TAXANE PRODUCTION IN CELL SUSPENSION CULTURES OF *TAXUS GLOBOSA* (SCHLTDL)

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Introduction. *Taxus globosa* Schltdl, known as "Mexican yew", is the only species of the *Taxus* genus found in Mexico. The yew produces various taxanes, including the anti-cancer agent taxol and three of its precursors, which accumulate in the foliage (1). To date, *in vitro* production of taxanes in *T. globosa* has been low in comparison with other *Taxus* species, some cell lines yielding only traces (1) or no taxol at all (2).

To stimulate the *T. globosa in vitro* production of taxanes (taxol, BIII, DABIII, DAT, Ceph) and their excretion into the culture medium, we evaluated the effects of two nutritional culture media (WPM and B5) and elicitation with methyl jasmonate (MeJ) (100µM).

Methods. Using cell suspension cultures of *T. globosa* previously established by Dr. Osuna (3), we evaluated different inoculum sizes (50, 100, and 150 g FW/L) for an optimum growth. Cells were cultured in WPM for 30 d. The flasks were shaken (110 rpm) at 25 ± 2 ° C in the dark. The effect of two basal media, WPM and B5, on cell growth and taxane production, with and without MeJ, was evaluated. Cell suspensions were maintained in the above-mentioned conditions for 20 days. The measured variables were: fresh and dry weight, growth index, viability, final volume of the medium and HPLC quantification of taxanes in the biomass and culture medium.

Results. The *T. globosa* cells cultured in WPM (50 g FW /L) elicited with MeJ produced 4 of the 5 taxanes by intracellular synthesis. Additionally, although production in B5 medium was lower than in WPM, taxane excretion increased by 74% (Fig. 1 and 2).

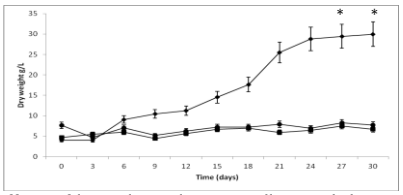

Fig 1. Effect of inoculum size on cell growth in *T. globosa* batch suspension cultures in WPM + PIC (2 mg/L), KN (0.1 mg/L) and GA_3 (0.5 mg/L). n=3±DE. p<0.0001*; Treatments: ♦ 50 g FW/L; ■ 100 g FW/L; • 150 g FW/L.

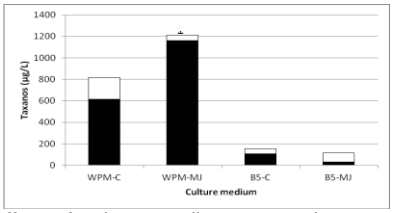

Fig 2. Effect of culture media WPM and B5 + 2,4-D (2 mg/L), KN (0.1 mg/L) and GA_3 (0.5 mg/L) (Control: WPM-C, B5-C) and MeJ elicitation (100µM) (WPM-MeJ, B5-MJ) on taxane production (■intra and □extracellular), in *T. globosa* batch suspension cultures. n=3±DE; p<0.0001*.

Conclusions. After determining the best inoculum size (50 g FW/L) for *T. globosa* cell growth in suspension culture, the best taxane production was achieved using WPM+MeJ and excretion to the medium was increased by B5+MeJ. As a result of this research, further experiments will be directed towards the use of two-phase batch culture to increase the production of taxanes with important anticancer activity.

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