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Schisandra lignans production regulated by different bioreactor type

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ABSTRACT

Schisandra chinensis (Chinese magnolia vine) is a rich source of therapeutically relevant dibenzocyclooctadiene lignans with anticancer, immunostimulant and hepatoprotective activities. In this work, shoot cultures of *S. chinensis* were grown in different types of bioreactors with the aim to select a system suitable for the large scale *in vitro* production of schisandra lignans. The cultures were maintained in Murashige-Skoog (MS) medium supplemented with 3 mg/l 6-benzylaminopurine (BA) and 1 mg/l 1-naphthaleneacetic acid (NAA). Five bioreactors differing with respect to cultivation mode were tested: two liquid-phase systems (baloon-type bioreactor and bubble-column bioreactor with biomass immobilization), the gas-phase spray bioreactor and two commercially available temporary immersion systems: RITA® and Plantform. The experiments were run for 30 and 60 days in batch mode. The harvested shoots were evaluated for growth and lignan content determined by LC-DAD and LC-DAD-ESI-MS. Of the tested bioreactors, temporary immersion systems provided the best results with respect to biomass production and lignan accumulation: RITA® bioreactor yielded 17.86 g/l (dry weight) during 60 day growth period whereas shoots grown for 30 days in Plantform bioreactor contained the highest amount of lignans (546.98 mg/100 g dry weight), with schisandrin, deoxyschisandrin and gomisin A as the major constituents (118.59, 77.66 and 67.86 mg/100 g dry weight, respectively).

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1. INTRODUCTION

Chinese magnolia vine (*Schisandra chinensis*) is a dioecious climbing plant. It grows naturally in north-eastern China, Korea, Japan, in the eastern part of Russia, in Primorsk, on the Kuril Islands and in the south of the Sakhalin Island (Hancke et al., 1999). Fruits of *S. chinensis* are well known in Traditional Chinese Medicine (TCM) and have been included as pharmacopoeial raw material in the Far East, America, and since 2008 also in European countries (European Pharmacopoeia 8.0, 2013; Szopa et al., 2016a).

S. chinensis owes its therapeutic properties to the presence of dibenzocyclooctadiene lignans (aka schisandra lignans), a specific group of secondary metabolites whose biosynthetic pathway has so far not been fully elucidated (Hancke et al., 1999; Opletal et al., 2004). The biological effects of schisandra lignans include hepatoprotective, adaptogenic, immunostimulant, anti-obesity and anticancer activities. They have been also proved to be promising therapeutic agents in cardiovascular and respiratory tract diseases (Park et al., 2012; Mocan et al., 2016; Szopa et al., 2016a). The compound of particular interest is schisandrin B which is capable of selectively inhibiting HIV-1 reverse transcriptase-associated DNA polymerase activity (Xu et al., 2015) and preventing the doxorubicin-induced cardiomyopathy (Thandavarayan et al., 2015). Biological properties of Chinese magnolia vine and its components have been recently reviewed by Szopa et al. (2016a).

Given the therapeutic relevance of dibenzocyclooctadiene lignans, efforts have been made to develop effective methods for their synthesis. However, difficulties have been encountered due to the complexity of the process, its low performance and high costs (Hancke et al., 1999; Opletal et al., 2004; Szopa et al., 2016a). Attempts to employ different types of *S. chinensis in vitro* cultures (callus, suspension and shoots) for the production of lignans have also been made, with yields ranging from traces to amounts comparable with parent plant material (Březinová et al., 2010; Kohda et al., 2012; Szopa et al., 2016b). Particularly promising results were obtained using agar, liquid stationary and agitated shoot cultures which were demonstrated to accumulate substantial amounts of dibenzocyclooctadiene lignans (Szopa et al., 2016b). However, large-scale *in vitro* production of plant chemicals requires the use of bioreactors, but no such studies on *S. chinensis* cell cultures have so far been conducted.

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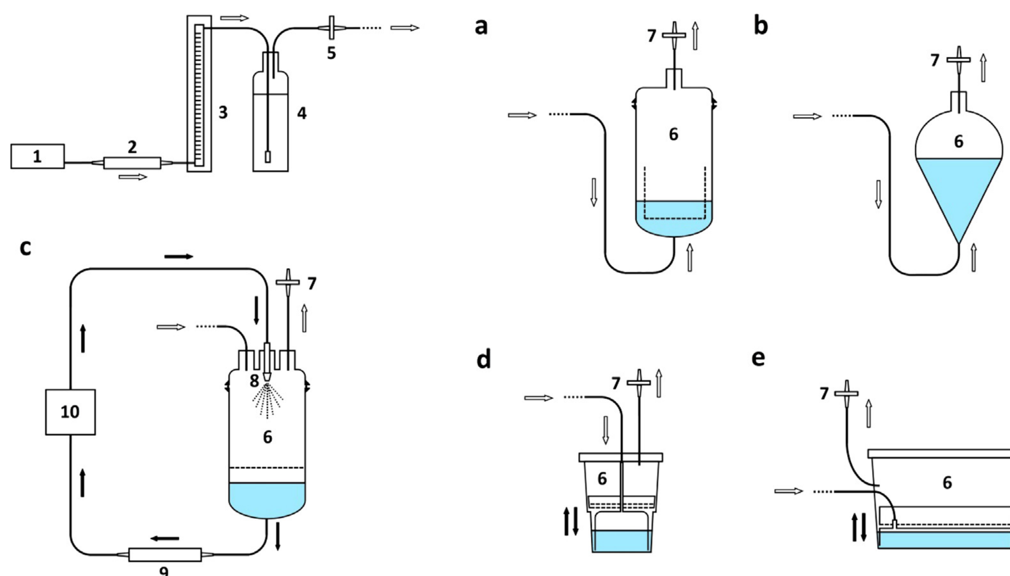

