

# Management of **microbial contaminants** in the tissue culture **laboratory**

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**Sanette Thiar is the laboratory manager of the *In Vitro* Gene Bank Laboratory at ARC-Roodeplaat. This gene bank maintains the open commercially grown potato varieties, varieties with sub-licenses and varieties maintained for clients. From this facility mother stock is sent to other tissue culture facilities and greenhouses for the production of mini-tubers.**

Tissue culture is an expensive process due to the intensive labor requirements and high cost of equipment, chemicals and electricity. However, losses due to systemic micro-organisms and contaminants can be crippling if not managed properly.

## **Contaminated cultures**

The most likely sources of contamination in a laboratory range from insufficient sterilization of the mother stock, endophytic cultivable micro-organisms to contaminants introduced in the laboratory during the handling of the cultures. It is therefore important to start with healthy disease-free material. Production of healthy material should be traceable during the whole process of multiplication.

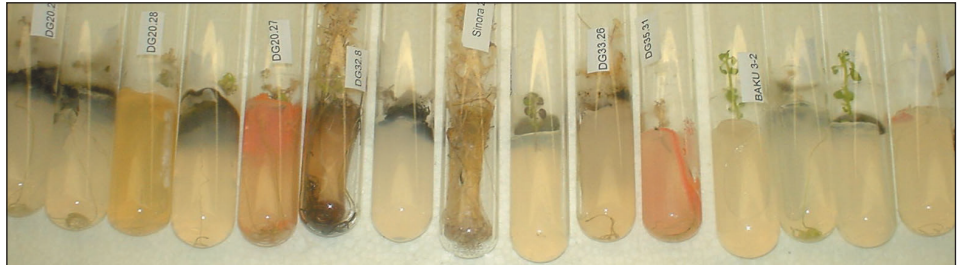
## **Operators sub-culturing plants**

Visual screening of plant material before cutting is extremely important. Contaminated cultures should be destroyed.

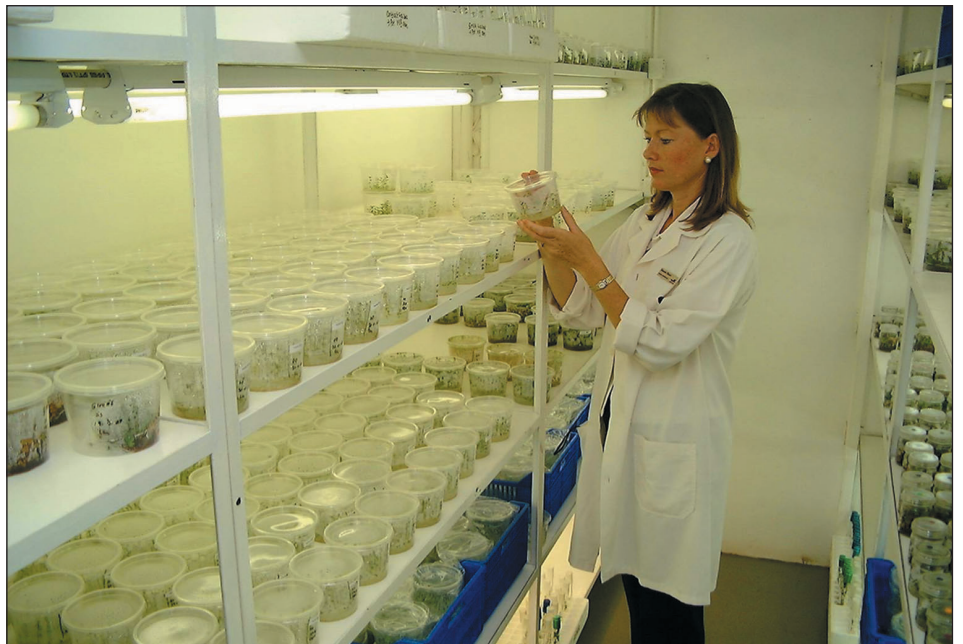
## **Visual screening for contaminants by laboratory manager**

A valuable tool in screening for bacterial contaminants is the use of an enriched nutrient broth. The base of the plant with roots is macerated and placed in the liquid nutrient broth.

The nutrient broth is a clear yellowish liquid enriched with nutrients preferred by bacteria. Bacterial growth will change this to a milky colour, which indicates the presence of bacteria. The contaminated cultures should be destroyed and one should only continue with the "clean" ones.



**Contaminated cultures.**



**Visual screening of contaminants by laboratory manager.**

## **Nutrient broth test - upper tube is contaminated with bacteria**

The use of antibiotics in tissue culture is discouraged as they only suppress the growth of the bacteria and do not eliminate the problem.

Once you start with healthy, clean material, the most likely cause of contamination is system failure or introduction of contaminants by humans in the laboratory.

Check the following to ensure proper sanitation:

- Fresh air introduced into the laboratory should be filtered.

- Access into the laboratory should be restricted.
- Laminar flow benches and autoclaves should be serviced and checked regularly.
- Air conditioners should be cleaned regularly.
- Handling procedures on the benches should be monitored e.g. use of tweezers, paper to cut on, etc.
- Cultures should be carefully screened for any sign of contamination before cutting.
- General sanitation of the facility should be done regularly e.g. washing

of floors and all surfaces with an antiseptic agent.

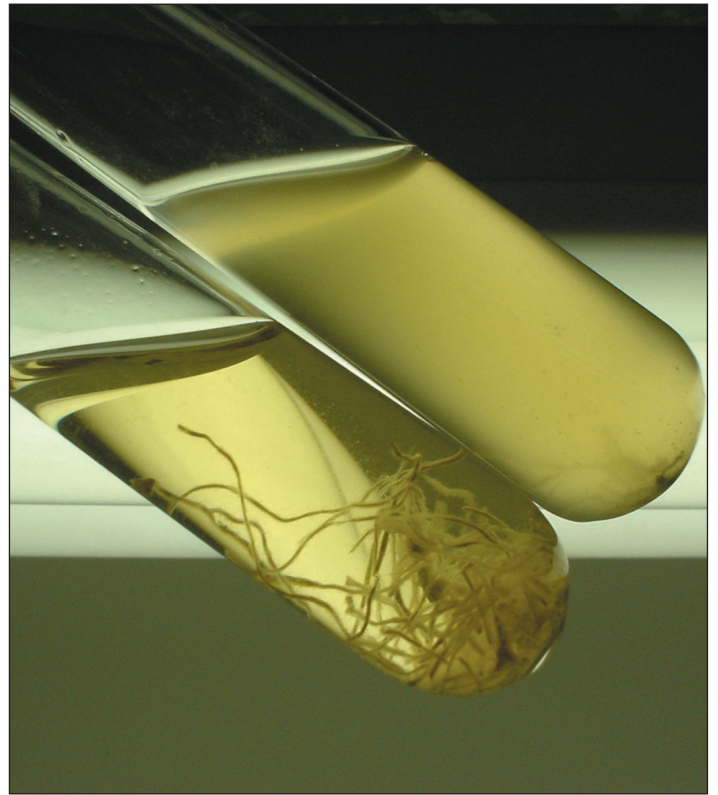
Systemic contamination reduces the multiplication rate of plants and can cause losses in the greenhouse. Currently, the effect of different types of contamination on survival and yield in the greenhouse is yet to be determined and would be worthwhile investigating.

Economic forces will dictate what quality standards would be acceptable. However, poor quality and contaminated tissue culture plants tend to tarnish the whole industry.

It is better to start healthy and stay healthy!



**Operators sub-culturing plants.**



**Nutrient broth test - upper tube is contaminated with bacteria.**