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ORIGINAL PAPER

Influence of the KDEL signal, DMSO and mannitol on the production of the recombinant antibody 14D9 by long-term *Nicotiana tabacum* cell suspension culture

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Abstract We have established two transgenic cell suspension culture lines of *Nicotiana tabacum* that express the catalytic antibody 14D9 as a secretory product (sec-Ab) or as a KDEL-tagged product in the endoplasmic reticulum (Ab-KDEL), respectively. After 3 years of culture, the performance improved to a production level of 0.15 ± 0.03 μg ml^{-1} on the seventh day of culture for the sec-Ab line and 0.48 ± 0.05 μg ml^{-1} on the third day for Ab-KDEL line. Analysis of the effect of osmotic stress using mannitol (90 g l^{-1}) as an osmolite revealed that there was a 12-fold increase in antibody yield (1.96 ± 0.20 μg ml^{-1}) on the seventh day of culture in line sec-Ab and a fivefold increase (2.31 ± 0.18 μg ml^{-1}) on the seventh day for line Ab-KDEL. The concentration of the antibody in the culture medium was not significant. Dimethyl sulfoxide used as a permeabilizing agent was not effective in increasing 14D9 yield, but it did cause distinctive cell damage at all concentrations tested.

Keywords Molecular farming · 14D9 · In vitro plant cell cultures · Recombinant proteins

Abbreviations

DMSO	Dimethyl sulfoxide
DW	Dry weight
ER	Endoplasmic reticulum
FW	Fresh weight
GI	Growth index
μ	Specific growth rate

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Introduction

The ability of plant systems to produce complex recombinant proteins, such as antibodies, has been demonstrated in many previous works (Hellwing et al. 2004; Ma Julian et al. 2005; Mett et al. 2008). The advantages of plant systems to produce biopharmaceuticals include, among others, their capacity to manage post-translational modifications, the simplicity a culture system (which simplifies the downstream processing and reduces costs), the absence of animal or human pathogens (virus, prions, oncogenes) and the feasibility of performing the production process following good manufacturing practices and good laboratory practices. In earlier studies, we have demonstrated the ability of *Nicotiana tabacum* whole plants and hairy roots to express the immunoglobulin GI-like murine antibody, 14D9 (Martínez et al. 2005; Petruccelli et al. 2006). The 14D9 antibody is a catalytic antibody that participates in the hydrolysis of enol-ethers with high stereospecifity (Shabat et al. 1998). Here, we report the establishment of undifferentiated *N. tabacum* in vitro cultures (calli and cell suspensions) that produce 14D9 and the results of our analysis of the effect of the endoplasmic reticulum (ER) KDEL retention signal on 14D9 production. We also present the effect of the long-term maintenance in the in vitro culture on the growth pattern and antibody production of the cultured lines. Finally, we report on the influence of dimethyl sulfoxide (DMSO) and osmotic stress induced by mannitol on culture performance.

Materials and methods

Plant material

Seeds from both the transgenic and wild-type *N. tabacum* strains were kindly provided by Dr. S. Petruccelli (Universidad Nacional de la Plata, Argentina). The transgenic lines investigated here express the 14D9 antibody using two different sorting signals to target the assembled protein to the secretory pathway (sec-Ab) or to retain it in the ER (Ab -KDEL). In the sec-Ab version, the sorting signal is the Tobacco Etch Virus leader sequence; in the Ab-KDEL strain, the ER retention signal KDEL is added to the construct (Petruccelli et al. 2006).

We obtained the seedlings following procedures currently in use in our laboratory (Martínez et al. 2005). Once established, we chose those lines having the highest amounts of the antibody as a starting material for the calli cultures.

Calli culture

Pieces of leaves (1 cm^2) from *N. tabacum* seedlings were used as explants for starting the calli cultures. The explants were placed on MSRT solid medium (Alvarez et al. 1993) supplemented with 1-naphthaleneacetic acid (2 mg l^{-1}) and kinetin (0.2 mg l^{-1}) as plant growth regulators. The cultures were maintained at 24 ± 2°C under a 16/8-h (day/night) h photoperiod (irradiance: 13.5 mmol m^{-2} s^{-1}) with periodical subcultures to fresh medium at 4-week intervals. Each subculture was sampled to evaluate both the biomass and 14D9 production.

Cell suspension cultures

Fresh friable calli (2 g) were transferred to 150-ml Erlenmeyer flasks containing 40 ml of MSRT culture medium with the same plant growth regulator combination as used for the calli cultures. After 1 month, the suspension cell cultures were transferred to fresh medium at 20-day intervals using an inoculum of 1:5 of the total final cell volume and maintained, as is described above, in a rotary shaker at 100 rpm. The procedure has been performed for more than 3 years.

