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ARTICLE *in* BIOTECHNOLOGY PROGRESS · SEPTEMBER 2008

Impact Factor: 2.15 · DOI: 10.1021/bp0101583 · Source: PubMed

Improved Paclitaxel and Baccatin III Production in Suspension Cultures of *Taxus media*

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A cell suspension culture of *Taxus media* was established from a stable callus line of this species. The growth rate and production of paclitaxel and baccatin III of this cell suspension were significantly increased during the shake flask culture in its respective optimum media for cell growth and product formation, which were selected after assaying 24 different culture media. The highest yields of paclitaxel (2.09 mg L^{-1}) and baccatin III (2.56 mg L^{-1}) in the production medium rose (factors of 7.0 and 3.0, respectively) in the presence of methyljasmonate (220 μg g^{-1} FW). When the elicitor was added together with mevalonate (0.38 mM) and *N*-benzoylglycine (0.2 mM), the increase in the yields of paclitaxel and baccatin III was even higher (factors of 8.3 and 4.0, respectively). Thereafter, a two-stage culture for cell suspension was carried out using a 5−l stirred bioreactor running for 36 days, the first stage being in the cell growth medium until cells entered their stationary growth phase (12 days) and the second stage being in the production medium supplemented with the elicitor and two putative precursors in the concentrations indicated above. Under these conditions, 21.12 mg L^{-1} of paclitaxel and 56.03 mg L^{-1} of baccatin III were obtained after 8 days of culture in the production medium.

1. Introduction

Paclitaxel (NSC-125973), a secondary metabolite of the *Taxus* species, has been recognized as the best anticancer drug to have emerged in the past 20 years. Due to the low yield of paclitaxel in nature and the environmentally destructive prospect of large-scale harvesting of yew trees, alternative sources of paclitaxel have been sought. Its complex chemical structure means that total chemical synthesis is not considered economically feasible (*1*). However, semisynthesis of paclitaxel from more abundant taxanes, for example, via the conversion of baccatin III isolated from the needles of yew trees, has provided an immediate and renewable source of the drug (*2*). Alternatively, the yield of paclitaxel and its synthetically useful progenitors in *Taxus* cell culture systems, described in several reports (*3*−*7*), offers another possibility, but yields need to be improved.

Secondary metabolite production in plant cell cultures is a process which is not usually dependent on growth. This is the case of paclitaxel yields in *Taxus* cell cultures, where the production of this secondary product mainly takes place when the lineal growth phase has finished and the culture is in its stationary growth phase (*7*−*9*). In such cases, a two-stage culture system would seem to be adequate in order to stimulate secondary metabolite production. First, plant cells are cultured in a medium that has been optimized for their growth, and second, after the medium has been removed, the cell biomass then continues its growth in a production medium that mainly stimulates the biosynthesis of secondary metabolites. At the same time, this system has the advantage of permitting the addition of biosynthetic precursors and elicitors when the secondary metabolite production is at its highest, that is, during the second stage of the culture.

In this work, the combined effects on paclitaxel and baccatin III yields from a *Taxus media* (*T. media*) cell suspension of the above-mentioned two-stage culture, together with elicitation and the feeding of putative precursors, were examined in a 5−l stirred bioreactor. Previous to this, shake flask experiments were carried out to evaluate the effects of different culture media and treatments on cell growth and the production of both taxanes.

2. Materials and Methods

2.1. Establishment and Maintenance of the Cell Culture. The cell suspension of *Taxus media* was established from a stable callus line (the variances in taxane content and growth rate of each subculture were <10%, data not shown), as reported earlier (*7*). The cell suspension was maintained in Gamborg's B5 medium (*10*) supplemented with 3% sucrose, 0.01% *myo*-inositol, 2 × B5 vitamins, 4 mg L^{-1} of 2,4-dichlorophenoxyacetic acid (2,4-D), 1 mg L^{-1} of kinetin, and 0.1 mg L^{-1} of gibberelic acid (GA_3). Routine maintenance of the culture was performed in 175-mL flasks (Sigma VO633) by transferring 10 mL of 10-day-old culture (cells plus medium) to 10 mL of fresh medium. All flasks were capped with Magenta B-Caps (Sigma 38648) and incubated in the dark at 25 °C and 100 rpm in a shaker incubator (Adolf Kühner AG, Schweiz).

