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Lecture 8 Plant Tissue Culture & Applications

What is it?

- Tissue culture is the term used for “the process of growing cells artificially in the laboratory” *(OSMS.otago.ac.nz/main/bursary)*
- Tissue culture involves both plant and animal cells
- Tissue culture produces clones, in which all product cells have the same genotype (unless affected by mutation during culture)

What's the Background?

Haberlandt

Carrel

- Tissue culture had its origins at the beginning of the 20th century with the work of Gottleib Haberlandt (plants) and Alexis Carrel (animals)

The Background, II

- The first commercial use of plant clonal propagation on artificial media was in the germination and growth of orchid plants, in the 1920's
- In the 1950's and 60's there was a great deal of research, but it was only after the development of a reliable artificial medium (Murashige & Skoog, 1962) that plant tissue culture really 'took off' commercially

Young cymbidium orchids

The Background, III

- A more recent advance is the use of plant and animal tissue culture along with genetic modification using viral and bacterial vectors and gene guns to create genetically engineered organisms

What is needed?

Tissue culture, both plant and animal has several critical requirements:

- Appropriate tissue (some tissues culture better than others)
- A suitable growth medium containing energy sources and inorganic salts to supply cell growth needs. This can be liquid or semisolid
- Aseptic (sterile) conditions, as microorganisms grow much more quickly than plant and animal tissue and can over run a culture

What is Needed, II

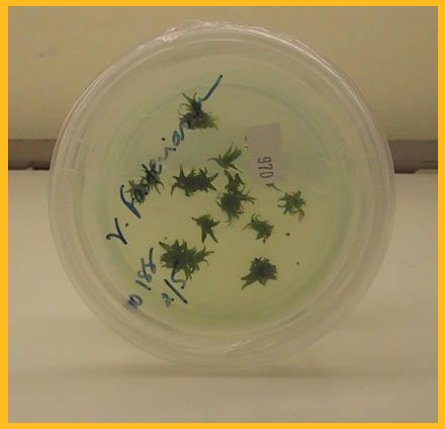
- Growth regulators - in plants, both auxins & cytokinins. In animals, this is not as well defined and the growth substances are provided in serum from the cell types of interest
- Frequent subculturing to ensure adequate nutrition and to avoid the build up of waste metabolites

Culturing (micropropagating) Plant Tissue - the steps

- Selection of the plant tissue (explant) from a healthy vigorous 'mother plant' - this is often the apical bud, but can be other tissue
- This tissue must be sterilized to remove microbial contaminants

The Steps, II

- Establishment of the explant in a culture medium. The medium sustains the plant cells and encourages cell division. It can be solid or liquid
- Each plant species (and sometimes the variety within a species) has particular medium requirements that must be established by trial and error



The Steps, III

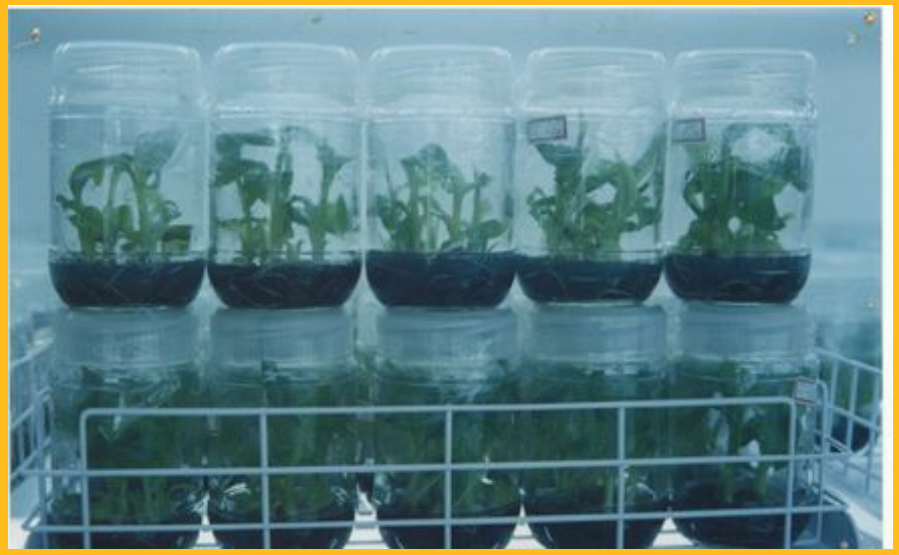
Dividing shoots

Warmth and good light are essential

- Multiplication- The explant gives rise to a callus (a mass of loosely arranged cells) which is manipulated by varying sugar concentrations and the auxin (low): cytokinin (high) ratios to form multiple shoots
- The callus may be subdivided a number of times

The Steps, IV

- Root formation - The shoots are transferred to a growth medium with relatively higher auxin: cytokinin ratios



The bottles on these racks are young banana plants and are
growing roots

The Steps, V

Tissue culture plants sold to a nursery & then potted up

- The rooted shoots are potted up (deflasked) and ‘hardened off’ by gradually decreasing the humidity
- This is necessary as many young tissue culture plants have no waxy cuticle to prevent water loss

Why do Plant Tissue Culture?