To study the kinetics of growth and antibody production, we placed an 8-day-old inoculum [approx. 2 g fresh weight (FW)] into 150-ml Erlenmeyer flasks containing 40 ml of the same culture medium as that described above. Triplicate samples for the analytical determinations were removed at 5-day intervals throughout the 30 days of culture.

Effect of DMSO

Once the behaviour of the culture in terms of growth and antibody production was established, we analysed the effect of DMSO on antibody production. sec-Ab and Ab-KDEL cell suspension cultures were transferred to Erlenmeyer flasks containing MSRT liquid medium, with or without the addition of 2.8 or 5.0% (v/v) DMSO on the 14th day of culture. Triplicate samples were removed at 5-day intervals up to the end of the batch culture. The flasks were incubated in a rotary shaker (100 rpm) under the same culture conditions as those described above.

Long-term expression of 14D9 in cell suspension lines

The parameters of growth and antibody accumulation in the biomass and in the culture medium were measured at the beginning culture initiation and after 3 years of culture maintenance to compare the behaviour of the respectively lines. Based on prior results (data not shown) we also performed a number of additional experiments to improve antibody yields. Lower concentrations of DMSO (1.5, 2.0 and 2.5% V/V DMSO) were tested on the third day of culture, and samples were taken at 5-days intervals until day 30. For the osmotic stress assay, mannitol was used as osmolite and added at the beginning of the culture at a concentration of 90 g l^{-1}. Samples were then taken at 3-day intervals for further testing.

Analytical methods

Estimation of cell growth

Biomass was estimated as FW and dry weight (DW). The cell suspension was filtered through a Whatman filter paper, under vacuum, and washed three times with distilled water to remove residual medium from the cell surfaces. The cells were then transferred to pre-weighed dishes and the total mass measured. Dry weight was estimated by transferring the harvested biomass to a drying oven at 60°C until a constant weight was achieved.

Growth index

We evaluated the growth index (GI) as the ratio of the final FW to the initial FW (Payne et al. 1991).

Protease activity

Briefly, 0.2 g of plant material was incubated in 1 ml of 10 mM buffer phosphate (pH 7.2), 2 mM cisteine and 5mM 2-mercaptoethanol at 4°C. The mixture was then centrifuged at 14,000 *g* for 20 min. The protease activity was measured in the supernatant according the technique described by Pena et al. (2006) and Vázquez and MacCormack (2002) using azocaseine as substrate.

Sucrose concentration

Sucrose measured in the culture medium according to the protocol of van Handel (1968). Briefly, 100 µl of the sample plus 100 µl of 30% NaOH was incubated in a water bath at 100°C for 10 min. Once the reaction had reached room temperature, 3 ml of anthrone solution was added, and the reaction volume was kept in a water bath at 40°C for 15 min. Absorbance was measured at 570 nm.

Biomass yield with respect to the carbon source

Biomass yield with respect to the carbon source (sucrose; $Y_{x/s}$) was determined as gram DW biomass per gram sucrose used.

14D9 Antibody analysis

To extract the antibody from the plant tissues, we first ground the samples using a cold mortar and pestle together with cold phosphate buffered saline (0.24 g KH_2PO_4 l^{-1}, 1.44 g Na_2HPO_4 l^{-1}, 0.2 g KCl l^{-1}, 8 g NaCl l^{-1}, pH 7.0–7.2) containing 10 µg leupeptin ml^{-1} and then centrifuged the mixture at 14,000*g* for 20 min at 4°C. The concentration of antibodies possessing both heavy (γ) and light (κ) chains were measure in the biomass extracts and in the culture medium using a sandwich enzyme-linked immunosorbent assay (ELISA). The ELISA test used goat anti-mouse antibodies specific for the γ and κ chains and a mouse immunoglobulin G (IgG) as standard (Sigma Chemical, St. Louis, MO). Only antibodies assembled into γ–κ chain complexes were measured. The ability of the antibodies to recognize the haptene was evaluated by direct ELISA and their integrity by Western blot. Western blot analysis was performed using goat anti-gamma mouse chain-conjugated peroxidase at 1:1,000 concentration (Southern Biotechnology, Birmingham, AL). The immune complexes were detected after incubation with Supersignal West Pico Chemiluminiscent Substrate (Pierce Chemical, Thermo Scientific Life Science, Rockford, IL). Total proteins were evaluated according to Bradford (1976).

