CHAPTER 5: BIOPROCESS CONSIDERATIONS IN USING ANIMAL & PLANT CELL CULTURES
Chapter 5.2: BIOPROCESS CONSIDERATIONS IN USING PLANT CELL CULTURES
General Structure of Plant Cell
Plant cell culture is a practice used to propagate plants under sterile conditions, often to produce clones of a plant.
Many of thousands of chemicals are produced only in plants.

These compounds are chemically complex & generally non-proteins-those that do not synthesize by microbes & chemical synthesis is not reasonable.

Some compounds of potential commercial interest from plant as following:

2. Food colors or dyes- Anthocyanins, shikonin, betacyanins.
3. Flavors- Vanilla, strawberry, grape, garlic.
4. Fragrances- Jasmine, lemon, mint
5. Sweeteners- Miraculin, monellin.
6. Agricultural chemicals (insecticides and herbicides)- allelopathic chemicals, azadirachtin

- Plant cell cultures provide alternative & complementary methods to whole plants extraction for the production of these compounds.
Advantages of Plant Cell Culture

1. Control of supply of product independent of the availability of plant itself (e.g. without plant cell culture technology, three 100-year-old of Pacific yew tree are required to supply enough paclitaxel to treat one cancer patient).

2. Cultivation under controlled and optimized conditions.

3. Strain improvements with programs analogous to those used for microbial systems.

4. No need of the use of harmful herbicides and pesticides.

5. Possibility of synthesizing novel compounds, not present in nature, by feeding of compounds analogous to natural substrates.

6. No dependence on climate, and geographical location etc.
## Plant Cell vs Microbial Cell

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Typical microbial (yeast) cell</th>
<th>Typical plant cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape</td>
<td>Spherical, cylindrical</td>
<td>Spherical, cylindrical</td>
</tr>
<tr>
<td>Size (μm)</td>
<td>2-10</td>
<td>50-100</td>
</tr>
<tr>
<td>Growth pattern</td>
<td>Single cells</td>
<td>Mostly aggregates</td>
</tr>
<tr>
<td>Doubling time (h)</td>
<td>1-2</td>
<td>20-100</td>
</tr>
<tr>
<td>Oxygen requirement (vvm)</td>
<td>1-2</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>Fermentation time</td>
<td>2-10 days</td>
<td>2-4 weeks</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>80</td>
<td>&gt; 90</td>
</tr>
<tr>
<td>Shear sensitivity</td>
<td>Insensitive</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Regulatory mechanisms</td>
<td>Complex, mostly known</td>
<td>Highly complex, mostly unknown</td>
</tr>
<tr>
<td>Genetic makeup</td>
<td>Stable</td>
<td>May be highly variable</td>
</tr>
<tr>
<td>Product accumulation</td>
<td>Often extracellular</td>
<td>Mostly intracellular</td>
</tr>
</tbody>
</table>
Laboratory requirements for plant cell culture

- Laminar air flow cabinets
- Autoclave
- Oven for dry sterilization
- Water distillation apparatus
- Incubator
- Shakers
- Fermenters or bioreactors
Materials and methods

- Plants – any part obtained from any plant species can be employed to induce callus tissue

- Media - inorganic salt
  - Carbon sources
  - Vitamins
  - phytohormones
  - organic supplements

- Or ready made medium – Murashige and Skoog, Gamborg, B5, Nitsch etc
Establishment of plant cell culture

- Callus and suspension cultures have been established from hundreds of different plants.
- A callus can be formed from any portion of the whole plant containing dividing cells.
- **Callus** — tissues arising by proliferation from explants on agar medium/Mass of undifferentiated cells produced in tissue culture is called callus. The callus is highly vacuolated and unorganised cells.
- **Suspension cultures** — cells or small cells aggregates growing dispersed in liquid medium.
Callus
Basic protocols for suspension culture

1. Whole Plant
   - Surface Sterilize
   - Suspension Culture
     - Remove Large Cell Clumps

2. Cutting from Plant
   - Solidified Medium for Rapid Growth
     - Callus Formation
       - Propagation as Callus
         - Organ Cultures (Root or Shoot)
           - Defined Medium with Agar
             - Sterile Seed
               - Plantlet
Methods Used for the Cultivation of Plant Cells

- Excised plant material is placed on solidified medium
- Callus forms can be quite large (\(> 1\) cm)
- Callus transfer to liquid medium (for propagation)
- Establishment of suspension culture from callus is straightforward if callus is friable
- A piece of callus is placed in liquid medium in shake flask.
- With moderate agitation, cells or small aggregates of cells will slough off
- A platform shaker is used to give a circular/orbital motion in a variable speed control (a range form 30-150 rpm).
Methods Used for the Cultivation of Plant Cells

- Cond. 25°C, pH5.5 in the dark
- These suspended cell then replicate
- After 2/3 weeks, suspended cells are transferred to fresh medium
- For large scale suspension cultures, bioreactors system is being used for the purpose of producing high value plant products.
In order to obtain products in high concentration, many effort have been made to stimulate or restore biosynthetic activities of cultured cells using various methods.