- A single explant can be multiplied into several thousand plants in less than a year - this allows fast commercial propagation of new cultivars
- Taking an explant does not usually destroy the mother plant, so rare and endangered plants can be cloned safely
- Once established, a plant tissue culture line can give a continuous supply of young plants throughout the year

Why do Plant Tissue Culture, II

- In plants prone to virus diseases, virus free explants (new meristem tissue is usually virus free) can be cultivated to provide virus free plants
- Plant ‘tissue banks’ can be frozen, then regenerated through tissue culture
- Plant cultures in approved media are easier to export than are soil-grown plants, as they are pathogen free and take up little space (most current plant export is now done in this manner)

Why do Plant Tissue Culture, III

- Tissue culture allows fast selection for crop improvement - explants are chosen from superior plants, then cloned
- Tissue culture clones are ‘true to type’ as compared with seedlings, which show greater variability

Culturing Animal Tissue- the Steps

- Animal tissue is obtained either from a particular specimen, or from a ‘tissue bank’ of cryo-preserved (cryo = frozen at very low temperatures in a special medium)
- Establishment of the tissue is accomplished in the required medium under aseptic conditions

Culture vessels and medium for animal cell culture

Culturing Animal Tissue, II

- Growing the cells / tissue requires an optimum temperature, and subculturing when required
- Human cells, for example are grown at 37degrees and 5% CO_2

Incubator

Animal tissue/cell culture - differences from plant tissue culture

- Animal cell lines have limited numbers of cell cycles before they begin to degrade
- Animal cells need frequent subculturing to remain viable
- Tissue culture media is not as fully defined as that of plants - in addition to inorganic salts, energy sources, amino acids, vitamins, etc., they require the addition of serum (bovine serum is very common, but others are used)

Animal tissue/cell culture - differences from plant tissue culture II

- Animal tissue cultures can pose biohazard concerns, and cultures require special inactivation with hypochlorite (e.g. Janola,Chlorox, etc.) and then incineration

Gloves and labcoat are always worn

The pipettes are disposable

Uses of Animal Tissue Culture

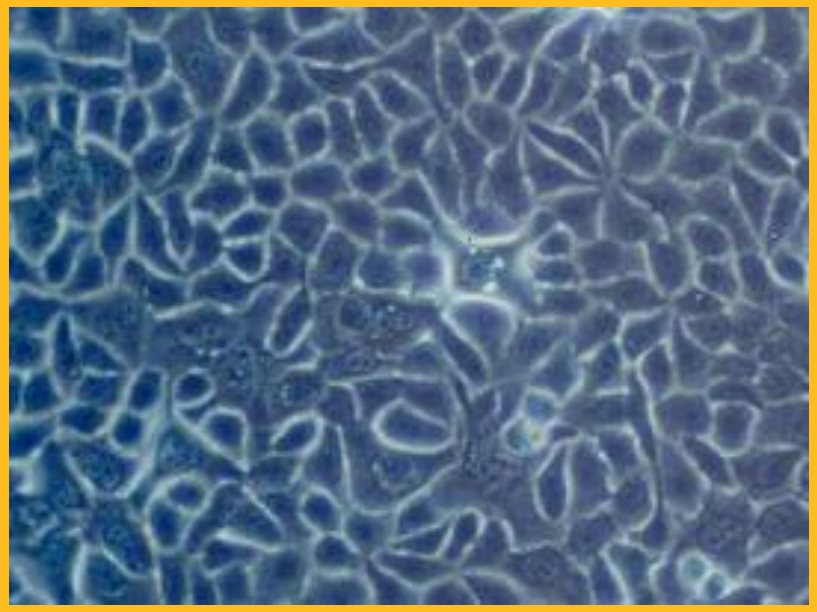


Photo courtesy of Sigma Aldrich

- Growing viruses - these require living host cells
- Making monoclonal antibodies, used for diagnosis and research
- Studying basic cell processes
- Genetic modification & analysis

Uses of Animal Tissue Culture II

- ‘Knockout’ technology - inactivating certain genes and tracing their effects
- Providing DNA for the Human Genome Project (and other species’ genome projects)

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