Reverse transcription–PCR analysis

Aliquots (150 mg) of suspension cell cultures from the wild-type, sec-Ab and Ab-KDEL clones were ground using the RNeasy Plant Mini Kit (Qiagene, Hilden, Germany) procedure. The integrity and size distribution of the purified total RNA were tested by gel electrophoresis under denaturing conditions. cDNA synthesis was performed using Superscript II Reverse Transcriptase (Invitrogen, Carlsbad, CA) and random primers (Invitrogen). The following primers were used for the PCR assay: ScFv-G4-1FW (5′-CAAAGACAACCATGGTCACG-′3 and Sc-FvG4-1Rev (5′-CGACAGTGTCAGAGGACGAA-3′). The positive control was a plant extract expressing 14D9, and the negative control was an assay in which water was used as the template. The PCR cycling parameters were 30 cycles of 94°C for 4 min (melting), 45°C for 1 min (annealing) and 72°C for 3 min (elongation), followed by a final elongation step at 72°C for 7 min. Three replicates were made in all determinations, and an analysis of variance (ANOVA) was performed in each test.

Results and discussion

Production of the 14D9 antibody by *N. tabacum*

The KDEL signal (Lys-Asp-Glu-Leu) is a C-terminal amino acid sequence responsible for protein ER retention or retrieval. The allocation of proteins into the ER protected the molecule from the reducing environment of the cytoplasm. Fusion with the KDEL signal is currently a common strategy to enhance the stability and yield of recombinant proteins in transgenic plants, but the results are still variable in terms of antibody production. Petruccelli et al. (2006) demonstrated that, relative to the sec-Ab line, the Ab-KDEL line showed a twofold increase in the accumulation of complete antibodies in transgenic plant leaves and that the increased accumulation of Ab-KDEL in seeds was tenfold higher than that in leaves. In our study, we analysed the influence of the KDEL signal on the behaviour of *N. tabacum* cell suspension cultures that express the 14D9 antibody. Over the years, we have established more than 20 independent calli lines of wild-type and sec-Ab and Ab-KDEL transgenic tobacco plants. Among these, only a few have been able to express a functional, intact recombinant antibody, with the yields being measured each month for 1 year. To establish cell suspension cultures, for each line (sec-Ab and Ab-KDEL), we selected those clones showing the best performance in terms of growth properties and antibody yield and analysed the growth kinetics and antibody production in both cases. There was no significant difference between the transgenic and wild-type lines in terms of final biomass (approximately 0.4 g FW ml^{-}). However, significant differences were found for the specific growth rate (μ) ($p < 0.05$). The highest μ was observed in line Ab-KDEL (0.149 day^{-1}) compared to the wild type (0.086 day^{-1}) and sec-Ab line (0.069 day^{-1}). The doubling time was 8.05, 10.07 and 4.62 days for the wild type, sec-Ab and Ab-KDEL lines respectively (Fig. 1a, b). The GI was not significantly different between these lines. The higher specific growth rate seen in the KDEL-tagged lines has been attributed to the insertion site of the foreign gene into the plant genome (Kohli et al. 2003).

