Plant Cell Culture for the Production of Fine Chemicals

Plants and useful chemicals

- Plants have long been recognised as a source of medicinal products.
- At first large portions of plants were used but as knowledge increased, specific compounds were advocated.
- Today, there is a wide variety of pharmaceutical and other chemicals which are extracted from plants. Others are produced synthetically to mimic their natural counterparts.

Value of Some Plant Compounds ($US)
Curtin (1983)

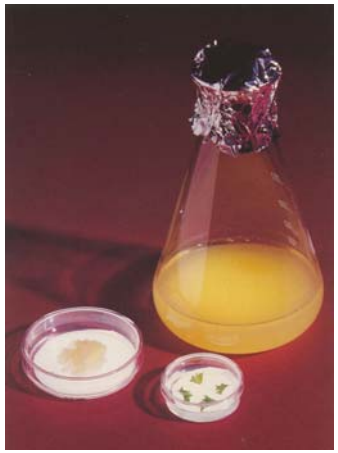
Compound	Use	Wholesale Price ($US)	Market ($US X 10^6)*
Vinblastine/Vincristine	Leukemia treatment	5000 g^{-1}	18 - 20
Digitalis	Heart disorders	3000 g^{-1}	20 - 55
Codeine	Sedative	650 kg^{-1}	50
Pyrethrins	Insecticide	300 kg^{-1}	20
Jasmine	Fragrance	5000 kg^{-1}	0.5 (W)
Spearmint	Flavor	30 kg^{-1}	85 - 90
Quinine	Antimalarial/flavor	100 kg^{-1}	5 -10

* Except (W): World market

Plant cell suspension culture

Consideration in this lecture given only to:

- Cell suspension culture (free or immobilized).
- *de novo* synthesis, not biotransformation and synthesis from precursors.



Fermentation technology and plant cell culture

Fermentation technology is applicable to the production of fine chemicals by plant cells because the plant cell is like a large microorganism.

Comparison of Microorganisms to Plant Cells
Brodelius (1985)

Parameter	Microbial Cell	Plant Cell
Size	approx. 2 μ^3	$>10^5$ μ^3
Genetic	Procaryotic	Eucaryotic
Shear	Insensitive	Sensitive
Doubling time	1 h	20 h
Fermentation time	1 - 2 days	2 - 3 weeks
Product	extra/intracellular	Mainly intracellular
Productivity	High	Low
Cost of media	approx. $6 m^{-3}	Approx. $50 m^{-3}

Advantages of plant cell culture over field cultivation or collection of plant material

- Production under highly controlled conditions.
- Potential for manipulation to increase productivity.
- Constancy of supply
- Potential for the production of new compounds.

Examples of useful compounds produced by plant cells in suspension culture

Compound	Plant
Antimicrobial	Catharanthus (protozoa)
	Lithospermum (bacteria)
Antitumour	Catharanthus
	Campotheca
Antispasmodic	Chamomile
	Isodon
	Valerian
Food flavors	Spearmint
	Asparagus
	Onion
	Mustard
Hydrocarbons	Asclepias
	Euphorbia
	Guayule
Tonics	Ginseng
	Cinchona
Insecticides	Pyrethrum
	Derris

Some factors limiting the industrial utilization of plant cells

1. Slow growth of cells

- Cost of biomass production will be high. In 1985, it as estimated that the value of a compound needs to be at least \$US1000 kg^{-1} for economic production.
- Contamination becomes a significant potential problem. In the time a plant cell replicates itself (20 - 60 h), a bacterium would have produced $10^{12} - 10^{36}$ progeny.

2. Low yield of product

Can be overcome by:

- Selection of high yielding cell lines.
- Two-stage culture to optimize the production of secondary metabolites.

Some factors limiting the industrial utilization of plant cells 2

3. Instability of cell lines

- Serial transfers of cell lines in to fresh media can lead to loss of synthetic capability.
- Reduction of cell division by using immobilized cells may be a way of overcoming the problem.

4. Requirement for differentiation for synthesis of product

- Does not appear to be an immediate solution to this problem.
- Genetic manipulations to decouple synthesis and differentiation may be the only solution.

5. Low shear resistance of cells

- Due to the size of plant cells.
- Can be overcome by the use of appropriate bioreactors, and/or immobilization of cells in protective matrices.