2.2. Development of Effective Growth and Product Formation Media. The first step in improving the

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10.1021/bp0101583 CCC: $22.00
Published on Web 05/02/2002

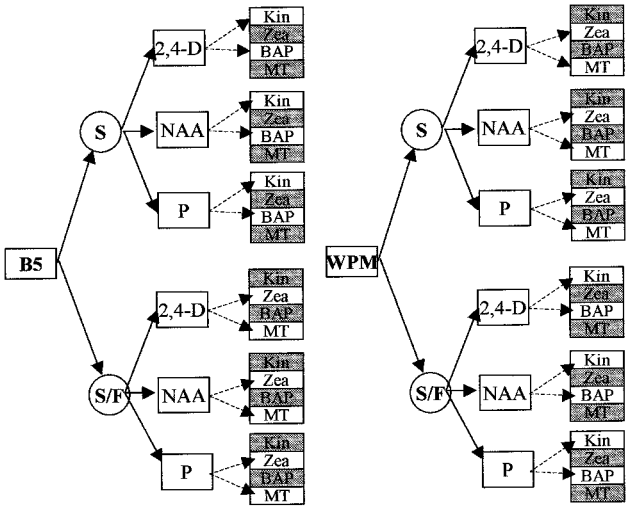

Figure 1. Schematic representation of the composition of 48 possible media resulting from the combination of 2 basal media (B5 and WPM) and 2 sources of sugar (S and S/F), 3 of auxin (2,4-D, NAA, and P), and 4 of cytokinin (Kin, Zea, BAP, and MT). As indicated by the broken arrows, the fractionary factorial design used allowed this number of media to be reduced to 24, with the cytokinins shown in the dark boxes being discarded. In all cases, the concentration of auxins was 2 mg L^{-1} and that of cytokinins 0.1 mg L^{-1}. S = 3% sucrose; S/F = 0.5% sucrose + 0.5% fructose; P = picloram; Kin = kinetin; Zea = zeatin; MT = *m*-Topolin.

productivity of the *T. media* cell suspension culture was to test the effects of different basal media [Gamborg's B5 and McCown's woody plant medium (WPM) (*11*)] and sources of sugar (3% sucrose and 0.5% sucrose + 0.5% fructose), auxin [2 mg $L^{-1}$of 2,4-D, 1-naphthaleneacetic acid (NAA), and Picloram, respectively] and cytokinin [0.1 mg $L^{-1}$of kinetin, zeatin, 6-benzylaminopurine (BAP), and *m*-Topolin, respectively] on growth and paclitaxel and baccatin III yield during the shake flask culture. Of the 48 possible culture media, only 24 were in fact tested. The selection, which is illustrated in Figure 1, was performed following a fractional factorial design based on an orthogonal array (*12*). For assays of all these culture media, cells were centrifuged at 1000*g* for 10 min and inoculated at a density of 100 g of cell fresh weight L^{-1} in 175-mL flasks containing 10 mL of medium and maintained in a shaker incubator at 100 rpm in the dark at 25 °C. Samples for analysis were taken after 24 days of culture. Our results (data not shown) revealed that McCown's WPM with 0.5% sucrose + 0.5% fructose, 2 mg L^{-1} of Picloram, and 0.1 mg L^{-1} of kinetin was optimum for cell growth (growth medium), while Gamborg's B5 medium (B5) with 3% sucrose, 2 mg L^{-1} of 2,4-D, and 0.1 mg L^{-1} of BAP (production medium) was optimum for the yield of both paclitaxel and baccatin III.

In a second series of experiments to evaluate and compare the effect of both of the selected culture media on cell growth and paclitaxel and baccatin III yield, cultivation in 175-mL flasks was performed as described, except that the samples for analysis were taken 2 days after subculturing and subsequently every 4 days over the culture period considered (24 days).