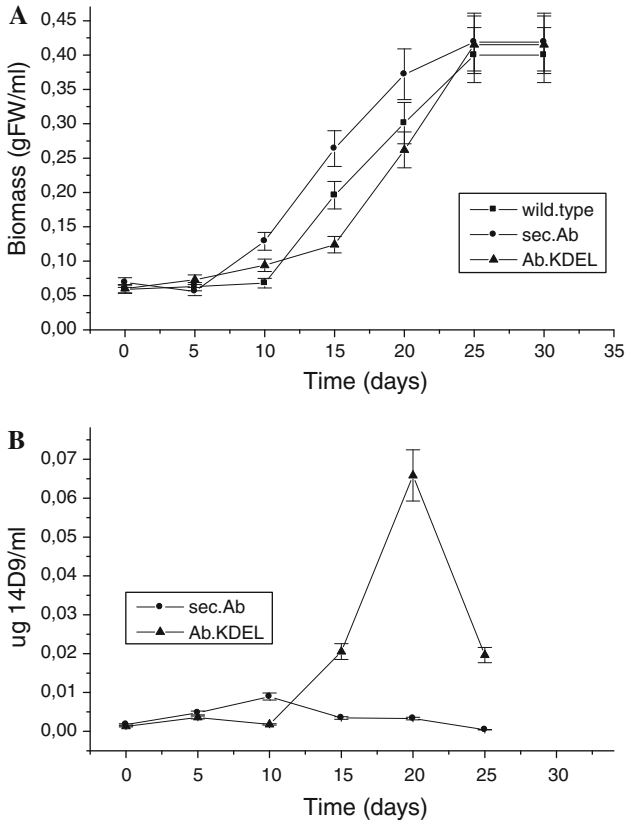

Fig. 1 Time course of growth (**a**) and antibody (**b**) accumulation into the biomass of wild-type (*filled square*), sec-Ab (*filled circle*) and Ab-KDEL (*filled triangle*) *Nicotiana tabacum* cell suspension cultures during a 30-day culture period. Culture conditions are as described in the Material and methods. Each *point* is the mean of three replicates ± standard deviation (SD). *sec-Ab* Transgenic *N. tabacum* line producing antibody 14D9 as a secretory product, *Ab-KDEL* transgenic *N. tabacum* line producing a KDEL-tagged 14D9 product in the endoplasmic reticulum, *FW* fresh weight

The content of 14D9 in the biomass increased up to day 10 in line sec-Ab and up to day 20 in line Ab-KDEL, with a sharp decline in antibody level after each peak in both cases. 14D9 yield was approximately fivefold higher in line Ab-KDEL than in line sec-Ab (0.066 ± 0.009 vs. 0.012 ± 0.002 mg ml^{-1}, respectively). The maximum antibody concentration calculated as a percentage of total soluble protein (%TSP) was 0.16 ± 0.02 for line sec-Ab and 0.62 ± 0.02 for line Ab-KDEL, which represents a 3.9-fold increase in line Ab-KDEL compared to line sec-Ab (Fig. 1b). This result is in accordance with the well-known stabilizing effect of the ER retention signal, as explained above. While the better productivity performance of the Ab-KDEL cell suspension line is in agreement with previous results in tobacco seeds and leaves (Petruccelli et al. 2006) and in hairy roots (Martínez et al. 2005), the antibody levels in the culture media in our study were in all cases not significant (data not shown). The profiles of growth and antibody production of both lines agree with our previous results on 14D9 production in hairy roots (Martínez et al. 2005). In terms of antibody integrity, the Western blots showed that both lines produced the 150-kDa 14D9-Ab assembly. Antibody functionality was tested by a direct ELISA. We did not identify any protease activity. The absence of 14D9 in the culture medium of line sec-Ab could be attributed to its retention in the apoplast, based on its molecular size, which is consistent with reports that secreted antibodies are preferentially localized in this organelle (Sharp and Doran 2001). The presence of whole antibodies in the media has been reported in some studies (e.g. de Wilde et al. 1996), while other authors claim that there is an exclusion limit for globular proteins of 40–60 kDa (Tepfer and Taylor 1981). However, the possibility that a different type of antibody or, more likely, a different type of protein would be retained by the cell wall due to chemical interactions cannot be excluded (de Wilde et al. 1996).

Influence of the addition of DMSO to the medium

The permeabilizing agent DMSO is commonly used for inducing the release of proteins from the cells into the culture medium. This process is achieved by destabilization of the membrane, which in turn induces the formation of pores in the membrane. It is surmised that proteins leak into the culture medium through these pores (Henderson et al. 1975; Wahl et al. 1995; Wongsamuth and Doran 1997; Doran 2006a). Our experiments clearly reveal that following the addition of DMSO on the 14th day of culture, and at all the DMSO concentrations tested, cell growth dropped almost immediately in the cell suspension cultures of both the sec-Ab and Ab-KDEL lines, and the cells ultimately died (Fig. 2). DMSO had not significant effect on the amount of antibody in the biomass (data not shown).

Long-term expression of the 14D9 monoclonal antibody in cell suspension lines

One of the most important features for of any biotechnological process is the stability of the productive lines (Huang and McDonald 2009). We studied the performance of our cultures over time for both growth behaviour and stability of 14D9-Ab production. Analysis of the growth pattern of each line (wild type and transgenics) revealed that it differed from that of 3 years previously (Fig. 3a). The growth kinetics of all three lines showed an exponential phase that started on day 3 of the culture, with establishment of the stationary phase around culture day 20. There was no significant difference in the μ in each line from that of 3 years previously. There was also no significant difference between the μ value of the wild type (0.098 day^{-1}) and that of the sec-Ab line (0.086 day^{-1}), the μ value of the former did differ from that of the

