CHITOSAN ELICITS MONO-GLUCOSYLATED STILBENE PRODUCTION AND RELEASE IN FED-BATCH FERMENTATION OF GRAPE CELLS•

Maura FERRI, Annalisa TASSONI

Department of Experimental Evolutionary Biology, University of Bologna, Via Irnerio 42, 40126 Bologna I.
E-mail: maura.ferri@unibo.it

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1. INTRODUCTION

Plants are an abundant source for numerous food additives, pharmaceutical and nutraceutical compounds. *Vitis vinifera* cv 'Barbera' petiole liquid cell cultures were previously optimised and several stilbene elicitors were tested with the aim to improve stilbene production (Tassoni *et al*., 2005; Righetti *et al*., 2007; Ferri *et al.*, 2007; 2009). Stilbenes are a family of antioxidant compounds derived from resveratrol that have important nutraceutical, pharmacological and medical applications (Baur *et al*., 2006). Extensive studies were previously focused on resveratrol effects in cardio- and chemoprotection. More recently mono-glucosylated stilbenes, such as piceid and resveratroloside, have attracted great attention due to the fact that they are physiologically as active as free resveratrol but more stable and bioavailable after ingestion through diet.

In the present research, the optimal conditions of grape (*Vitis vinifera* cv 'Barbera') cell suspensions in batch and fed-batch bioreactor cultures were studied to specifically improve the production of mono-glucosylated stilbenes and the elicitor chitosan was tested.

2. MATERIALS AND METHODS

Vitis vinifera cv 'Barbera' petiole liquid cell cultures in laboratory scale (20 mL cultures in 200 mL jars) were previously optimised (Tassoni *et al.*, 2005) and 50 mg L^{-1} chitosan was added to elicit stilbene production (Ferri *et al*., 2009). Scale-up processes were performed and different bioreactor batch culture conditions were tested in 1 L (working volume of 800 ml) stirred tank bioreactor (Applikon Biotechnology). The best condition (MS medium with 30 g L^{-1} sucrose and 50 mg L^{-1} rifampicin, 40 g L^{-1} fresh weight inoculum concentration, 24 °C temperature, 0.2 L min^{-1} air flow, 100 rpm stirring rate with marine impeller, pH and dO_2% monitored and not adjusted) was selected for

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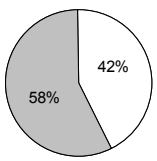
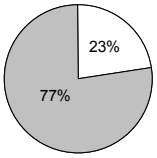
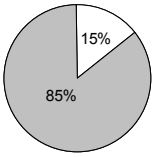
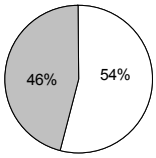
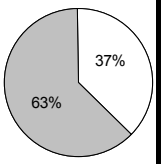
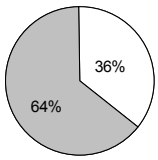
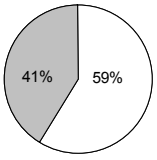
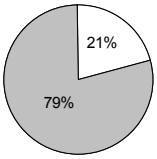
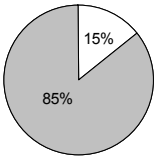
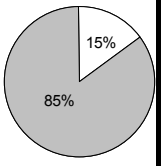
further fed-batch experiments (Ferri *et al*., 2010). Fed-batch fermentations were performed supplying fresh medium (with and without the addition of 50 mg L^{-1} chitosan) after 14 days of batch process (Ferri *et al*., 2010).

Polyphenols were extracted from biomass and culture broths and analysed by reverse-phase HPLC-DAD separation (Ferri *et al*., 2009).

3. RESULTS AND DISCUSSION

In the 'Barbera' cell suspensions in jar scale (Ferri *et al*., 2009), chitosan treatment slightly elicited stilbene production, in particular the accumulation of mono-glucosides, but reduced the percentage of total release in the medium (tab. 1).

Tab. 1 -. Stilbene production in five different culture processes. The yields (μmol gDW^{-1}) of stilbenes, the percentages of free and mono-glucosylated resveratrol and of endogenous and released levels, are reported at the day of maximum production of each process. DW, dry weight.

process	jar	jar + chitosan	batch	fed-batch	fed-batch + chitosan
maximum yield (μmol/gDW)	1.57	1.61	14.02	11.29	32.72
free resveratrol / mono-glucosylated resveratrol					
endogenous / released					

The scale-up from jars (20 mL) to bioreactor (800 mL culture volume) in a 14 days batch process, determined a 9-fold increase in the stilbene yield, with higher mono-glucoside production and total stilbene release (tab. 1). In fed-batch fermentation, notwithstanding a large biomass growth, stilbenes were a little reduced with respect to the batch process, while catechin accumulation was improved reaching its highest level of production (data not shown). The addition of 50 mg L^{-1} chitosan in fed-batch system (28 days of culture) determined a large increase of stilbenes, 63 % of which represented by resveratrol mono-glucosides. Of the total produced stilbenes, 85 % were released in the medium. The highest stilbene production was therefore reached in fed-batch bioreactor process supplied with chitosan with a maximum total stilbene yield of 32.72 μmol gDW^{-1} (day 28), equivalent to 48 mg L^{-1}. In the fed-batch cultures added with

chitosan, the catechin accumulation was reduced, with 90% of the total amount represented by epigallocatechin-gallate, one of the most active antioxidants.

4. CONCLUSIONS

The optimised bioreactor cultures of *Vitis vinifera* cv 'Barbera' allowed the accumulation of stilbenes and catechins. In fed-batch conditions, most of the produced polyphenols were represented by highly valuable compounds used for medical and pharmaceutical purposes, such as mono-glucosylated stilbenes and epigallocatechin-gallate.

Highest stilbene production was reached in fed-batch process supplied with the elicitor chitosan, demonstrating its efficacy in inducing stilbenes (both free resveratrol and mono-glucosylated derivatives) production also in bioreactor system. Moreover, most of the compounds were released into the culture media, this being a relevant advantage for the recovery of specific molecules or of polyphenol-enriched mixtures.

These results represent a further step toward the employment of grape cell cultures in fed-batch bioreactor, as a promising alternative to whole plant extraction, for the industrial production of plant polyphenols, also noting the necessity of developing suitable sustainable cultural processes.

Abstract

In the present study, the optimal conditions of grape (*Vitis vinifera* cv 'Barbera') cell cultures in batch and fed-batch bioreactor processes were studied to specifically improve the production of mono-glucosylated stilbenes.

With respect to jars, bioreactor system induced higher polyphenol yields and largely increased their release in the culture medium. The highest stilbene production was reached in fed-batch bioreactor process supplied with 50 mg L^{-1} chitosan, that improved the accumulation of both free and mono-glucosylated resveratrol derivatives.

Moreover, the vast majority of the compounds was released in the culture media, this being a considerable advantage for the bioreactor production and easier recovery of specific molecules or of polyphenol enriched mixtures as a promising alternative to whole plant extraction.

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