficiencies of gas and nutrients in a bioreactor. In general, increasing agitation speed or aeration rate can improve the mass transfer and mixing characteristics. However, these approaches have several limitations, such as power consumption, cell damage due to shear stress (Langhansová *et al*. 2005, Thanh *et al*. 2006). So, the research of how to reduce the rear stress and improve the mass transfer would be interesting. Considering growth characteristics of plant cells, a novel internal loop airlift bioreactor with sifter draft tube (ILABSDT) was designed. Due to liquid axial flow in riser/downcomer and radial flow through the sifter holes, ILABSDT has better mixing performance and lower shear stress. Improving gas transfer and reducing the shear stress may promote the cell growth and the biosynthesis of secondary metabolites. In this paper, the cell growth and syringin biosynthesis of *Saussurea medusa* in ILABSDT were investigated.

S. medusa cell line was established from the leaf explants and maintained in our laboratory (Yuan *et al*. 2002). The callus were subcultured every 15 d. The cell culture medium is Murashige and Skoog (1962; MS) medium supplemented with 2 mg dm^{-3} α-naphthalene-acetic acid (NAA), 0.5 mg dm^{-3} 6-benzylaminopurine (BAP) and 30 g dm^{-3} sucrose. The medium pH was adjusted to 5.85 - 5.90. Then the ILABSDT containing 2 dm^3 liquid medium was autoclaving at 1.06 kg cm^{-2} for 20 min. For comparison, suspension culture in 500 cm^3 shake flask containing 200 cm^3 liquid medium on a rotary shaker (130 rpm) and 2 dm^3 bubble column bioreactor

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Abbreviations: BAP - 6-benzylaminopurine; BCB - bubble column bioreactor; d.m. - dry mass; f.m. - fresh mass; HPLC - high performance liquid chromatography; ILABSDT - internal loop airlift bioreactor with sifter draft tube; NAA - α-naphthaleneacetic acid
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(BCB) were performed.

The schematic diagram of the ILABSDT is illustrated in Fig. 1. Air agitation is achieved by aeration by an air compressor and the air is sterilized through membrane air-filters. A concentric gas sparger with pore size of about 60 μm is fixed on the bottom of the riser. The aeration rate is monitored by a flowmeter. All cells were cultured under irradiance of 27 μmol m^{-2} s^{-1} provided by white cool fluorescent tube lamps in a 16-h photoperiod and at temperature of 25 ± 1 °C.

Before the cell fresh mass (f.m.) determinations, the cell suspensions were filtered through a filter paper and the cells were washed with distilled water. After the cell f.m. was recorded, the cells were dried at 60 °C to constant mass to determine the cell dry mass (d.m.). For syringin content determination, smashed dry cells (0.1 g) were extracted with 10 cm^3 70 % (v/v) methanol for 24 h and centrifuged at 5 000 *g* for 10 min. Then, the supernatant was filtered through a 0.2 μm membrane filter (*Waters Corporation*, Massachusetts, USA). 20 mm^3 sample was injected into a HPLC system equipped with a diamonsil C18 column (250 × 4.6 mm, 5μm particle size), fitted with a C18 guard cartridge. The mobile phase used for separation consisted of solvent A (water : acetic acid : triethylamine, 99.1:0.8:0.1) and solvent B (methanol). The elution profile was: 0 min A:B (8:2), 10 min A:B (7:3), 15 min A:B (4:6). The flow rate was 1.0 cm^3 min^{-1}, column temperature was 30 °C and pH was 3.8. UV detection was at a wavelength of 270 nm.

All experiments were repeated three times. The data obtained were statistically analyzed by *PROC ANOVA* of *SAS* version *6.12*. Data were submitted to analysis of variance and mean were then compared with Duncan's Multiple Range Test, and the term significant has been used to denote the differences for which $P \leq 0.05$.

