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Current Opinion in Biotechnology

Prospects for the use of plant cell cultures in food biotechnology

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Plant cell cultures can offer continuous production systems for high-value food and health ingredients, independent of geographical or environmental variations and constraints. Yet despite many improvements in culture technologies, cell line selection, and bioreactor design, there are few commercial successes. This is principally due to the culture yield and market price of food products not being sufficient to cover the plant cell culture production costs. A better understanding of the underpinning biological mechanisms that control the target metabolite biosynthetic pathways may allow the metabolic engineering of cell lines to provide for economically competitive product yields. However, uncertainty around the regulatory and public acceptance of products derived from engineered cell cultures presents a barrier to the uptake of the technology by food product companies.

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Current Opinion in Biotechnology 2014, **26**:133–140

This review comes from a themed issue on **Food biotechnology**

Edited by **Mattheos AG Koffas** and **Jan Marienhagen**

0958-1669/$ – see front matter, Published by Elsevier Ltd.

http://dx.doi.org/10.1016/j.copbio.2013.12.010

Introduction

The totipotency of most plant cells means that they readily lend themselves to cell culture approaches. The long history of plant cell culture, starting from the beginning of the 20th Century, has established a tremendous baseline of knowledge and technologies. This ranges from the plant media and hormone manipulations through to bioreactor engineering and the availability of tools such as the root pathogen *Agrobacterium rhizogenes*. The potential of plant cell cultures as sources of nutraceutical and medicinal compounds also has a long history of research, dating back at least 50 years [1••,2]. Cell cultures can offer several potential advantages over the extraction of compounds from field grown plant material, the principal ones being:

- Continuous production systems independent of geographical or environmental variations and constraints.
- Products of uniform quality produced from defined Good Manufacturing Practice (GMP) systems that can be guaranteed free of agrochemicals.
- The possibility for high-concentrations of target compounds and rapid production of biomass. In nature the target compounds may be only found at low concentrations or in specific plant tissues.
- Easier extraction protocols, reducing the need for use of aggressive solvents.

Furthermore, extraction from cell cultures can have significant advantages over full chemical synthesis of target compounds, including avoiding issues around synthesis of complex structures and specific stereoisomers, toxic chemicals used in synthesis, and labelling requirements. For example, despite the achievement of total chemical synthesis of the anti-cancer drug paclitaxel it is also being produced on a commercial scale in plant cell cultures by the company Phyton Biotech using fermenters up to 75,000 litres [3] (the original source from the Pacific yew tree was unsustainable). Genetic modification (GM) of whole plants can potentially offer some of these advantages via the metabolic engineering of biosynthetic pathways for consistent high-level production of the target compounds. However, some of the target compounds have complex biosynthetic pathways that are challenging for metabolic engineering. Additionally, GM plants require regulatory approval before they can be commercially grown.

The advantages offered by cell culture production systems should be appealing to the food ingredient and dietary supplement industries. Guaranteed GMP and consistency of product are both key issues for these industries. Moreover, some of the most desirable compounds are found only in small amounts in specific plant tissues, such as saffron spice compounds in the stigmas of *Crocus* flowers [4]. Microbial cell cultures are already an established part of food industries, whether for biotransformation in dairy fermentation or the production of recombinant chymosin for the cheese industry [5]. In a notable recent advance, metabolically engineered yeast has been used to produce commercial omega-3 eicosapentaenoic acid products, to substitute for those derived from fish [6••]. Biotransformation applications have also been demonstrated using plant cell cultures, for example the use of *Vanilla planifolia* or *Capsicum frutescens* cultures to transform the ferulic acid substrate to vanillin [7]. Thus, in theory, plant cell cultures offer a route to controlled large-scale production of food ingredients.

Table 1

Examples of food and health compounds that are potential targets for production in plant cell cultures.

