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ORIGINAL RESEARCH PAPER

Guggulsterone production in cell suspension cultures of the guggul tree, *Commiphora wightii*, grown in shake-flasks and bioreactors

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Abstract Cell suspension cultures of *Commiphora wightii*, grown in modified MS medium containing 2,4-dichlorophenoxyacetic acid (0.5 mg l^{-1}) and kinetin (0.25 mg l^{-1}), produced ~5 μg guggulsterone g^{-1} dry wt. In a 2 l stirred tank bioreactor, the biomass was 5.5 g l^{-1} and total guggulsterone was 36 μg l^{-1}.

Keywords Cell culture · *Commiphora wightii* · Guggulsterone · Stirred tank bioreactor

Introduction

Guggulsterone (as its ***E***- and ***Z***-isomers) is an effective anti-hyperlipidemic agent obtained from the gum resin of guggul tree, *Commiphora wightii* (Abnott.) Bhandari (syn. *C. mukul*) (Dev 1999; Wang et al. 2004). Over-exploitation and slow growth of the tree, which is associated with poor seed set, make this plant an endangered species (Kumar and Shankar 1982; Kumar et al. 2003). The biotechnological production of guggulsterone has received attention as a promising alternative production source. Attempts have also been made to develop a micropropogation method through cloning (Barve and Mehta 1993) and somatic embryogenesis (Singh et al. 1997; Kumar et al. 2003) to increase the tree population. Guggulsterone is an effective antagonist of the bile acid receptor (Wu et al. 2002), farnesoid X receptor (Urizar et al. 2002) and a ligand-dependent transcription factor that regulates the expression of *CYP 7A1* gene involved in maintaining the cholesterol/bile acid homoeostasis through the bile salt export pump (Owsley and Chiang 2003).

Production of metabolites in cell cultures and development of scale-up technology require standardization of various growth parameters for optimal yield (Ramawat and Mathur 2007; Merillon 2007). Guggulsterone production in callus (0.09–0.22%) and cell cultures (0.18–0.32%) of *C. wightii* was reported (Phale et al. 1989) but without details of age of cultures, subculture period and procedure for guggulsterone extraction. The callus cultures raised in our laboratory contained 2–8 μg guggulsterone g^{-1} dry wt, which could be enhanced by morphactin up to 20 μg g^{-1} (Tanwar et al. 2007). This is the first report describing details of cell cultures grown in shake-flask and stirred tank bioreactor and production of guggulsterones in cell suspension cultures as influenced by growth and inoculum density.

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Materials and methods

Cell cultures

Cell suspension cultures of *C. wightii* were grown in modified Murashige and Skoog (1962) medium containing 2,4-dichlorophenoxyacetic acid (0.5 mg l^{-1}), kinetin (0.25 mg l^{-1}) and 3% (w/v) sucrose, referred to as CN4 medium with NH_4NO_3 at 825 mg l^{-1}, KNO_3 at 475 mg l^{-1} and $CaCl_2 \cdot 6H_2O$ at 220 mg l^{-1}. This medium was adopted from results obtained with callus cultures (Mathur et al. 2007). A 250 ml Erlenmeyer flask containing 100 ml medium was shaken at 100 rpm at 26°C in the dark. The subculture period and inoculum size were usually 15 days and 125 mg dry wt/ 100 ml medium (10% v/v), respectively. Growth and guggulsterone contents were determined periodically using vessels of different sizes and stirred tank bioreactor as given in the results. Six replicate flasks were used in each treatment and experiments were conducted twice. The details of bioreactor conditions are given in Table 2 (see below).

Guggulsterone extraction and HPLC analysis

The cell cultures were harvested after 15 days, the lyophilized cells (1 g) were finely grounded, extracted overnight with 25 ml methanol as described earlier (Tanwar et al. 2007). In brief, the methanol was evaporated under vacuum; the residue was extracted with ethyl acetate from which a sample was injected in HPLC after filtration through syringe filters (0.45 μm, 4 mm nylon filter). All the results are average of at least two separate analyses. Separation was accomplished on a 250 × 4 mm (C_{18} 5 μm) reverse phase column protected by a guard column of same material using a modified gradient (Tanwar et al. 2007) based on earlier method (Mesrob et al. 1998). The elute was monitored at 245 nm. ***E***- and ***Z***-Guggulsterones were obtained from Chromadex, U.S.A. and Natural Remedies, Bangalore, respectively.

Results and discussion

Cell cultures of *C. wightii* were established and scaled up to a 2 l bioreactor. Growth of the cell cultures and guggulsterone production was maximal after 15 days (Fig. 1). The ratio of the ***E***- and ***Z***-isomers was approximately constant at 4:1. Best results for cell growth and guggulsterone production was achieved using an inoculum of 10% (v/v) (see Table 1). This was then used in further experiments with cell being continuously harvested at 15 days as the optimum time.

