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Review

The safety assessment of food ingredients derived from plant cell, tissue and organ cultures: A review

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ABSTRACT

Plant cell, tissue and organ cultures (PCTOC) have become an increasingly attractive alternative for the production of various high molecular weight molecules which are used as flavourings, fragrances, colouring agents and food additives. Although PCTOC products are cultivated *in vitro* in a contamination free environment, the raw material produced from PCTOC may contain many components apart from the target compound. In some cases, PCTOC raw materials may also carry toxins, which may be naturally occurring or accumulated during the culture process. Assessment of the safety of PCTOC products is, therefore, a priority of the biotech industries involved in their production. The safety assessment involves the evaluation of starting material, production process and the end product. Before commercialisation, PCTOC products should be evaluated for their chemical and biological properties, as well as for their toxicity. In this review, measures and general criteria for biosafety evaluation of PCTOC products are addressed and thoroughly discussed.

Contents

1. Introduction

Plant cell, tissue and organ cultures (PCTOC) have been used to produce a wide range of phytochemicals, which are used as flavourings, colourants, essential oils, sweeteners, antioxidants and nutraceuticals (Georgiev, Eibl, & Zhong, 2013; Murthy, Lee, & Paek, 2014; Murthy, Georgiev et al., 2014). In recent years, various PCTOC strategies have been developed for the improvement of biomass and phytochemical production. Bioreactor technologies have also been developed for large-scale cultivation of plant cells and organs for the production of phytochemicals (Murthy, Dandin, Zhong, & Paek, 2014; Murthy, Kim, Georgiev, & Paek, 2014;

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Murthy, Kim, Park, & Paek, 2014a, 2014b; Murthy, Lee et al., 2014; Murthy, Georgiev et al., 2014; Paek, Murthy, Hahn, & Zhong, 2009). Various natural and synthetic growth regulators are supplied to the culture medium aiming to promote the growth of cells/organs *in vitro*, for the modification of morphogenetic events and for the accumulation of metabolites. Diverse chemicals and physical agents are also used as elicitors to boost the production of bioactive compounds (Namdeo, 2007).

Biomass produced through PCTOC is used as a raw material for procuring food and medicinal ingredients (Murthy, Lee et al., 2014; Murthy, Georgiev et al., 2014; Murthy, Kim, Georgiev et al., 2014; Murthy, Kim, Park, & Paek, 2014a, 2014b; Paek et al., 2009). These ingredients are often extracted from the raw material without going through stringent purification. The raw material produced from PCTOC may contain a mixture of many components, even including toxic by-products. This has raised concern within the food and pharmaceutical industry about the biosafety and efficacy of the PCTOC raw material and ingredients therein. It also prompted considerations from regulatory agencies around the world to look at the safety standards of PCTOC products.

An approach taken by many regulatory agencies for safety evaluation of PCTOC-derived products was based on substantial equivalence, that is, to determine if the culture derived food/medicinal ingredients are substantially equivalent to their whole plant counterparts (Fu, 1999). Nevertheless, when the PCTOC products are commercialised, biosafety regulations come into force. Standard protocols on safety, efficacy, standardisation and documentation of botanicals and herbal preparations (Abdel-Rehaman et al., 2011; Kroes & Walker, 2004; Mosihuzzaman & Choudhary, 2008), natural food additives (Anonymous, 2007, 2012a, 2012b) and genetically modified crops (Anonymous, 2008; Cockburn, 2002; Kok, Keijer, Kleter, & Kuiper, 2008; Kok & Kuiper, 2003; Konig et al., 2004; Kuiper, Kleter, Noteborn, & Kok, 2002) are available, however, such protocols and safety measures are not accessible for PCTOC products. In this review, we have discussed safety issues of PCTOC products as food supplements. The precautionary measures to be taken for the initiation of cultures to harvest raw material and subsequent methods for biosafety assessment of PCTOC raw materials and products have also been presented.