The highest biomass [11.2 g(d.m.) dm^{-3}] and syringin production (179.2 mg dm^{-3}) were observed when the inoculation size was 2.0 and 1.5 g(d.m.) dm^{-3} (Table 1). Statistic analyses showed that no significant differences were observed on biomass and syringin production between inoculation size 1.5 and 2.0 g(d.m.) dm^{-3}. However, the cell growth ratio under 1.5 g(d.m.) dm^{-3} inoculation size was higher than that under 2.0 g(d.m.) dm^{-3}. Hence, inoculation size 1.5 g(d.m.) dm^{-3} was determined as optimal inoculation size for syringin

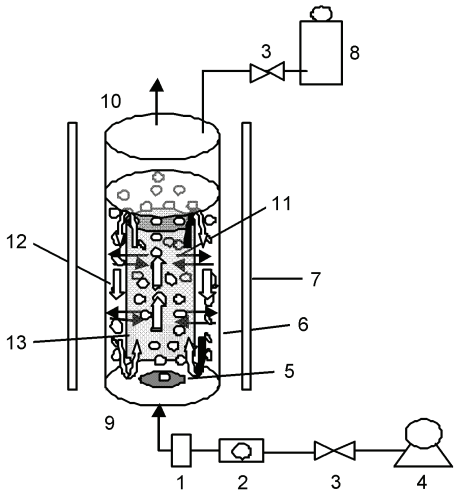

Fig. 1. Internal loop airlift bioreactor with sifter draft tube system (ILABSDT). 1 - gas filter, 2 - flowmeter, 3 - valve, 4 - gas pump, 5 - gas sparger, 6 - glass column, 7 - fluorescent lamp, 8 - water reservoir, 9 - gas inlet, 10 - gas outlet, 11 - riser, 12 - downcomer, 13 - sifter draft tube.

Table 1. Effects of inoculation size, aeration rate and sifter mesh number on the *S. medusa* growth ratio (final f.m. per explant f.m.) and syringin content and production in ILABSDT. All cells were cultured under irradiance of 27 μmol m^{-2} s^{-1}, 16-h photoperiod and temperature of 25 ± 1 °C. Means ± SD of 3 replicates.

		Biomass [g(d.m.) dm^{-3}]	Growth ratio	Syringin content [mg g^{-1}(d.m.)]	Syringin production [mg dm^{-3}]
Inoculation size [g(d.m.) dm^{-3}]	0.5	5.3 ± 0.22	10.60 ± 0.92	12.7 ± 1.61	67.1 ± 3.7
	1.0	8.6 ± 0.28	8.60 ± 0.79	16.0 ± 1.54	137.3 ± 5.9
	1.5	10.8 ± 0.38	6.73 ± 0.49	16.6 ± 1.19	179.2 ± 4.8
	2.0	11.2 ± 0.29	5.60 ± 0.32	15.3 ± 2.01	171.8 ± 5.6
	2.5	9.1 ± 0.43	3.64 ± 0.37	14.6 ± 1.10	132.7 ± 4.9
Aeration rate [dm^3(air) dm^{-3}(medium) min^{-1}]	0.2	10.7 ± 0.36		16.4 ± 2.41	175.6 ± 6.55
	0.3	11.6 ± 0.27		17.7 ± 2.74	204.9 ± 6.07
	0.4	9.8 ± 0.41		14.8 ± 2.32	144.7 ± 5.04
	0.5	9.2 ± 0.43		13.0 ± 1.91	119.8 ± 5.71
Sifter mesh number	6	10.1 ± 0.53		13.8 ± 1.27	140.2 ± 4.15
	10	10.3 ± 0.42		15.0 ± 2.05	154.8 ± 6.68
	14	11.7 ± 0.71		17.7 ± 2.07	206.6 ± 10.66
	20	11.6 ± 0.66		17.2 ± 1.79	199.7 ± 6.47
	24	9.8 ± 0.43		16.7 ± 2.42	163.5 ± 6.04

Table 2. Time courses of *S. medusa* biomass and syringin production in shake flask, BCB and ILABSDT. The cells were cultured in 500 cm^3 shake flask containing 200 cm^3 liquid medium on a rotary shaker (130 rpm) or in BCB and ILABSDT containing 2 dm^3 liquid medium. For BCB, the aeration rate was 0.4 dm^3(air) dm^{-3}(medium) min^{-1}. For the ILABSDT, the mesh number of sifter was 14 and the aeration rate was 0.3 dm^3(air) dm^{-3}(medium) min^{-1}. The inoculation size of three culture modes was 1.5 g (d.m.) dm^{-3}. All cells were cultured under irradiance of 27 μmol m^{-2} s^{-1}, a 16-h photoperiod and 25 ± 1 °C. Means ± SD of 3 replicates. Syringin productivity [mg dm^{-3} d^{-1}] was 9.85 ± 1.1, 9.65 ± 1.2 and 12.41 ± 1.6 in shake flasks, BCB and ILABSDT, respectively.