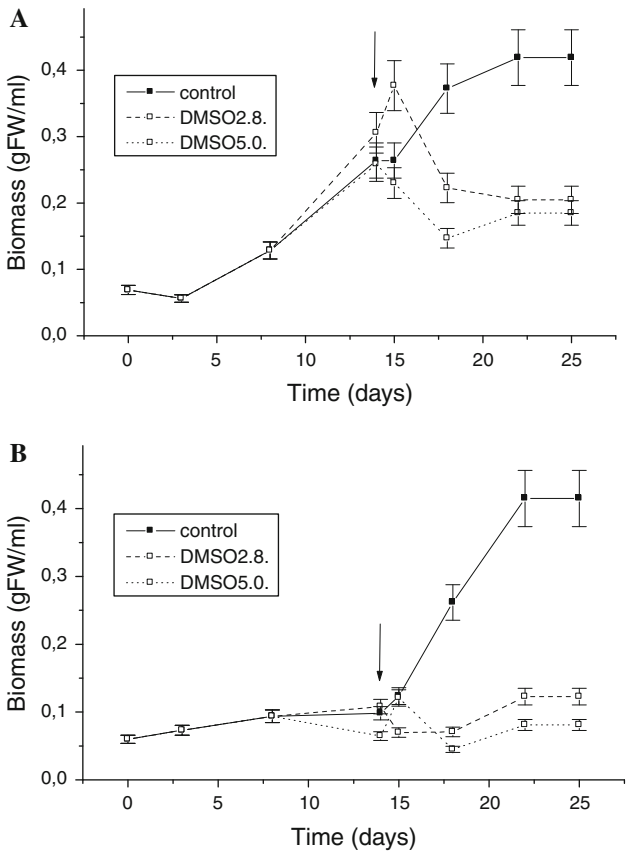

Fig. 2 Time course of growth of sec-Ab (**a**) and Ab-KDEL (**b**) *N. tabacum* cell suspension cultures following the addition of dimethyl sulfoxide (*DMSO*) at 2.8% v/v (*open square* + *broken line*) or 5% v/v (¨*open square* + *dotted line*¨) on the 14th day of culture. The cells were cultured for 30 days. Culture conditions are as described in the Material and methods. Each *point* is the mean of three replicates ± SD

Ab-KDEL line (0.123 day^{-1}). The biomass yield at the end of the culture was not significantly different between the three lines, nor was it different from the amount determined at the end of the batch culture period3 years previously. The $Y_{x/s}$, biomass yield with respect to the carbon source (sucrose) was 0.711, 0.419 and 0.467 g DW biomass g^{-1} sucrose for the wild type and lines sec-Ab and Ab-KDEL, respectively. These data are in accordance with the $Y_{x/s}$ previously determined in plant cells (normal levels: 0.3–0.7 g DW biomass g-^{1} sucrose; Payne et al. 1991). In contrast, the performance was different when the 14D9 levels in the biomass were analysed. In the sec-Ab line, 14D9 production presented a one-peak production profile, with the highest concentration (0.16 ± 0.03 μg ml^{-1}) reached on the 7th day of culture. In line Ab-KDEL, the maximum amount (0.48 ± 0.05 μg ml^{-1}) was reached on the third day of culture, which represents a threefold increase as well as a shift from production in the late exponential phase of growth to the earliest one compared with the result obtained 3 years previously (Fig. 3b). The production peak associated with the early phase of intensive growth is clearly evident, which is in accordance with the shortened lag phase. The whole phenomenon (increased antibody yield and shift of the production peak) could be attributed to the habituation of the cultures to the in vitro condition and to the systematic methodology followed to maintain them during a long-term period. The level of 14D9 in the culture medium was not significant.

Analysis of transgenic gene expression

The analysis of the transgenic genes (sec-Ab and Ab-KDEL) was carried out by reverse transcription (RT)–PCR. The integrity and distribution size of the total RNA obtained was checked by denaturing agarose gel electrophoresis as described in the Materials and Methods (Fig. 4a). The ribosomal bands obtained appeared as sharp bands (28S and 18S). RT using random primers was performed to obtain the mRNA for the detection of multiple species of cDNAs from the total RNA. The RT reaction was amplified with a PCR and appropriate specific primers.