Fig. 1. Schematic diagrams of bioreactor systems employed in the study: a) bubble column bioreactor (BCB), b) balloon bioreactor (BB), c) spray bioreactor (SB), d) RITA® bioreactor and e) Plantform bioreactor. 1-air pump, 2-air prefilter, 3-flowmeter, 4-air humidifier, 5-inlet air sterilizing filter, 6-growth vessel, 7-outlet filter, 8-spray nozzle, 9-polypropylene fiber filter, 10-peristaltic pump. The elements 1–5 (left upper part of the figure) are common for bioreactors a–e. Dashed lines indicate stainless steel mesh (b and c) or perforated parts (d and e). White and black arrows indicate the flow direction of air and growth medium, respectively.

The aim of this work was to establish bioreactor cultures of *S. chinensis* shoots and evaluate their potential for lignan biosynthesis. The shoots were maintained in Murashige-Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 3.0 mg/l 6-benzyladenine (BA) and 1.0 mg/l 1-naphthaleneacetic acid (NAA) which was previously shown to provide best growth and lignan accumulation in *S. chinensis in vitro* cultures (Szopa et al., 2016b; Szopa and Ekiert, 2015, 2013). Since biomass growth and and secondary metabolite content depend strongly on the type of bioreactor used (Liu et al., 2003; Schumann et al., 2012 Zobayed et al., 2004a, 2004b), the experiments involved systems differing with respect to the way of medium application and aeration mode. *S. chinensis* shoots were maintained in liquid-phase, gas-phase and temporary immersion bioreactors, all regarded as suitable for differentiated plant cultures (Paek et al., 2005; Steingroewer et al., 2013; Towler et al., 2008). The harvested shoots were evaluated for biomass yield and lignan accumulation, in order to select the best system for dibenzocyclooctadiene lignan production and further scale-up studies.

2. MATERIALS AND METHODS

2.1. Plant material

The shoot cultures of *Schisandra chinensis* (Turcz.) Baill. (Schisandraceae), grown on agar-solidified MS medium supplemented with 3.0 mg/l BA and 1.0 mg/l NAA (further referred to as MS_{Sch} medium), were chosen for the experiments. The cultures were maintained as described previously (Szopa et al., 2016b; Szopa and Ekiert, 2011).

2.2. Bioreactor setup

The experiments included five bioreactors: three custom-made installations: bubble column bioreactor (BCB), balloon bioreactor (BB) and spray bioreactor (SB), as well as two commercially available temporary immersion systems: RITA® (fr. Récipient à Immersion Temporaire Automatique, VITROPIC, Saint-Mathieu-de-Tréviers, France) and Plantform (Plant Form AB, Lomma, Sweden). The schematic diagrams of bioreactors were presented in Fig. 1. In all systems, the air supply consisted of Optima air pump (Hagen, Montreal, QC, Canada), polypropylene fiber prefilter, flowmeter, air humidifier and inlet air sterilizing filter (PTFE membrane, 0.2 μm pore size, Cole-Parmer, Vernon Hills, US-IL). The bioreactors were aerated at 1 vvm (constant aeration for BB, BCB and SB, periodic (5 min every 90 min) aeration for Plantform and RITA®). The details of BCB (Fig. 1a), which was equipped with stainless steel mesh basket for biomass immobilization, were given previously (Jaremicz et al., 2014; Raj et al., 2015). The BB system (Fig. 1b) included the pear-shaped glass growth vessel (diameter: 120 mm, height: 180 mm), aerated from the bottom. The SB system (Fig. 1c) employed the same glass growth vessel as BCB, but the plant material was supported on the stainless steel mesh (aperture: 1 x 1 mm), positioned above the medium surface. The growth medium was pumped (Masterflex peristaltic pump, Cole-Parmer) successively through polypropylene fiber prefilter (diameter: 16 mm, length: 200 mm), 200 mesh stainless steel filter and dispersed onto shoots for 30 s at 10 min intervals (100 ml/min, TN 1.5 hydraulic nozzle, Spraying Systems Co, Wheaton, US-IL). The details of RITA® (Fig. 1d) and Plantform bioreactors (Fig. 1e) are available on manufacturers' websites. The immersion frequency, in temporary immersion systems, was 5 min every 90 min.

All bioreactors were steam sterilized (1 bar, 120°C, 20 min) before use. The working volume was 500 ml (BCB, BB, SB and Plantform bioreactors) or 200 ml (RITA® system).

2.3. Experimental procedures

S. chinensis in vitro shoots (acc. *2.1*) grown for 60 days on agar MS_{Sch} medium were used as inoculum for bioreactor experiments. All systems were inoculated at 3:100 (w/v) ratio (15 g of shoots per vessel in BCB, BB, SB and Plantform bioreactors; 6 g of shoots per RITA® container) and maintained for 30 and 60 days in a batch mode. Agitated cultures (3 g of shoots per 100 ml MS_{Sch} medium, 250 ml Erlenmeyer flasks with silicone sponge closures, Eppendorf INNOVA 2300 shaker operated at 120 rpm) grown for 30 and 60 days served as the control group (Szopa et al., 2016b). Harvested shoots and medium samples (40 ml) were freeze dried (Lyovac GT2 apparatus, Finn-Aqua-Santasalo-Sohlberg, Finland) and subjected to chromatographic analysis. All experiments were performed in four replicates. The cultures were grown under con-

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