Elimination of contaminating bacteria from plant tissue cultures

T. Orlikowska, T. Malinowski, L. Ogórek
Research Institute of Horticulture, 96-100 Skierniewice, Teresa.Orlikowska@inhort.pl

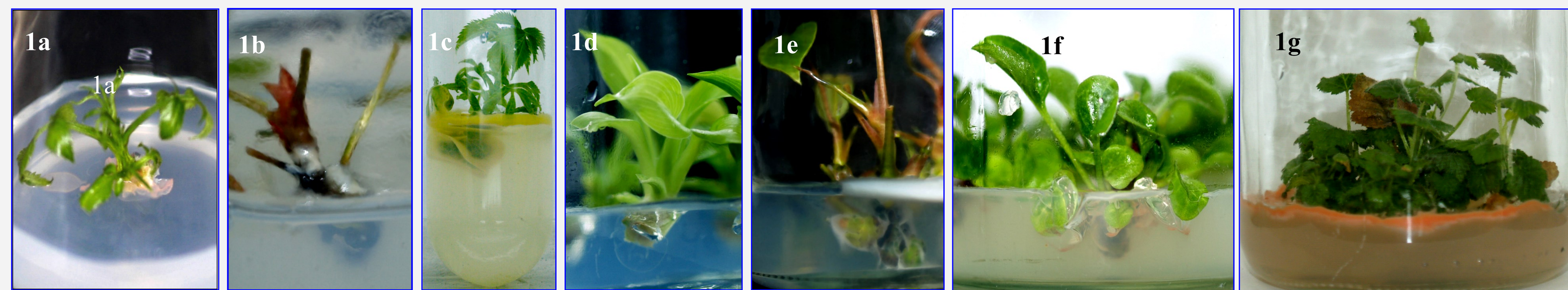


Fig. 1. a-g. Plant tissue cultures contaminated with different bacteria
Fig. 2. Growth of bacteria on different media, from left top to right down (King B, NA, R2A, 523, K, 1/2 MS).
a. *Bacillus* sp. after 48 h, b. *Methylobacterium lusitanum* after 98 h
Fig. 3. Effect of bacterial contamination on rooting cultures (from left to right). a. gerbera: control, with *Bacillus* sp., *Pseudomonas* sp., b. gerbera: control, with *Serratia* sp., *Staphylococcus* sp., c. hosta: control, with *Bacillus* sp., *Pseudomonas* sp., d. hosta: control, with *Serratia* sp., *Staphylococcus* sp.

Plant-associated bacteria form both epiphytic and endophytic populations through plants and their complete elimination from plant tissues is not possible despite of superficial sterilization of initial explants. Microorganisms, even in cryptic stage, may have detrimental effects (called vitropathy) on the cultures concerning multiplication or stability. They can also influence the results of experiments affecting metabolism of explants either directly or through changing the media and the atmosphere composition in the vessels.

Remedia for initial explants

Sanitation protocol should eliminate as much as possible bacteria from initial explants, including: cultivating donor plants at the high phytosanitary conditions, isolating the smallest possible shoot tips for culture initiation, effective superficial sterilization and inner sanitation with bactericidal preparations, culture of initial explants in the media with the addition of organic nitrogen, and checking the presence of cultivable bacteria by cultivating of explant fragments on bacteriological medium.