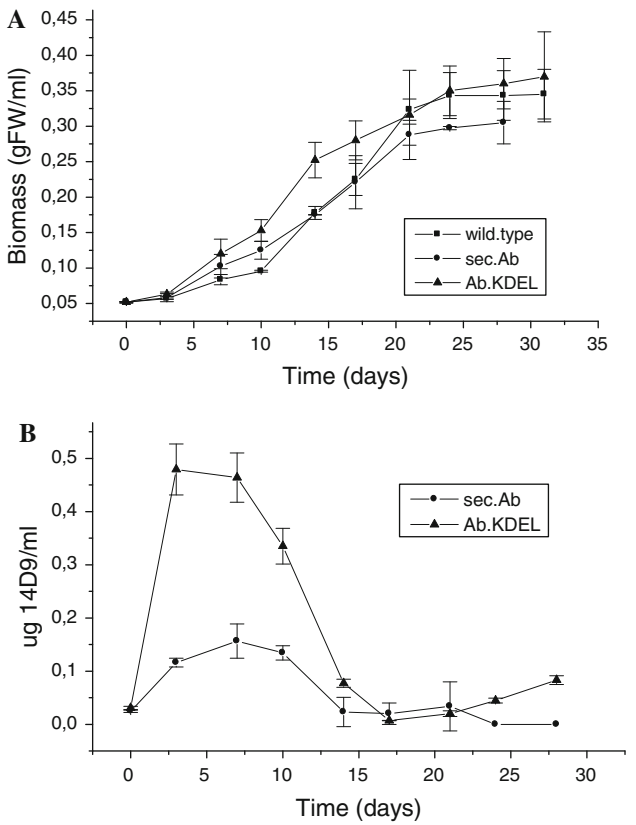

Fig. 3 Time course of growth (**a**) and antibody accumulation in the biomass (**b**) of wild-type (*filled square*), sec-Ab (*filled circle*) and Ab-KDEL (*filled triangle*) *N. tabacum* cell suspension cultures maintained for 3 years in in vitro culture. Culture was performed for 30 days under the conditions described in the Material and methods. Each *point* is the mean of three replicates ± SD

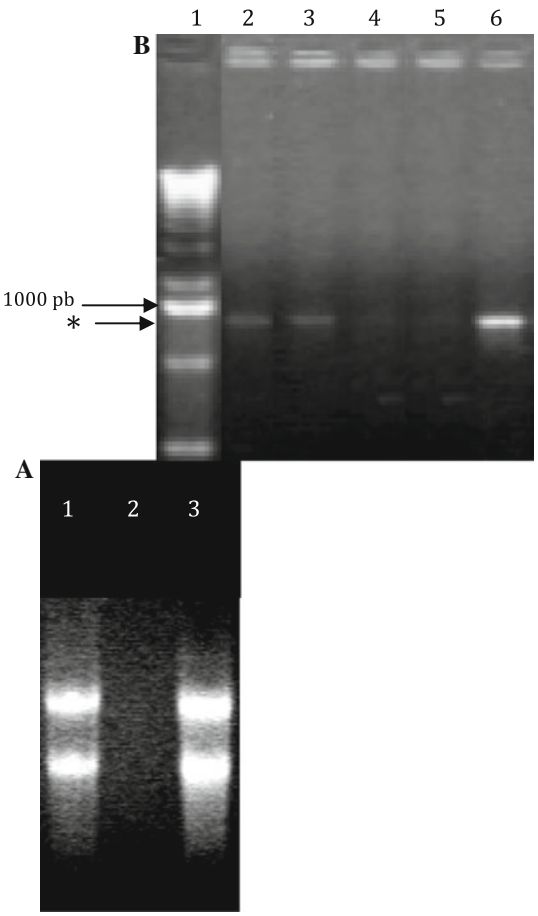

Fig. 4 RNA analysis of the wild-type (*WT*) and the transgenic lines sec-Ab and Ab-KDEL of *N. tabacum* cell suspension cultures. **a** Total RNA from transgenic lines sec-Ab (*lane 1*) and Ab-KDEL (*lane 3*). **b** Reverse transcription–PCR analysis of the total mRNA extracted from sec-Ab (*lane 2*), Ab-KDEL (*lane 3*), negative control (*lane 4*), wild type (*lane 5*), positive control (*lane 6*), 1-kb molecular marker (*lane 1 arrow*). *Asterisk* 14D9 molecular size

Figure 4b shows the amplification product from multiple samples and the positive control (lane 6). The molecular mass of this band was estimated to be 846 bp, corresponding to amplification of the 14D9 fragment in the transgenic lines. No bands were observed in the negative controls where water was used as template (lane 4).

