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Research review paper

Bioreactor systems for *in vitro* production of foreign proteins using plant cell cultures

Ting-Kuo Huang, Karen A. McDonald *

Department of Chemical Engineering and Materials Science, University of California, Davis, One Shields Avenue, Davis, CA 95616, United States

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ABSTRACT

Plant cells have been demonstrated to be an attractive heterologous expression host (using whole plants and *in vitro* plant cell cultures) for foreign protein production in the past 20 years. In recent years *in vitro* liquid cultures of plant cells in a fully contained bioreactor have become promising alternatives to traditional microbial fermentation and mammalian cell cultures as a foreign protein expression platform, due to the unique features of plant cells as a production host including product safety, cost-effective biomanufacturing, and the capacity for complex protein post-translational modifications. Heterologous proteins such as therapeutics, antibodies, vaccines and enzymes for pharmaceutical and industrial applications have been successfully expressed in plant cell culture-based bioreactor systems including suspended dedifferentiated plant cells, moss, and hairy roots, etc. In this article, the current status and emerging trends of plant cell culture for *in vitro* production of foreign proteins will be discussed with emphasis on the technological progress that has been made in plant cell culture bioreactor systems.

Contents

* Corresponding author. Tel.: +1 530 752 8314; fax: +1 530 752 1031.
E-mail address: kamcdonald@ucdavis.edu (K.A. McDonald).

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1. Introduction

Biomanufacturing of heterologous protein products has gained increasing importance due to the expanded market demand for therapeutic and industrial applications leading to the development of alternative safe and cost-efficient expression platforms and emerging technologies (Hacker et al., 2009; Karg and Kallio, 2009). At present, several gene expression systems have been developed for foreign protein production. Each system offers distinct features consisting of protein expression enhancement, ease of genetic manipulation, and protein quality (Durocher and Butler, 2009). In the past decade, the plant cell-based expression platform as a bioreactor system (whole plants and *in vitro* plant cell, organ and tissue cultures) have been investigated as a potential alternative for the large scale production of foreign proteins including plant-made pharmaceuticals (PMP) and plant-made industrial products (PMIP) (Davies, 2010; Sharma and Sharma, 2009). Plant cell bioreactors provide attractive features over traditional expression systems that utilize microbial and mammalian host cells, including their intrinsic safety (plant cells do not propagate mammalian viruses and pathogens and can be easily grown without any animal-derived components which are important considerations for therapeutic applications), cost-effective biomanufacturing that leads to lower production costs (Shadwick and Doran, 2005), and the capability for post-translation modifications (ability to produce glycoproteins and complex multimeric proteins with similarity to their native counterparts in terms of *N*-glycan structure compared to mammalian cells) (Gomord et al., 2010). The features of various host cell-based protein expression systems have been discussed extensively (Demain and Vaishnav, 2009; Yin et al., 2007).

Compared to the use of whole plants as a production platform, *in vitro* plant cell cultures (suspended plant cells, tissue and organ cultures, etc.) grown in controllable bioreactor systems offer additional features for economical, sustained foreign protein production (Hellwig et al., 2004) such as 1) shorter production cycles in that the timescale for foreign protein production in plant cell culture requires days or weeks compared to months in transgenic plants; 2) more consistency in batch-to-batch product yield, quality and homogeneity of the target protein N-glycan pattern due to the homogeneous culture of plant cells under controlled bioreactor conditions (De Muynck et al., 2010; Lienard et al., 2007); 3) cheaper and simpler downstream recovery and purification particularly for products secreted into the extracellular medium and lower secondary metabolite and host cell protein concentration (Rawel et al., 2007); 4) elimination of the need for intensive labor for cultivation of greenhouse or field-grown plants; 5) reduced contamination from endotoxin and mycotoxin; 6) safer production platform in a closed bioreactor system, avoiding gene flow in the environment and contamination of the food chains (Franconi et al., 2010), and 7) ease of compliance with cGMP requirements and product registration process, etc.

Remarkable progress for improving foreign protein production yield and quality through bioreactor engineering (Huang and McDonald, 2009) and genetic engineering approaches (Desai et al., 2010; Streatfield, 2007), such as enhanced transgene transcription, post-transcription stability, and translation efficiency, has demonstrated that *in vitro* plant cell culture is become an enabling technology for biomanufacturing foreign proteins. In this article, the recent technological progress in bioreactor-based plant cell cultures for *in vitro* production of foreign proteins will be discussed.

2. Foreign protein production using *in vitro* plant cell culture

Currently, foreign proteins including therapeutic proteins (antibodies, antigens, vaccines and human blood proteins, etc.), specialty proteins (e.g. gelatin and collagen for drug capsules), and industrial enzymes (such as cellulases and lipases for biofuel applications) can be expressed in plant cell cultures, including dedifferentiated plant cells (such as suspended tobacco, rice, and carrot cells, etc.) and differentiated plant tissue and organ cultures (such as moss and hairy root, etc.) (Holland et al., 2010). An ideal plant cell culture bioreactor system for large scale foreign protein production should exhibit the following features: 1) ease of genetic manipulation by either stable transformation or transient expression, 2) high protein expression level, 3) low endogenous proteolytic activity, 4) high product stability in the heterologous expression environment (inside and outside of the cells), 5) low concentration of secondary metabolites (may cause changes in protein structural and biological properties and/or complicate downstreaming processes), 6) post-translational modification capability, uniform glycosylation pattern and proper protein folding, and 7) homogeneous dispersion in a bioreactor, etc. Table 1 shows examples of foreign protein expression by stably transformed plant cell culture systems. In this section, the current status and characteristics of different types of bioreactor-based plant cell cultures for foreign protein production will be discussed.

2.1. Foreign protein production in suspended dedifferentiated plant cells

Dedifferentiated plant cell suspension cultures, driven by a variety of gene expression systems, are commonly used for foreign protein production (Huang and McDonald, 2009; Lico et al., 2008) due to the fact that they are more amenable to cGMP regulations and can be cultivated and optimized easily in large scale bioreactors (Shih and Doran, 2009). Plant cell suspension cultures are usually derived from stably transformed plant tissues by *Agrobacterium*-mediated transformation. Callus cells initiated from explants from transgenic plants can be grown in a chemically defined media to establish transgenic cell suspension cultures (Rao et al., 2009). Generally the addition of plant growth regulators is required in the medium to promote rapid cell growth and maintain cell morphology.

Recently Protalix Biotherapeutics in Israel and Pfizer in the US announced a collaboration to develop and market the plant-made recombinant glucocerebrosidase (prGCD) using a transgenic carrot suspension cell culture bioreactor platform as a biologic therapeutic protein drug for the treatment of Gaucher's disease in EU and USA (Ratner, 2010). This represents an exciting milestone for recognizing plant cell culture-based biomanufacturing as a bio-equivalent and economical alternative to mammalian production of human biopharmaceuticals, further suggesting the possibility of biosimilar products for existing protein drugs. In another promising development, a recombinant animal vaccine against Newcastle Disease Virus (NDV) produced in transgenic tobacco cell cultures by Dow Agrosciences was approved by USDA in February 2006 (Travis, 2008). Both of these represent breakthroughs for developing plant cell culture as a biomanufacturing platform for large scale production of foreign protein products.

Protalix Biotherapeutics has developed a transgenic carrot suspension cell culture platform (called ProCellEx™) for prGCD production for patients with the genetic disorder Gaucher's disease, a rare lysosomal disease, who are not able to degrade glucosylceramides in the body due to the fact that GCD is absent or nonfunctional (Shaaltiel et al., 2007). Currently, patients are treated with either Ceredase® by Genzyme, a