2.3. Treatments with Elicitors and Biosynthetic Precursors. Cells were cultivated for 24 days in a 175-mL flask containing 10 mL of the selected production medium in the same conditions indicated above. Inoculi were 1 ± 0.2 g of cells (fresh mass) harvested from a donor suspension grown for 12 days in the growth medium, which was the length of time necessary for it to enter the stationary growth phase (see section 3.1). The elicitors assayed were arachidonic acid (5 μg g^{-1} FW), methyljasmonate (220 μg g^{-1} FW), and vanadyl sulfate (81.5 μg g^{-1} FW). These concentrations were those previously established as optimum for paclitaxel biosynthesis by Ciddi et al. (*13*), Ketchum et al. (*14*), and Cusidó et al. (*7*), respectively. Elicitors were added to the cultures in 2.5 μL of ethanol/(mL of culture) (*6*). Equal volumes of ethanol were added to all cultures. With regard to biosynthetic precursors, we tested different concentrations of mevalonate (0.19, 0.38, and 0.76 mM) and *N*-benzoylglycine (0.1, 0.2, and 0.5 mM) all of which were previously dissolved in water. All compounds were sterilized by filtering through 0.22-μm sterile filters (Millipore) and added to the production medium prior to inoculation to give the final concentrations considered. For analysis, three flasks from each treatment were harvested at day 2 and then at 4-day intervals in the case of elicitors and at days 12 and 24 in the case of biosynthetic precursors.

2.4. Bioreactor Culture. The bioreactor used was a commercially available 5−l turbine stirred tank bioreactor (Applikon Dependable Instruments, Schiedam, The Netherlands). The culture was aerated through a sintered steel sparger. The flow was set at 0.8 L min^{-1} at the beginning of the experiment and then gradually increased up to 1.5 L min^{-1} and maintained at this level until the end of the culture period with a mass flow control system (Brooks, Veenendaal, The Netherlands). Exhaust gas was led through a glass condenser cooled by a cryostat (Lauda Messgeräte, Lauda, Germany) at 4 °C in order to minimize evaporation. The working volume was kept at 3.5-L culture medium, and the temperature, at 25 °C. In all cases the inoculum consisted of 100 g of cell fresh weight L^{-1}. To carry out the two-phase culture the bioreactor was kept running for 36 days. The inoculum cells were first cultured for 12 days in the growth medium, which as previously indicated is the length of time necessary for them to enter the stationary growth phase. After this, the medium was removed and the resulting cell biomass continued growing in the production medium for a further 24-day period either with or without the addition of the best elicitor/biosyntetic precursor combination for the improvement of paclitaxel and baccatin III yield, according to our previous shake flask culture results. To replace the growth medium with the production medium, the stirring action of the bioreactor was switched off to allow the cells to settle thus facilitating the operation. The production medium supplemented or not, as indicated above and sterilized by filtration, was added, and the process was repeated twice to ensure the total elimination of the previous medium. For analysis, samples of approximately 20 mL from three separate cultures were taken aseptically 6 h after subculturing (day 0) and subsequently every 4 days over the culture period considered.

2.5. Biomass Accumulation and Viability Assay. Fresh weight was determined by suction filtering of suspension cultures using Miracloth filters (Calbiochem, CA). Then the cells were washed with several volumes of distilled water to remove residual medium, frozen at −20 °C, and freeze-dried for 3−4 days. Dry weight and cell-associated paclitaxel and baccatin III were subsequently determined. Cell viability was followed using the method described by Duncan and Widholm (*15*).

2.6. Paclitaxel and Baccatin III Measurements. Paclitaxel and baccatin III were extracted from freeze-dried cells and culture media as described by Cusidó et al. (*7*). To quantify both taxanes in the different extracts, an indirect competitive enzyme immunoassay (CIEIA) method described by Grothaus et al. (*16*) was followed.