Culture time [d]	Shake flask		BCB		ILABSDT	
	biomass [g(d.m.) dm^{-3}]	syringin [mg dm^{-3}]	biomass [g(d.m.) dm^{-3}]	syringin [mg dm^{-3}]	biomass [g(d.m.) dm^{-3}]	syringin [mg dm^{-3}]
0	1.50 ± 0.30	22.74 ± 4.4	1.50 ± 0.26	23.02 ± 4.1	1.50 ± 0.22	22.81 ± 4.0
3	2.13 ± 0.41	33.40 ± 5.9	2.42 ± 0.38	37.97 ± 6.3	2.33 ± 0.36	33.61 ± 5.9
6	3.34 ± 0.55	50.20 ± 7.0	3.81 ± 0.51	51.77 ± 7.7	4.26 ± 0.56	63.99 ± 8.5
9	4.95 ± 0.71	77.79 ± 9.8	5.36 ± 0.59	84.21 ± 8.6	6.92 ± 0.71	113.44 ± 8.9
12	7.41 ± 0.51	121.50 ± 12.0	7.67 ± 0.63	125.73 ± 11.4	9.71 ± 0.62	152.50 ± 11.4
15	9.33 ± 0.46	146.56 ± 11.0	9.85 ± 0.72	144.79 ± 13.2	11.54 ± 0.61	186.20 ± 13.5
18	11.62 ± 0.98	174.56 ± 13.3	9.22 ± 0.66	138.02 ± 12.7	10.21 ± 0.58	150.79 ± 13.0
21	12.97 ± 0.62	206.60 ± 10.7				
24	10.22 ± 0.87	153.53 ± 11.1				

production and the further research. When *S. medusa* cells were cultured in ILABSDT, it was founded that the cells were sensitive to aeration rate. At low aeration rate, the cell growth and syringin production increased with the increase of aeration rate. The maximum biomass (11.6 g(d.m.) dm^{-3}), syringin content (17.7 mg g^{-1}) and syringin production (204.9 mg dm^{-3}) were obtained at airflow rate of 0.3 dm^3(air) dm^{-3}(medium) min^{-1}. When the aeration rate was over 0.3 dm^3(air) dm^{-3}(medium) min^{-1}, the biomass, syringin content and syringin production showed a sharp decrease (Table 1). It may be due to the cell growth and second metabolite biosynthesis being destroyed by the higher aeration rate (Pan *et al.* 2000). Mesh number of the sifter had also significant effect on cell growth, syringin content and production. When the mesh numbers of sifter was lower than 14, the cell biomass, syringin content and production increased with the increase of mesh number. The highest biomass [11.7 g(d.m.) dm^{-3}], syringin content (17.7 mg g^{-1}) and production (206.6 mg dm^{-3}) were obtained when the mesh number was 14 (Table 1). Statistic analyses showed that no significant differences of biomass and syringin production were observed between 14 and 20 mesh of the sifter. Lower or higher mesh number made the marked difference of the sifter holes diameter, hereupon, affected the air bubbles and liquid across the net, thus higher shear stress or poor oxygen transfer happened due to the changes of air bubble size and liquid flow rate in the riser (Fu *et al.* 2003). Therefore, 14 mesh was optimal for syringin biosynthesis.

On the 15th day of cultivation, the biomass and syringin production reached the maximum in BCB and ILABSDT (Table 2). However, the culture time when the biomass and syringin production reached the maximum in the shake flask was 21 d. In the shake flask, both the biomass and syringin production were higher than those in BCB and ILABSDT (Table 2). But, the cell growth and syringin production in ILABSDT were better than those in BCB. In general, the production of second metabolites was reduced when the cell cultures were transferred from shake flasks to bioreactors (Schlatmann *et al.* 1993). However, bioreactor culture had significantly shorter culture time than shake flask culture, and had higher second metabolite productivity. The most suitable culture time of the shake flask culture was longer than those of the two style bioreactors. No significant difference on syringin productivity was obtained between shake flask culture and BCB culture. Due to large bubble was sliced into smaller bubbles when it crossed the sifter, ILABSDT possessed better mixing efficiency and oxygen transfer rate than BCB (Wu *et al.* 1990). Hence, among three culture modes, the syringin productivity in ILABSDT was the highest and reached 12.41 mg dm^{-3} d^{-1} (Table 2). Compared to shake flask culture, ILABSDT had shorter culture time. ILABSDT are superior to BCB in terms of mixing performance, oxygen transfer and higher productivity. Due to the advantages than other culture modes including shorter culture time, lower shear stress, better mass transfer, and higher second metabolites productivity, ILABSDT has a very high potential for industrial applications.

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