An increase in the size of the flask and corresponding volume of medium decreased the biomass and total guggulsterone yield l^{-1} (Table 2). No guggulsterone was detected in the spent medium. The total guggulsterone content slightly increased in cells grown in a 2 l stirred tank bioreactor as compared to 2 l flasks. The decrease in biomass production was also associated with a low guggulsterone yield in the medium. The low biomass production might be due to low aerobic conditions in large vessels as vessels of all sizes were agitated at same rpm. About 60% increase in biomass production and about 20% decrease in guggulsterone production were recorded in the cultures during twelve months growth on the medium of same composition. For the initial six months, cultures were almost stable in terms of growth and guggulsterones production, thereafter an increase in growth was recorded.

The cultures of *C. wightii* are maintained on MS medium with varying salt (Mathur et al. 2007) and plant growth regulator combinations (Tanwar et al. 2007) to find out the best combination of growth medium. The apparent high guggulsterone

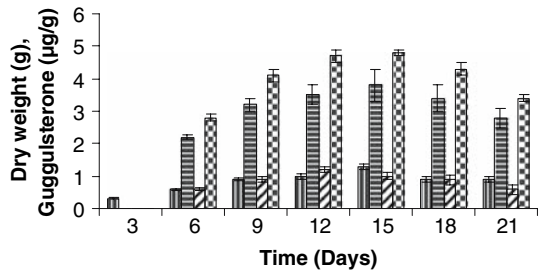

Fig. 1 Time course of guggulsterone production and growth (dry weight) in cell cultures of *C. wightii* grown in shake flasks containing CN4 medium up to 3 weeks. ▮ Dry weight, ▤ guggulsterone-E, ▨ guggulsterone-Z, ▪ total guggulsterone

Table 1 Effect of inoculum size on the growth and guggulsterone production in cell cultures of *C. wightii* grown in 250 ml flasks containing 100 ml CN4 medium

Inoculum (% v/v)	Culture (days)	DW (g ± SD)	Biomass productivity (g DW l^{-1} day^{-1})	Guggulsterone content μg g^{-1} ± SD		
				GS-*E*	GS-*Z*	Total
5	20	1.0 ± 0.07	0.5	2.9 ± 1.5	0.9 ± 0.01	3.8
10	15	1.3 ± 0.08	0.9	3.9 ± 1.0	1.0 ± 0.02	4.9
20	10	0.9 ± 0.07	0.9	3.4 ± 1.8	0.8 ± 0.02	4.2
30	8	0.8 ± 0.09	1.0	2.7 ± 0.9	0.9 ± 0.01	3.6

The cultures were harvested at different days when they become turbid (DW = dry weight, GS-E = guggulsterone **E**, GS-Z = guggulsterone **Z**, SD = standard deviation)

Table 2 Growth of *C. wightii* cell suspension cultures grown in CN4 medium in different sized vessels and 2 l glass stirred tank bioreactor (Inceltech, France) contained 1.5 l medium, 10% v/v inoculum and harvested at fifteen days growth

Vessel / medium (volumes)	Dry Wt Yield (g l^{-1} ± SD)	Guggulsterone content μg g^{-1} ± SD		
		GS-*E*	GS-*Z*	Total yield l^{-1}
250 ml/100 ml	13 ± 0.7	3.9 ± 0.1	1.0 ± 0.01	63.7
500 ml/200 ml	8.5 ± 0.22	3.6 ± 0.06	0.9 ± 0.02	38.2
1 l/400 ml	8.5 ± 0.4	3.9 ± 0.09	1.2 ± 0.02	43.3
2 l/800 ml	5.9 ± 0.4	4.1 ± 0.08	1.3 ± 0.02	31.7
2 l Bioreactor/1.5 l	5.5 ± 0.35	5.1 ± 0.09	1.5 ± 0.01	36.3

The bioreactor was set at 80 rpm agitation with 15% v/v/min aeration (Total yield l^{-1} = Total GS g^{-1} × DW l^{-1})

content recorded by Phale et al. (1989) might be due to density of other metabolites present in the extract used in spectrophotometric method. The HPLC method based on Mesorb et al. (1998) clearly separated the resin components. In several plant cell cultures, improvement in accumulation of metabolites with high cell densities has been recorded (Zhang and Zhong 1997, Wang et al. 1997), however the mechanism underlying this effect is far from clear. In the present work with cell cultures of *C. wightii*, increased vessel/medium volume and inoculum size grown in growth medium resulted in low net biomass and guggulsterone accumulation. The present results will be helpful in further deciding the medium and time period for cultivation of the cells in the growth and production media.

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