Compound	Compound type	Use	Common plant sources
Anthocyanins	Flavonoids	Colourant (e.g. E163)	Grape (*Vitis vinifera*) skins, sweet potatoes, various fruits
Betacyanins	Betalains	Colourant (e.g. E162)	*Beta vulgaris* roots, *Amaranthus* flowers
Carthamin		Colourant (Natural Red 26)	*Carthamus tinctorius* (safflower) petals
Capsaicin	Capsaicinoid	Flavour	*Capsicum* fruits
Ferulic acid	Phenylpropanoid	Food ingredient preparations	Many sources, for example, cereal brans
Ginsenosides	Triterpene saponins	Health food extract	*Panax* spp. roots
Lavender oil	Terpenes and other compounds	Flavour	*Lavandula* spp. flowers
Noni root extract	Anthraquinones, alkaloids	Health food extract	*Morinda citrifolia* roots
Nootkatone	Sesquiterpene	Flavour	*Citrus* × *paradisi* (grapefruit) fruits
Phytosterols	Phytosterols such as β-sitosterol	Health ingredient	Seed oils, especially from legumes
Resveratrol	Stilbene	Health food extract	Grape seed extract, legume shoots
Saffron (crocetin, safranal)	Apocarotenoids	Flavour and colourant	*Crocus sativus* (saffron) stigmas and styles
Vanillin	Phenylpropanoid	Flavour	Seed pods of *Vanilla planifolia*

The priority food targets for cell culture production would be expected to be those ingredients that are of high value but which are either present only in low levels in the source plant material or for which cultivation of the plant species is challenging and wild sources limited. They are more likely to be plant secondary metabolites (SMs) rather than recombinant proteins, as microbial systems are well established for several important food industry proteins. Some examples of potential targets are given in Table 1, including products for both the food ingredient and health food supplement industries. Individual food ingredients are the basis of major global industries, for example the annual world market for plant-derived vanilla flavour is estimated at more than US$250 million, while the total annual worldwide herbal extracts market was recently estimated at US$17.5 billion [8]. Many flavours in particular, which represent more than a quarter of the world market for food additives, are produced by chemical synthesis or chemical transformation of natural substances. Yet despite significant research and commercial efforts over a long period, very few food or health products produced by plant cell cultures have been commercialized. Nosov [1••] lists 14 production facilities that at some point had commercial plant cell culture operations, and there are other examples beyond that list, such as the Swiss biotech company ROOTec Bioactives GmbH that is producing both pharmaceuticals and cosmetics commercially from hairy root cultures (http://www.rootec.com). However, the only food-related product among these examples is the manufacture of ginsenosides-based health supplements (ginseng triterpene saponins) [1••,9,10]. Moreover, very few have had ongoing commercial success, specifically those producing ginsenosides, paclitaxel, berberine alkaloid-based health supplements, and the dye shikonin [7,10–12]. There are many plant cell culture lines producing other food components which have not reached commercial production, such as those producing the natural food colourants anthocyanins [2] and betalains [13,14], the sweetener stevioside [15], and aroma and flavour compounds like vanillin [16], nootkatone (grapefruit flavour) [17], 2,3-butanedione [18], valencene (citrus aroma) [19] and basmati flavour [7]. The reasons for the commercial failure of these is perhaps illustrated by examining the basis of the few successes; agronomic difficulties in growing the source plant combined with either high cell culture productivity (e.g. shikonin) or high market value (e.g. paclitaxel). In general, the key limitation of plant cell cultures for commercial food applications is the price of the product versus the cost of production. The plant-derived anticancer drugs vincristine and paclitaxel are priced at around US$20,000 and US$400,000 per kg, respectively, while vanillin extracted from pods, which is considered an expensive food ingredient, only costs around US$4,000 per kg. Overall, the lack of significant commercial success for plant cell cultures in food industries is due to:

- Failure of the dedifferentiated cell lines to produce the desired SMs with the structures or abundance found in the organs of the parent plants.
- The price of the product versus the cost of production.
- Lack of clarity on the regulatory status of cell culture derived foods.

Without a step-change in the current plant cell culture technologies it seems unlikely that this current limited use will change in the near future. The traditional culture technologies, based on many years of work by cell biologists and engineers, have somewhat plateaued in terms of reducing the cost of production, and this is reflected in the literature of the last decade. More effort is now focusing on incorporating new technologies, in particular metabolic engineering, although this may further complicate the regulatory pathway for potential products. In