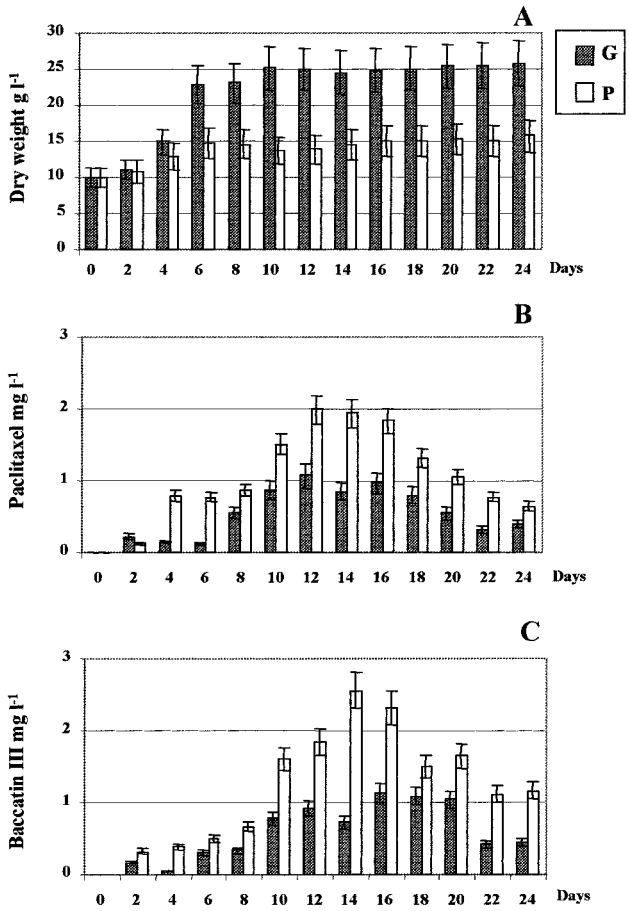

Figure 2. Time courses of biomass accumulation (A) and total yield (cell-associated + extracellular) of paclitaxel (B) and baccatin III (C), by a cell suspension of *T. media* cultured in a 175-mL shake flasks containing 10 mL of culture medium. In all cases, the inoculum consisted of 100 g of cell fresh weight L^{-1}. G = growth medium; P = production medium. Data represent average values from 3 replicates ± SD.

Paclitaxel and baccatin III protein coating antigen, anti-paclitaxel and anti-baccatin III monoclonal antibody, and the corresponding standards were obtained from Hawaii-Biotechnology Group.

3. Results and Discusion

3.1. Shake Flask Experiments. ***3.1.1. Cell Biomass and Taxane Yield in Growth and Production Media.*** The effects of the culture media giving optimum growth and paclitaxel and baccatin III yield in the *T. media* cell suspension cultured in shake flasks are shown in Figure 2. As mentioned in section 2.2, both media (growth and production) were selected after assaying 24 different culture media. In the *T. media* cells cultured in the growth medium (Figure 2A), the linear growth phase appeared to begin almost immediately and lasted for 10 days, and the stationary growth phase lasted for 14 days. A biomass production of 25.20 g of dry weight L^{-1} was measured on day 10, and a maximum of 25.80 g of dry weight L^{-1} on day 24. As can be deduced, this represented a growth rate of 0.25 day^{-1} at the end of the linear growth phase (which conditioned an inoculum doubling time of 7.8 days) and a growth rate of 0.11 day^{-1} at the end of the culture. These growth rates are quite good in comparison to reports of 17-day doubling time in cell cultures of *T. cuspidata* (*3*) or 14-day doubling time in cell cultures of *T. media* (*17*). In contrast, when the cell suspension

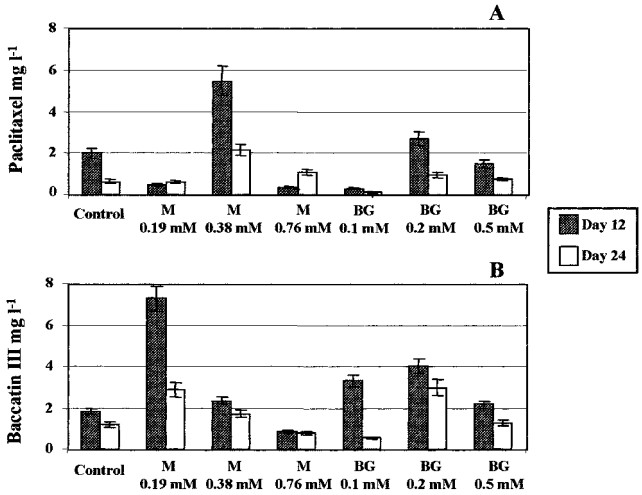

Figure 3. Comparison of total content (cell-associated + extracellular) of paclitaxel (A) and baccatin III (B) in cell suspension of *T. media* grown for 24 days in 175-mL shake flasks containing 10 mL of production medium with mevalonate or *N*-benzoylglycine and without these biosynyhetic precursors (control). In all cases, the inoculum consisted of 100 g of cell fresh weight L^{-1}. M = mevalonate; BG = *N*- benzoylglycine. Data represent average values from 3 replicates ± SD.

grew in the production medium (Figure 2A), although there was a slight increase with respect to inoculum cell biomass (<50%) during the first 6 days of the culture, after this point, the cells appeared to be entering the stationary growth phase. A biomass yield of 14.80 g of dry weight L^{-1} was reached on day 6 and one of 15.70 g of dry weight L^{-1} on day 24, which represented a growth rate of 0.19 and 0.05 day^{-1}, respectively. The lower growth rate after day 6 was not a consequence of cell lysis, since the viability percentage of cultured cells was more than 85% from day 10 to day 24 (data not shown).