2. Safety considerations of plant cell, tissue and organ culture processes

Fu (1999) elaborated the safety considerations of PCTOC manufacturing process in four main steps, namely cell line development, process scale-up, production and purification. Several types of cell and organ cultures have been used for the production of food and pharmaceutical ingredients, including cell suspension cultures and organ cultures, such as transformed shoot cultures and hairy root cultures. In recent years, embryo and adventitious root cultures have also been used for the production of food ingredients (Paek et al., 2009; Park, Ahn, Lee, Murthy, & Paek, 2005; Shohael, Khatum, Murthy, & Paek, 2014; Shohael, Murthy, & Paek, 2014). Organ cultures are comparatively more stable than cell cultures in terms of genetic stability (Choi et al., 2000; Paek et al., 2009). The safety measures to be followed during the initiation of cultures for the production of biomass and secondary metabolites from PCTOC are as follows (Fig. 1).

1. Selection of suitable plant material: careful selection of source material, collection of information on Latin name (genus, species, and authority), common name(s) of the material used, chemotype and geographic origin are essential at this stage.
2. PCTOC method for selection of raw material: selection of suitable explants for induction of callus, cell and organ lines.
   (i) Production of cell or organ lines for the production of biomass.
   (ii) Optimised culture conditions/parameters for *in vitro* cultivation, such as medium, salt strength, growth regulators and its concentration, medium pH, temperature, illumination, light intensity and quality.
   (iii) Type of culture vessel (bioreactor) used, agitation, aeration, mode of operation, bioreactor conditions during growth and production cycles.
   (iv) Elicitation methodology used, type and concentration of elicitor used, time of addition of elicitor and duration of exposure.
   (v) Biomass types such as cells, adventitious roots, hairy roots, embryos, shoots and harvesting of bioactive ingredients from the medium, method of harvesting.
3. Method of processing PCTOC raised raw materials: drying and processing of biomass, storage conditions.

Further, additional requirements during biomass production are: (1) maintenance of uniform, optimised chemical and physical parameters in order to ensure batch-to-batch production consistency (Paek et al., 2009), (2) assessment of genetic stability of cultured cells and organs preferably through molecular biology techniques such as randomly amplified polymorphic DNA (RAPD) analysis, (3) analysis of target phytochemicals by refined quantitative techniques such as high pressure liquid chromatography (HPLC), gas chromatography (GC) analysis at least on a batch by batch basis.

Recently, large-scale cultures are operated using bioreactors for biomass production and a reduction in productivity has been observed when cell and organ cultures are transferred from shake flask to bioreactor cultures (Murthy, Lee et al., 2014). The decrease or shift in production was attributed to the different physical conditions, such as degree of mixing, shear stress and gas phase composition. Therefore, all these parameters should be investigated in small-scale bioreactors before adopting large–scale bioreactor cultures. Bioreactor design and selection, and maintenance of bioprocess parameters are equally important for obtaining a proper yield and maintaining the quality of product (Georgiev et al., 2013).

Techniques that have been used to increase the yield of products during the production stage of a cell or organ culture include addition of precursors to the cultures, elicitation and *in situ* product removal. For example, addition of methyl jasmonate as an elicitor during the *Panax ginseng* adventitious root culture has improved the accumulation of ginenosides by 7-fold (Kim, Hahn, Murthy, & Paek, 2004; Paek et al., 2009; Yu, Gao, Hahn, & Paek, 2002). Various types of chemical and physical elicitors have been tested and used for enhanced accumulation of bioactive compounds in PCTOC (Namdeo, 2007; Zhao, Davis, & Verpoorte, 2005). In view of the biosafety of products, it is recommendable to avoid toxic elicitors, such as heavy metals, detergents, xenobiochemicals, fungicides, herbicides and other harmful chemicals. In addition, elicitors of biological origin (methyl jasmonate, salicylic acid) as well as physical elicitors (UV irradiation, differential light sources) can be used for the improvement of product accumulation.

In situ product removal techniques have been used in the second phase of plant cell and organ cultures with the objective of removing the products from the cultured cells and organs. Application of an *in situ* product removal makes the recovery of products easier and it often leads to increased productivity. For example, Buitelaar, Langenhoff, Heidstra, and Tramper (1991) studied the effect of an organic phase on cell growth and thiopene production in *Tagetes patula* hairy root cultures. The authors observed significant improvement of thiophenes productivity with the addition of hexadecane to the cultures, however, the compositions of the