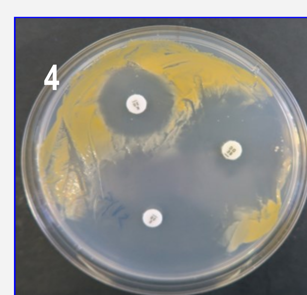
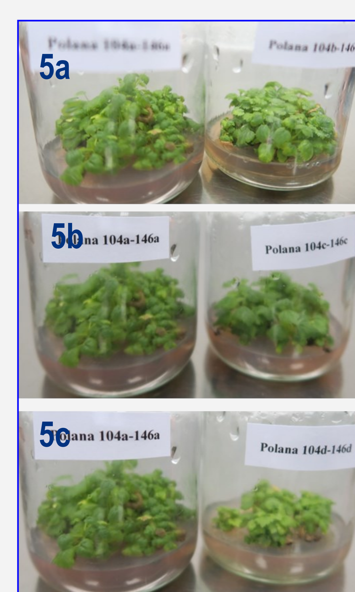


Fig. 4. Antybiogram
Fig. 5. Effect of biocydes on raspberry explants, control left: a. Rifampicin 50 mg/l, b. PPM 2 mg/l, c. PPM 4 mg/l.

Biocydes	Subculture I		Subculture II	
	Shoot number	Shoot length (cm)	Shoot number	Shoot length (cm)
Control	4,3	1,5	4,9	1,5
PPM 0,2%	4,6	3,3	4,6	1,9
PPM 0,4%	5,0	2,0	4,8	2,2
PPM 1%	2,3*/**	1,6	4,7	2,4
PPM 2%	1,9*/**	1,4	2,9*/**	1,4



Remedia for long term cultures

1. Systematic removal of cultures with symptoms of bacteria on/in the medium
2. Using screening tests to reveal cultivable bacteria in the cryptic state
3. In the case of mass contamination:
   a. isolating bacteria and make antybiogram to find out the most effective biocyde (Fig. 4)
   b. testing of harmfulness of chosen biocide for plant explants (Table 1 , Fig. 5)
   c. including not harmful biocide in the medium in every subculture or every second subculture
   d. applying as the first aid PPM, which is most known biocide aganst the broad bacteria spectrum and and safe for plant

Experimental

Material and methods. To eliminate endophytic bacteria (*Pseudomonas* sp.) from long term raspberry 'Norna' shoot cultures, we applied an inner sterilization by infiltration of defoliated plantlets with different biocides: Rifampicin 50 mg/l; PPM 0.1 and 0.2%; NaOCl 0.1 and 0.05%, $HgCl_2$ 0.1 and 0.05%), at ambient and lowered pressure atmosphere 30 milibars. Leaf-free 1.5 cm shoots taken from the propagation medium were placed in tens into tubes with the biocide solution; distilled water was the control. The open tubes were placed in a desiccator and with the vacuum pump the pressure was reduced to 50-30 milibars. Such pressure was maintained for 30 min or 2 x 15 min, and then the pressure was equalized rapidly. The shoots were 2 x rinsed in sterile water, drained on the sterile filter paper and placed on the propagation medium. Every 5 days the bottom segments of shoots were placed upon the NA bacterial medium and the number of dead shoots was recorded every 10 days. Also, initial explants were treated in the similar manner. The control explants were decontaminated superficially as usual (ethanol 70% for 1 min., $HgCl_2$ 0.1% for 3 min. and 3 x sterile water).

Results. All biocides applied at normal pressure, were able to decrease bacteria population to some extent, which was demonstrated by the absence of bacterial leaks in the plant medium but bacteria were detected in the bottom parts of shoots on the NA bacterial medium in the each term. In the subsequent subcultures on the plant medium with no biocide added, bacteria became visible in the medium. From the tested biocides applied at the lower pressure only $HgCl_2$ in both concentrations caused permanent elimination of bacteria, as they did not grow from bottoms of explants on the NA medium thorough two months. The most effective approach was the use of 2 x 15 min. 0.05% water solution of $HgCl_2$, under 30 milibars pressure because this treatment was less detrimental to shoot explants than 0.1% because the shoots getting better quickly and started multiplying. Percentage of died shoots was 50-60%. The attempt to release from the bacteria of the initial explants succeeded in half because the shoots were free of bacteria (in the test on NA medium) but most died because of the massive oxidation of phenols, which was visible as the blackening of the explants and the medium around them. This study will be continued.

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