When comparing total paclitaxel and baccatin III levels (cell-associated + extracellular) during the culture of the *T. media* cells in their respective growth and production media (Figure 2B,C), it can be seen that the most productive states in both media coincided on day 12 for paclitaxel and day 14 for baccatin III. The maximum yields of paclitaxel and baccatin III in the production medium were 2.09 and 2.56 mg L^{-1}, respectively. As can be deduced, in the case of paclitaxel the increase was twice as high in the production medium as in the growth medium, while in that of baccatin III the increase was more than 2-fold. These results show the suitability of the production medium for improving the yields of the taxanes considered in the conditions of our work.

3.1.2. Production Medium Modifications and Cell Growth and Taxane Content. The time course of growth and total paclitaxel and baccatin III content (cell-associated + extracellular) of the *T. media* cell suspension was followed for 24 days in the various treatments with the addition of elicitors and biosynthetic precursors to the production medium (see section 2.3). Compared to the control (in the production medium), cell growth was not significantly affected by the various supplements during the period of the experiment (data not shown). However, considerable differences in total paclitaxel and baccatin III content (compared to the control) were observed in the *T. media* cells grown in the production medium supplemented with the biosynthetic precursors mevalonate and *N*-benzoylglycine and with the elicitors arachidonic acid, methyljasmonate, and vanadyl sulfate. In the case of the biosynthetic precursors (Figure 3A,B), it was observed that although *N*-benzoylglycine at 0.2

mM was the most effective concentration tested for stimulating the biosynthesis of the two taxanes considered, mevalonate at 0.38 and 0.19 mM were the most effective concentrations in stimulating the biosynthesis of paclitaxel and baccatin III, respectively. The highest total paclitaxel content in the presence of 0.38 mM mevalonate (5.46 mg L^{-1}) was reached on day 12, though that observed on day 24 (2.16 mg L^{-1}) was still considerable. When compared with the control, these contents were 3.0-fold greater on day 12 and 3.3-fold greater on day 24. Regarding the total baccatin III content in the presence of 0.19 mM mevalonate, it was greater on day 12 (7.41 mg L^{-1}), being 4.0 times higher than in the control. In the presence of 0.2 mM *N*-benzoylglycine, the total content of paclitaxel content (2.69 mg L^{-1}) and of baccatin III (4.00 mg L^{-1}), both reached on day 12, was 1.4- and 2.2-fold greater, respectively, than the corresponding control values.

From the above results it may be inferred that the total content of both taxanes was affected not only by the nature of the biosynthetic precursor added to the production medium but also by its concentration and the growth stage of the cells. Results obtained by other researchers (*18*) corroborate the fact that each biosynthetic precursor has an optimum concentration for promoting the biosynthetic pathway in which it is involved. The stimulatory effect of *N*-benzoylglycine on taxane accumulation in *Taxus* cell cultures has been reported by Fett-Neto et al. (*3*), who suggested that this could be due to *N*-benzoylglycine hydrolysis, yielding a benzoyl moiety and a glycine residue. These components could lead to the synthesis of phenylalaline and/or benzoic acid. With respect to the stimulatory effect of mevalonate on paclitaxel and baccatin III content observed in our *T. media* cell cultures, it is understandable if we consider that these diterpenic compounds are formed through the traditional mevalonate pathway. However, [^{13}C]-labeling studies with higher plants have unambiguously established the existence of a nonmevalonate pathway for terpenoid biosynthesis (*19*). According to Eisenreich et al. (*20*), their studies on the paclitaxel biosynthesis show conclusively that the taxane ring system is not synthesized via mevalonate. This leads us to consider that, under the conditions of this experiment, the two previously mentioned metabolic pathways could be operative in the synthesis of paclitaxel and baccatin III. Previous work carried out by Lansing et al. (*21*) and Zamir et al. (*22*) showed that when *Taxus* plants are supplied with labeled mevalonate, high rates of radioactively labeled paclitaxel are obtained.