6. Cell aggregation

- Plant cells tend to aggregate in clumps. Resulting diffusional gradients cause differences in synthetic performance directly related to aggregate size.
- A large range in aggregate size means a wide range in synthetic capability.

The potential of plant cell culture

Despite the limitations towards industrial utilization, suspension cultures have been successfully used to obtain yields of compounds higher than those found in whole plants.

Some Selected Compounds Formed in Plant Cell Culture With a Yield Equal to or Higher Than That of the Parent Plant (Brodelius, 1985)

Plant	Compound	% Dry Weight		Culture/Plant	Reference
		Culture	Plant		
Coleus blumei	Rosmarinic acid	18	2.2	8.2	Zenk *et al.,* 1975
Panax ginseng	Ginsengoside	27	4.1	6.7	Furuya & Ishii, 1972
Thalictrum minor	Berberine	10	0.01	1000	Nakagawa *et al.,* 1984
Catharanthus roseus	ajmalicine	1.0	0.3	3.3	Matsumoto *et al.,* 1982
Lithospermum erythrorrhizon	Shikonin	14	1 - 2	7 - 14	Fujita *et al.,*1981

An overview of the development of the first commercial process for a plant cell compound

Shikonin

Synthesized in roots of the *shikon* plant, *Lithospermum erythrorrhizon.*

Shikonin

Key determinants of viability of commercial production

- Traditional medicine in Japan
- Has anti-bacterial and anti-inflammatory properties
- Is bright red in color
- Plants take 5 -7 years before shikonin concentration reaches 1 -2% in the roots.
- Problems in cultivation in Japan meant the importation of 10 tonnes p.a. from China and Korea.
- Pure shikonin costs about $US1000 kg-1 (1980s).

The success of the shikonin story is partially due to

- the high price of the collected product
- the ability to improve productivity to the extent that the following comparison can be made:

A Comparison of Shikonin Production From Intact Plants and Cultivated Plant Cells

	Time Before Harvest	Shikonin (% Dry Weight)
Whole plant	2 - 3 years	1 - 2
Cell suspension	3 weeks	14

Shikonin: Improvement in productivity

1. Manipulations in cultural practice

Recognition of the product being a secondary metabolite. Separation of cell growth and product synthesis stage.

One-Stage Versus Two-Stage Culture for Shikonin
Fujita *et al.* (1982)

	One-Stage Culture	Two-Stage Culture
Medium	White	LS/White
Culture time (d)	14	23
Productivity ratio	1	4.6

2. Improvement in formulation of media

Effects of Improvement of in Media
Fujita *et al.* (1982)

	Old Media	New Media
Medium	LS/White	MG-5/M-9
Culture time (d)	23	23
Shikonin (%)	1.07	13.6
Productivity ratio	1	12.7

Two-Stage Culture With MG-5 and M-9
Yamada and Fujita (1983)

	Medium		Total
	MG-5	M-9	
Culture time (d)	9	14	23
Growth rate (times)	7.5	3.6	27
Shikonin (mg L^{-1})	0	1500	1500

Immobilization of plant cells

Many products produced by plant cells are stored in vacoules. This is a limitation to their release when cells are immobilized to take advantage of such cultures and to overcome some of the problems previously alluded to.

Product release from vacoules of plant cells

For products to be released, the plasma membrane and the tonoplast (membrane surronding the vacoule) have to be passed.

Permeabilization of plant cells for product release

Chemicals can be used to make membranes within plant cells permeable to various compounds.

Concentration of Various Permeabilization Agents Required for Release of 50 and 90% of Intracellularly Stored Products From Cultivated Plant Cells (Brodelius, 1986)

	Plant	*Chenopodium rubrum*		*Thalictrum rugosum*	
	Product	Betanin		Berberine	
Permeabilization Agent	Release (%)	50	90	50	90
DMSO (% v/v)		10	35	13	30
PEA (% v/v)		0.86	0.98	0.60	0.80
Chloroform (% sat.)		54	64	50	67
Triton X-100		185	230	140	210
HDTMAB (ppm)		22	84	24	60

DMSO Dimethylsulfoxide
PEA Phenethyl alcohol
HDTMAB Hexadecyltrimethylammonium bromide