Stability of IgG1 in the culture medium

We observed a sharp fall in antibody accumulation in both the cytoplasm and the culture media after each production peak, suggesting that an analysis of the stability of a commercial immunoglobulin IgG in the culture media would provide useful information. We added a standard IgG1 (Sigma I5381) into an Erlenmeyer flask containing 40 ml of a *N. tabacum* wild-type cell suspension and into a second Erlenmeyer flask containing only 40 ml of MSRT culture media. In both cases, the IgG1 disappeared from the culture medium within 24 h. This phenomenon was reported by Doran (2006a, b) in a study with a mouse monoclonal IgG_1 in transgenic tobacco suspension cells and hairy root cultures. Two hypotheses have been proposed: (1) that some component of the medium could have a deleterious effect on the IgG; (2) that the vessel walls, due to electrochemical forces, could adsorb the antibody molecule. In future experiments, we will analyse the possibility of coating the vessels in order to eliminate any possible adsorption effect.

Influence of osmotic stress

Osmotic stress in plant cells caused by the addition of mannitol to the medium has been reported to modify genetic expression and increase protein synthesis (Soderquist and Lee 2005). A minor protein-stabilizing effect has also been found (Wimmer et al. 1997). This led us to test the possibility that mannitol would be able to increase the amount of 14D9 in the biomass and, therefore, its yield. Figure 5 shows that at the concentrations tested in our study, mannitol had a negative influence on cell growth, producing a sharp fall in the μ in both transgenic lines (0.052 vs. 0.046 day^{-1} for lines sec-Ab and Ab-KDEL, respectively). Conversely, the final biomass reached at the end of the culture period was 0.074 and 0.092 g FW ml^{-1} for the sec-Ab and Ab-KDEL lines, respectively, which is remarkably lower than that of the controls (Fig. 5). The biomass yield relative to the carbon source was 0.5 ± 0.01 and 1.88 ± 0.22 g DW g^{-1} sucrose for the sec-Ab and Ab-KDEL lines, respectively, while total protein content was 2.2-fold higher in the sec-Ab line and 2.4-fold higher in the Ab-KDEL line (relative to the controls). Antibody production had increased by 12-fold (1.96 ± 0.20 µg ml^{-1}) in line sec-Ab and by fivefold (2.31 ± 0.18 µg ml^{-1}) in line Ab-KDEL (Fig. 5) by day 7 of culture. The western blot analysis reveals that the bands corresponding to 14D9 were more intense in cultures supplemented with the addition of mannitol (Fig. 5e, lanes 4 and 5). These results confirm our hypothesis of mannitol having a positive effect on 14D9 production and suggest that the fall in biomass was probably related to the stress caused by the osmolite, which also influenced protein synthesis. Measurement of the pH indicated a distinct acidification of the culture medium.

Influence of DMSO on optimized *N. tabacum* 14D9: producing in vitro cultures

We also tested the effect of DMSO at lower concentrations than those used in the first year of culture (1.5, 2.0 and 2.5% v/v), expecting that a lower level of DMSO would cause less cell damage. Taking into account the shift forward in the production of 14D6 in the optimized cultures, DMSO was added on the third day of culture instead of on