Elicitation was tested using arachidonic acid (5 μg g^{-1} FW), methyljasmonate (220 μg g^{-1} FW), and vanadyl sulfate (81.5 μg g^{-1} FW). As shown in Figure 4A, the maximum total paclitaxel content was 13.76 mg L^{-1} on day 18 in the presence of the assayed methyljasmonate concentration in the production medium, though those observed on days 20−24 were almost as high. These quantities of paclitaxel were significantly ($p < 0.001$, *t*-test) greater than the maximum achieved in the treatments with arachidonic acid (3.93 mg L^{-1} on day 24) and vanadyl sulfate (5.58 mg L^{-1} on day 20). Compared to the maximum total paclitaxel content in the untreated control (1.99 mg L^{-1} on day 12), the increase was up to 7-fold in the presence of methyljasmonate and up to 2-fold and 3-fold in the presence of arachidonic acid and vanadyl sulfate, respectively. As shown in Figure 4B, the maximum total baccatin III content was 7.10 mg L^{-1} on day 16 in the presence of methyljasmonate, being 2.82 mg L^{-1} in the presence of arachidonic acid on day 16 and

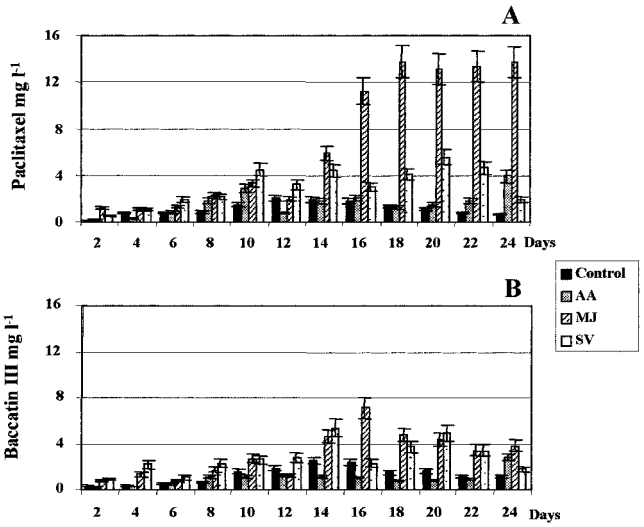

Figure 4. Comparison of total content (cell-associated + extracellular) of paclitaxel (A) and baccatin III (B) in cell suspension of *T. media* grown for 24 days in 175-mL shake flasks containing 10 mL of production medium with arachidonic acid (5 μg g^{-1} FW), methyljasmonate (220 μg g^{-1} FW), or vanadyl sulfate (81.5 μg g^{-1} FW) and without these elicitors (control). In all cases, the inoculum consisted of 100 g of cell fresh weight L^{-1}. AA = arachidonic acid; MJ = methyljasmonate; SV = vanadyl sulfate. Data represent average values from 3 replicates ± SD.

5.36 mg L^{-1} in the presence of vanadyl sulfate on day 14. Compared to the maximum total content of this taxane in the control (2.56 mg L^{-1} on day 14), it seems evident that only the presence of both methyljasmonate and vanadyl sulfate in the production medium significantly ($p < 0.001$) increased the yield of baccatin III.

Under the conditions assayed, it is clear that the addition of methyljasmonate (220 μg g^{-1} FW) to the production medium was the best single strategy for increasing the production of paclitaxel and baccatin III. However, the fact that the highest levels of both compounds were achieved in the latter phase of the culture suggested that the stimulatory action of this elicitor on the biosynthesis of the considered taxanes could not take place until the cells had formed sufficient precursors in response to its presence in the culture medium. For this reason, we tested the combined effect of adding methyljasmonate to the production medium together with the most effective concentrations of assayed biosynthetic precursors for increasing paclitaxel yield, i.e., mevalonate and *N*-benzoylglycine at 0.38 and 0.20 mM, respectively. As shown in Figure 5A,B, in the trials where methyljasmonate was combined with the indicated concentrations of mevalonate and *N*-benzoylglycine, the maximum total paclitaxel and baccatin III content (for both taxanes on day 12) was 15.72 and 10.38 mg L^{-1}, respectively. Although these increases were not very important with respect to previous trials with the elicitor alone (factors of 1.2 and 1.5, respectively), these contents of paclitaxel and baccatin III were achieved in a much shorter period of time (6 and 4 days earlier, respectively). It is of interest that the paclitaxel content of 15.72 mg L^{-1} obtained in this study by day 12 is among the highest concentrations of this taxane reported in recent years by academic laboratories. Hirasuna et al. (*23*) reported paclitaxel concentrations of up to 13 mg L^{-1} by day 27 in shake flask cultures of *T. baccata* cells, Hezari et al. (*2*) reported 18.7 mg L^{-1} by day 27 in shake flask cultures of *T. canadensis* cells and Yukimune et al. (*6*) reported 110.3 mg L^{-1} by day 14 in shake flask cultures of *T. Media*.