Several typical approaches that may increase productivity of cultured plant cells:

a) Optimization of cultural condition
b) Selection of high-producing strains
c) Addition of precursors
d) Biotransformation
e) Elicitor treatment
1) Optimization of cultural conditions

**Medium**

-The most important is the medium that influence both the growth of cells and yield of desirable products

-Various basal medium have been used and Murashige & Skoog (MS) is among the most widely applicable

-Sucrose and glucose are carbon source for plant tissue cultures and affects cell growth and yield of products

-Phytohormones such as auxins and cytokinin have shown the most remarkable effects on growth and productivity of plant metabolites.
Surrounding environment (temp, pH, light & O₂)

- Generally, a temperature of 17-25°C is normally used for induction of callus tissues and growth of cultured cells
- The pH is usually adjusted between 5-6 before autoclave
- The use of light depends on the type of culture and the desire products
- Oxygen is not critical for plant cultures but still has an effect on the growth and production
Cell density

- The use of high cell density cultures in a suitable bioreactors found to increase yield in some cultures.

TWO-STAGE CULTURE

- Some plant cells needs different media for the cell growth and secondary metabolite production.
- This cultural condition is called two-stage culture.
  - It means that 2 types of culture medium are used.
  - both culture medium may differ in the concentration or types of nutrient, hormone or vitamins used.
two types of medium were used because the medium used for promoting growth is not suitable for promoting metabolites production.

In such system, the first medium is used to promote the growth of cells in the culture system.

the second culture is to promote the production of metabolites in the culture.

Cells of plant will be first cultivate in the first medium and after certain period, they will be transferred into the second growth until harvesting period.
Two-stage culture for shikonin production

1st stage tank
200 L, 9 days

2nd stage tank
750 L, 14 days
2) Selection of high-producing strains

- The physiological characteristics of individual plant cells are not always uniform
- Therefore a rapid assay method is crucial in the selection of a high yielding cell line
- The specific cell line is obtained from the selection of a number of strains producing high level of desirable product
- The strains then were subjected to further cell cloning to increase the level of secondary metabolites
Fig. 1. Outline of the selection method. A The original *E. millii* calluses were divided into 128 segments, and a segment was placed on agar-medium in one section of a 9-section Petri dish and coded. B Segments were cultured at 28 °C under light (6000 lux) for 10 days. C Each grown segment was then divided into two cell-aggregates; one (*D*₁) for subculture and the other (*D*₂) for quantitative analysis of the pigment. D₁ The reddest of the nine aggregates were removed and placed in an empty Petri dish (*E*). E Each of these red aggregates was divided into several segments, of which the reddest pieces were removed, then coded and placed on agar-medium (*B*). ○ = unselected segment; ● = selected segment; □ = unselected aggregate; ⊗ = grown aggregate
3) Addition of precursors

- Precursor – a compound that participate in the chemical reaction that produce another compound
- Addition of precursors to the culture media sometimes stimulates secondary metabolite production
- This approach is advantageous if the precursors are inexpensive
For exp: **Phenylalanine** is one of the biosynthetic precursors of rosmarinic acid. Addition of this amino acid to *Salvia officinalis* suspension cultures stimulated the production of rosmarinic acid and shortened the production time as well.
4) Biotransformation

- A suitable substrate compound may be biotransformed to a desired product using plant cell
- Biotransformation has been extensively applied in the fermentation using microorganisms and their enzymes
- For example, L-aspartic acid and L-malic acid can be biotransformed from fumaric acid using microorganisms
Using plant cells, for exp. the biotransformation of $\beta$-methyldigitoxin to $\beta$-methyldigoxin using *Digitalis lanata* has been investigated.

- Digoxin has a large market as a cardiac glycoside.

- This approach beside precursors feeding are the most commercially realistic approaches because of economic reasons.
5) Elicitor treatment

- Elicitor is an agent of microbial infections on intact plants that cause the synthesis of specific secondary metabolite.
- Some studies reviewed possible correlations between stress and secondary metabolism in cultured cells.
- Some suggested that upon infection, plants show their defense mechanism by secreting secondary metabolite.
Elicitors that have been used in plant cell cultures are yeast extract, chitosan, inorganic and organic molecules and many more.

Plants grow under stress condition also show elicitation effects. Phosphate limitation in hairy root cultures of *Hyoscyamus muticus* had increased production of the sesquiterpene solavetivone.

Examples of inorganic compounds used are sodium chloride, potassium chloride, sorbitol and abscisic acid.

For economical use of the elicitors, they should be cheap and easy to obtain.