Microbial Cell Factories

Poster Presentation

Characterization of *Medicago truncatula* cell suspension cultures producing valuable recombinant proteins

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Background

Nowadays, the use of plants for large-scale production of recombinant proteins is gaining wider acceptance because of their many practical, economic and safety advantages, compared with traditional microbial and animal production systems.

However, production systems that use whole plants to express recombinant proteins may lack several of the intrinsic benefits of cultured cells, such as the precise control over growth conditions. Plant cell cultures may combine the merits of plant systems with those of microbial and animal cell cultures [1]. Optimization of environmental conditions in culture could be used to enhance foreign protein synthesis and stability, reducing the total cost of protein production. Moreover, a great advantage of synthesizing recombinant proteins in plant cell cultures is the very simple procedure of product purification, especially when the product is secreted into the liquid culture medium. As well as the potential commercial benefits, in vitro systems also represent an important tool for studying the process of foreign protein synthesis, assembly, secretion and turnover in plant cells and tissue.

Results

Recently, we have proposed the legume model plant *Medicago tuncatula* as a promising production system [2]. In this work, specifically, several suspension cell lines were established from transformed *M. truncatula* plants expressing two valuable recombinant proteins from different sources, human Erythropoietin (EPO) and fungal phytase. For both proteins two versions were available, one where the protein is secreted and the other where it is retained in the Endoplasmic Reticulum (ER).

Callus induction was achieved through incisions using a sharp razor blade (perpendicular to the mid-vein of the folioles) and the abaxial side of the folioles was maintained in contact with the medium with appropriate growth regulators. Calli were kept at 23°C in the dark on solid media for approximately two months. When calli reached the appropriate size, cells were transferred to Erlenmeyer flasks with shosen medium, kept with agitation in the dark at 24°C, and subcultured to fresh medium every week (A.S. Pires, unpublished results).

Identification of recombinant EPO or phytase was performed by Western blot analysis, showing that different cell suspension lines exhibit different expression levels of recombinant protein. Interestingly, we observed that in some cell lines with higher expression levels of the secreted version of recombinant protein, part of it is not being secreted to the medium. On the other hand, in suspension cells generated from transgenic plants engineered to produce recombinant protein targeted to the ER, the proteins are not being totally retained. These results are probably related with stress imposed to the cell by high expression levels of a foreign protein.

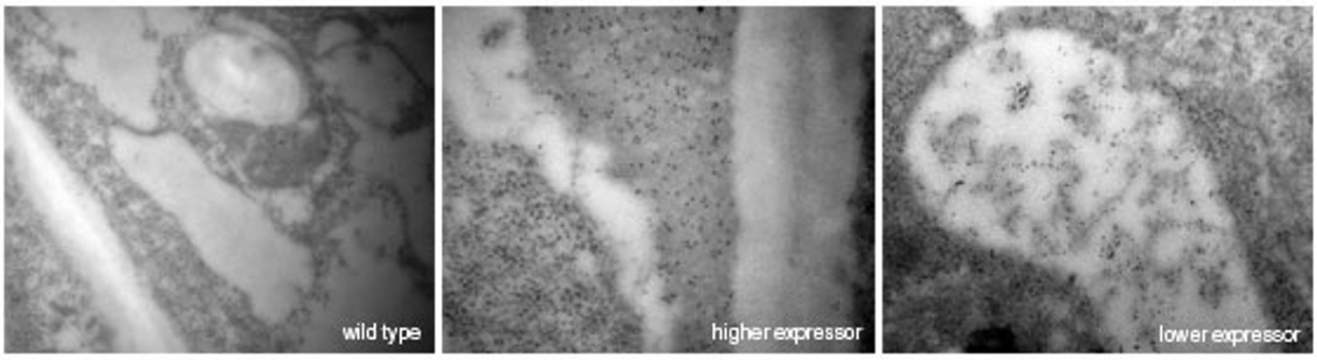

Figure 1
Electron microscope images of subcellular localization of recombinant phytase in plant cells harvested from three different suspension cell lines established from *M. truncatula* plants: a wild type, a higher expressor and a lower expressor of this recombinant protein.

Preliminary immunolocalization analysis, using electron microscopy, revealed that in those cell lines with different expression levels, the subcellular localization of the recombinant protein is significantly different as well as the subcellular structure itself. As we can see in Figure 1, in phytase higher expressor cells, anti-phytase signal is located on unidentified structures under the cell wall but in the lower expressor cells the labelling is concentrated in apparently lytic vacuoles. We hope these studies will help us to answer the following question: is it the cell morphology that determines the subcellular fate of the product or the opposite?

In order to compare the obtained results with other plant based systems, leaves from transgenic *M. truncatula* original plants and transgenic BY2 Tobacco Suspension Culture expressing the same recombinant proteins were also analysed.

Conclusion

Similarly to *M. truncatula* leaves [2], results obtained for cell suspension cultures established from this plant also indicate that this alternative could be highly suited for the production of recombinant proteins, with all the advantages widely recognized for plant cell cultures. Further studies, including the systematic analysis of pos-translational modifications of the recombinant proteins produced by this system, are still required to improve its potential to compete with other expression platforms for production of valuable recombinant proteins.

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