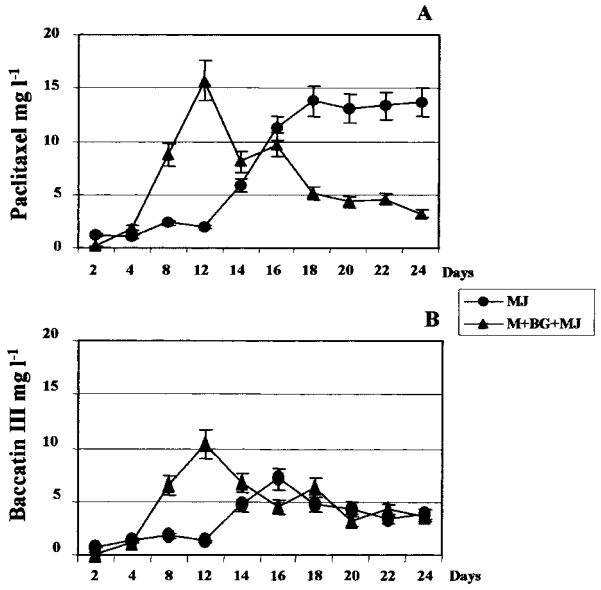

Figure 5. Comparison of total content (cell-associated + extracellular) of paclitaxel (A) and baccatin III (B) in cell suspension of *T. media* grown for 24 days in 175-mL shake flasks containing 10 mL of production medium without supplement (control) and supplemented with a combination of methyljasmonate (220 μg g^{-1} FW), mevalonate (0.38 mM), and *N*-benzoylglycine (0.20 mM). In all cases, the inoculum consisted of 100 g of cell fresh weight L^{-1}. MJ = methyljasmonate; M + BG + MJ = mevalonate + *N*-benzoylglycine + methyljasmonate. Data represent average values from 3 replicates ± SD.

3.2. Bioreactor Experiments. The cell biomass and taxane production values obtained during our shake flask experiments clearly showed that the growth medium used was suitable for cell biomass yield and the production medium was suitable for paclitaxel and baccatin III yield, and that, at the same time, a good way to improve the yield of both taxanes was to supplement the production medium with methyljasmonate (220 μg g^{-1} FW) together with mevalonate (0.38 mM) and *N*-benzoylglycine (0.20 mM). For this reason, a two-stage culture, the first stage being for cell growth and the second for paclitaxel and baccatin III yield, was carried out in a 5−l stirred bioreactor. *T. media* cells were cultured in growth medium for the first 12 days, which is when the stationary growth phase begins (according to previous results; see section 3.1). Subsequently, the medium was removed and the resulting cell biomass continued growing in the production medium for the additional 24 days under control conditions or supplemented with the elicitor and two precursors in the concentrations indicated above.

As shown in Figure 6A, under the control conditions, the cell biomass increased more than 2-fold during the 12 days in the growth medium, while the resulting biomass (19.13 g of dry weight L^{-1}) increased only 1.2-fold during the additional 24 days in the production medium, as can be deduced from the maximum value of biomass yield (24.17 g of dry weight L^{-1}) reached at the end of the 36-day culture period. It seems evident from these results that the growth of cells in the resulting cell biomass was significantly inhibited ($p < 0.001$) in the production medium, suggesting that their stationary growth phase began almost inmediately. Compared to the control, cell growth was barely affected by the addition of methyljasmonate (220 μg g^{-1} FW), mevalonate (0.38 mM), and *N*-benzoylglycine (0.20 mM) to the production medium. However, notable differences in total paclitaxel and baccatin III content (cell-associated + extracellular) were observed (Figure 6B,C). The highest total content

