Taxus globosa S. LC3 CELL LINE CHARACTERIZATION: GROWTH, PACLITAXEL and BACATIN III PRODUCTION

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Introduction. Taxane diterpenoids are characteristic molecules of *Taxus* spp. (yew trees). Paclitaxel and baccatin III are two outstanding molecules of this group, the first one an FDA approved antitumoral agent since 1992 and the second a natural precursor for paclitaxel semi-synthesis. In order to attend the supply of this anticancer drug and at the same time to preserve its natural source, several strategies have been developed, plant cell culture (1, 2) being one of them. Nowadays, this drug is mainly produced by Phyton Biotech using *T. baccata* cells and Samyang Genex *T. chinensis* cells. *Taxus globosa* S., the Mexican yew, has been reported (3) with one of the highest paclitaxel contents (400-500 μg/g dried needles) while *T. chinensis* needles exhibit the lowest (26 μg/g dried needles). For reproduction or enhancement of both paclitaxel and other important taxoid concentrations in *Taxus globosa* S., cell cultures imply challenges to any multidisciplinary research group. Our group started a research program establishing three *Taxus globosa* cell lines (4) and continues performing different biological-chemistry and engineeering studies on them.

The aim of this particular work is for *Taxus globosa* S. LC3 cell growth characterization, as well as paclitaxel and baccatin III production.

Methods. *T. globosa* LC3 cell line was aseptically subcultured each 10d in supplemented Gamborg B5 with 2x vitamins and 20g/L sucrose. Kinetics were made in 50mL Erlenmeyer flasks with 20mL of same media for 30d, 25° C, 120rpm, darkness, in duplicate. Dry weight was determined by gravimetric method. Extracellular products were extracted with acidified methanol (0.01% acetic acid) and quantified by HPLC (Alltima Phenyl Rocket column, 30°C, mobile phase: TFA 0.5M: Methanol: CH_3CN 50:19:31, 1.5mL/min, UV det. λ 230mn). Intracellular products were enzymatically released and then extracted with methanol and CH_2Cl_2 and finally quantified by HPLC.

Results. Figure 1 shows cell growth, adaptation phase ending around 8d. Maximum paclitaxel production (1.2015mg/L) appears during this phase around 1d, with 75% excretion, the second highest production appearing during exponential phase at 16d (0.9947mg/L), 70% excretion; baccatin III maximum production (0.7385mg/L) takes place on 28th day (stationary phase) with an excretion of 98%.

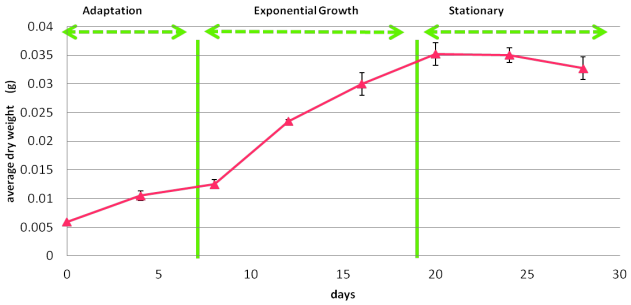

Fig.1 Dry cell growth from *T. globosa* S. LC3 suspension cell culture.

Table 1 presents kinetic parameters of *T.g.* LC3 cell line.

Table 1. *T. globosa* S LC3 cell line: paclitaxel and baccatin III production kinetic parameters.

t (d)	Bacatin III		Paclitaxel	
	$Y_{P/X}$ (g/g)	Q_P (mg/Ld)	$Y_{P/X}$ (g/g)	Q_P (mg/Ld)
1	0.00012	0.043	0.02906	1.201
16	0.00022	0.040	0.00055	0.0621
28	0.00025	0.026	0.00145	0.013

Conclusions. *T. globosa* S. LC3 cell line is able to produce baccatin III and paclitaxel, extracellularly rather than intracellularly, under the experimental conditions studied. The effect of larger inoculum sizes on these current findings needs to be studied.

References.

1. Majumder A., Jha S., (2009) *Ejournal of biotechnological sciences*. 1: 46-69
2. Expósito O., Bonfill M., Moyano E., Onrubia M., Mirjalili O., Cusidó R.M., Palazón J., (2009). *Anti-Cancer Agents in Medicinal Chemistry* **9:**109-121
3. van Rozendaal, E.L.M., Lelyveld, G.P. , van Beek, T.A. (2000). *Phytochemistry* 53:383-389
4. Barradas-Dermitz, D.M., Hayward-Jones P.M., Mata-Rosas M., Palmeros-Sánchez B., Platas-Barradas O.B.J., Velásquez-Toledo, R.F. (2010) *BIOCELL* 34(1):1-