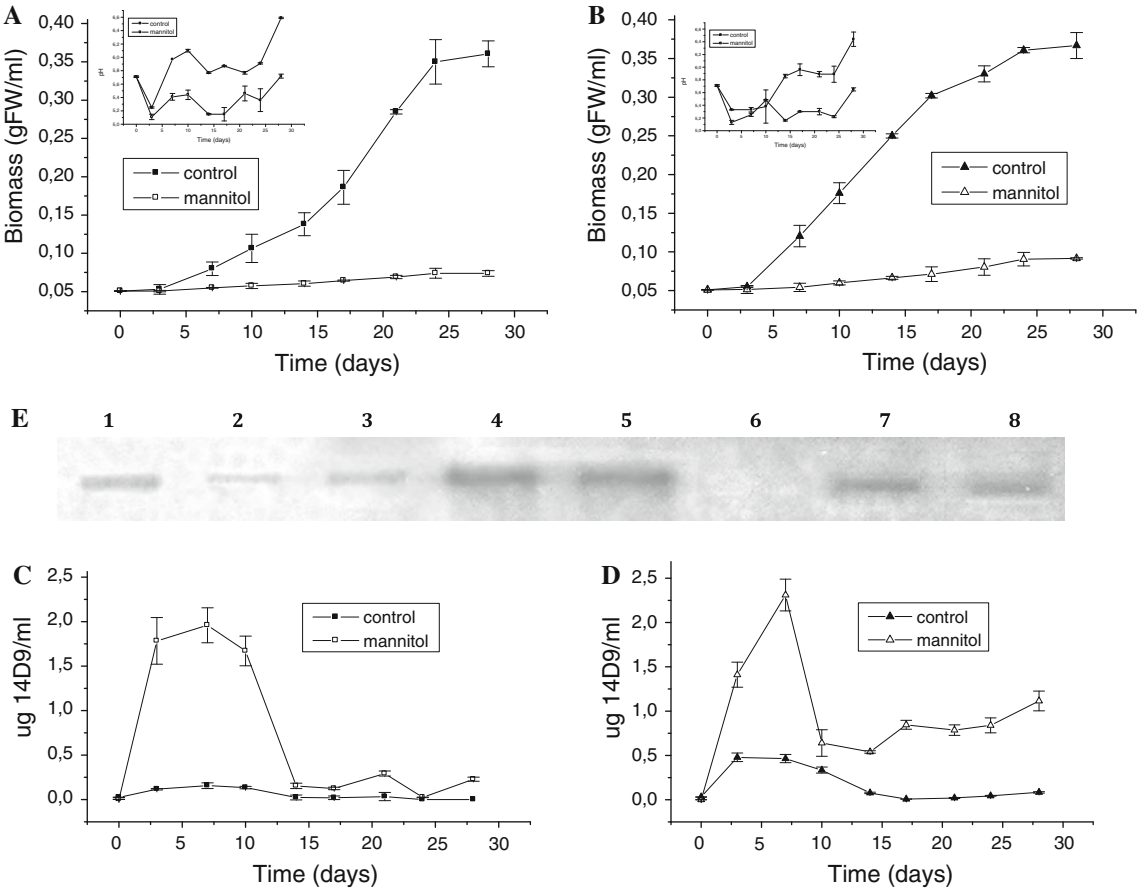

Fig. 5 Time course of growth (**a**, **b**), medium pH (inset) and antibody accumulation (**c**, **d**) in the biomass of sec-Ab (**a**, **c**) and Ab-KDEL (**b**, **d**) *N. tabacum* cell suspension cultures following the addition of mannitol (90 g l^{-1}) at the beginning of the culture period. Cells were cultured for 30 days under the conditions described in the Material and methods. Each *point* is the mean of three replicates ± SD. **e** Immunoblot analysis of cell suspensions (*lanes*: *2*, sec-Ab, *3* Ab-KDEL, *4* sec-Ab with 90 g/l mannitol, *5* Ab-KDEL with 90 g/l mannitol) leaves (*lanes*: *7* sec-Ab, *8*) Ab-KDEL) and *N. tabacum* wild type (*lane 6*). *Lane 1* 100 ng immunoglobulin G standard (Sigma I5381), antibody: anti-mouse immunoglobulin-specific serum, non-reduced 9% polyacrylamide gel

the 14th day. Even though the DMSO concentrations tested were lower, cell damage was evident. The very low antibody concentration detected in the culture medium is probably associated to that cell damage which causes antibody leakage. There was no significant difference in the 14D6 level in the biomass.

Conclusions

One remarkable finding of our study is that we determined that it is possible to optimize the performance of our *N. tabacum* cell suspension cultures through periodic subculturing at the exponential phase of culture. Doing this, we were able to establish cell suspension lines that have both a shortened lag phase and, in the case of the transgenic lines, earlier 14D9 production peaks. Also, antibody levels were higher in the optimized cultures, probably due to the habituation of the cultures to the in vitro condition. From a biotechnological point of view, these changes are beneficial to their use in a production process in bioreactors. We also demonstrated that the presence of the ER-KDEL retention signal, which protects the molecule from the oxidative environment of the cytoplasm, is as beneficial for the 14D9-Ab production by *N. tabacum* suspension cell cultures as it is in whole plants and hairy roots (Martínez et al. 2005; Petruccelli et al. 2006).

The commercial IgG added to the culture media disappeared from the system within a few hours, suggesting that the sharp fall in antibody that we observed 24 h after its production could be attributed to a phenomenon of adsorption by the vessel walls or to protein degradation produced by culture media components.

The addition of DMSO to the culture media did not have a beneficial influence on antibody yield, and its inhibitory effect on cell growth is evident.

The effect of the osmotic stress on antibody yield was markedly improved by the addition of mannitol to the

medium at a concentration of at 90 g l^{-1}, with a maximal 14D9 yield in the Ab-KDEL transgenic line (0.48 mg l^{-1}). This result is lower than those reported by Zheng et al. (2003) in recombinant *Escherichia coli* expressing the 14D9 single chain Fv (3 mg l^{-1}).

In future studies, we will analyse the combined effect of osmotic stress and permeabilization on antibody production.

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