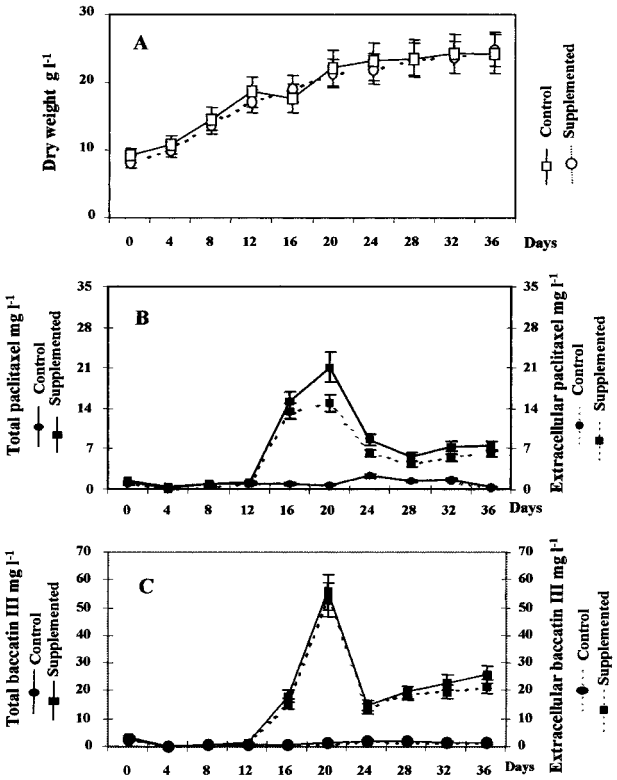

Figure 6. Time courses of biomass accumulation (A) and total (cell-associated + extracellular) and extracellular paclitaxel (B) and baccatin III (C) content by a two-stage culture of *T. media* cell suspension in a 5−l stirred bioreactor running for 36 days. The first stage was in the growth medium for 12 days, and the second stage was in the production medium without supplement (control) and supplemented with a combination of methyljasmonate (220 μg g^{-1} FW), mevalonate (0.38 mM), and *N*-benzoylglycine (0.20 mM). The cultivation parameters are given in section 2.4. Data represent average values from 3 replicates ± SD.

of paclitaxel (21.12 mg L^{-1}) and baccatin III (56.03 mg L^{-1}), both reached on day 20 (after 8 days of culture in the production medium), was 9- and 26-fold higher, respectively, in the supplemented trials. It should also be mentioned that the two biosynthetic precursors and the elicitor tested are relatively cheap compounds that provide a way to manipulate the production of paclitaxel and baccatin III by *Taxus* cell cultures without negatively affecting biomass production, which is one of the requisites for the commercial viability of a large-scale plant cell culture.

Unlike in previous work (*2*), under the conditions of our bioreactor experiments, paclitaxel and baccatin III were continuously secreted into the culture medium. *T. media* cells excreted an average of 94% paclitaxel and 97% baccatin III when their yield was maximum (from day 16 to 36). The excretion of both taxanes was not a consequence of cell lysis, since the viability percentage of cultured cells was as high as 85% throughout the experiment. The capacity of *Taxus* cells to excrete accumulated taxanes into the culture medium is an important factor since this accumulation may limit biosynthesis (*24*), possibly by means of a feedback inhibition mechanism. Moreover, it could be important for industrial application, since it facilitates downstream processing.

4. Concluding Remarks

Taken as a whole, our results show how the combined effect of the different strategies used in this work can

clearly stimulate the production of paclitaxel and baccatin III by a cell suspension of *T. media* and that this can be especially valuable in the bioreactor culture system. In this context, the fact that there was a greater accumulation of paclitaxel and baccatin III in the bioreactor culture than in the shake flask culture (factors of 1.4 and 5.4 respectively) when both culture types were in the highest productive state confirms the suitability of the selected growth conditions used in the bioreactor culture. It should be borne in mind that in the production processes using plant cell cultures, the scale-up from a shake flask to a stirred bioreactor has very often resulted in reduced productivities (*25*). The paclitaxel content of 21.12 mg L^{-1} obtained in this study by day 20 in the bioreactor culture exceeds that reported by academic laboratories for other *Taxus* species cultures in bioreactors where the highest values were 1.50 mg L^{-1} by day 28 (*5*), 2.94 mg L^{-1} by day 21 (*26*), and 3.00 mg L^{-1} by day 27 (*27*). With regard to the production of baccatin III, the highest total levels reached by our bioreactor culture (56.03 mg L^{-1} by day 20) greatly exceeded the 0.10 mg L^{-1} by day 21 reported by Vanìk et al. (*26*) for a cell suspension of *T. baccata* cultured in a 6−l bioreactor.

Acknowledgment

This research has been partly supported by two grants from the Spanish CICYT (95-0274-OP and BIO99-0503-CO-02). A.N.-O. is grateful for his research grant from CIRIT.

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Accepted for publication February 21, 2002.

BP0101583