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City Water Can Contaminate Tissue Culture Stock Plants

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Abstract. **Stock plants of 'Shepody' and 'Yukon Gold' potato *(Solarium tuberosum* L.) were grown in a greenhouse and irrigated with city water. Contamination rate of stem explant tissue cultures excised from these stock plants was 50% to 100%. A comparison of the microorganisms isolated from the contaminated cultures and from 0.22-µm filter disks through which 20 liters of city water had passed revealed the presence of similar bacterial floras. Five genera of bacteria *(Listerium* spp., *Corynebacterium* spp., *Enterobacter* spp., *Pasteurella* spp., and *Actinobacillus* spp.) were isolated from contaminated cultures and cultured filter disks. Watering greenhouse-grown stock plants with filtered city water decreased contamination of stem explant cultures 30% to 50%. Installing an ultraviolet light water-sterilizing unit at the greenhouse inlet point effectively reduced contamination.**

Microbial contamination of plant tissue cultures (Knox and Smith, 1980; Tanaka et al., 1983) is usually caused by fungi or bacteria (Deberg, 1987; Knauss and Millar, 1978; Leifert and Waites, 1990; Long et al., 1988; Phillips et al., 1981; Young et al., 1984) that exist on or in the tissue of the original explant and are not destroyed by disinfestation. In the tissue culture environment, these microorganisms outgrow the explant tissues and cause culture loss (De Fossard, 1976; Herman, 1990). Even if the contaminant is slow-growing and does not damage tissues visibly, it may affect the physiological reactions of infected tissues (George and Sherrington, 1984).

We experienced considerable difficulty in establishing contaminant-free stem internode explant cultures of potato. Losing cultures to microbial contamination wastes time and resources. Because plant tissues may be adversely affected by antibiotics, eliminating bacteria was a more expedient option. We report that bacterial contamination in potato stem explant tissue cultures was reduced by watering greenhouse stock plants with sterilized water.

Establishing greenhouse stock plants from tubers. 'Yukon Gold' and 'Shepody' potato tubers were green-sprouted and planted in a greenhouse under a 16-h photoperiod supplemented by an equal combination of very-high-output (VHO) Grolux (Sylvania F96T12, Grolux, VHO) and cool-white fluorescent lamps (F96T12, cool white, high output). Stock plants were grown in 2.6-liter pots containing a soil mix of two parts disinfested clay loam soil and one part ASB potting mix (Greenworld Garden Products, Pointe Sapin, N. B., Canada). A 20N–8.7P–16.6K liquid fertilizer was applied at 2 g·liter^{-1} every 14 days. Stock plants were watered with city water at soil level unless otherwise indicated. The water supply for the city of Fredericton is chlorinated; the water leaving the treatment plant contains ≈ 1 ppm chlorine. Days and nights ranged between 26 and 36C and 15 and 19C, respectively. Only Safer's soap and parasitic wasps were used for pest control.

Tubers to be watered with sterile water were surface-disinfested in 10% Javex 5 (5.25% sodium hypochlorite; Bristol-Myers, Belleville, Canada) before being planted in the disinfested soil mix described above.. Plants were watered only at soil level with city water that had been sterilized by filtering through a 0.2-µm minicapsule (Gelman Sciences, Montreal) and stored in an autoclave carboy.

Establishing tissue-culture-derived stock greenhouse plants. Plantlets derived from single-node cuttings grown in vitro on a Murashige and Skoog (MS) (1962) agar-solidified medium were transferred to soil and placed in shade in an intermittent-mist chamber that used unfiltered city water. Stock plants were watered as described above. Another group of plantlets was transplanted into a propagation tray and watered by hand exclusively with sterile water. After 4 weeks, all plants were transferred to 2.6-liter pots containing disinfested soil and watered with city water or sterile water, respectively.

Disinfesting stock plants. Stem internode sections were excised from 9-week-old stock plants with a presterilized, one-sided razor blade. A new blade was used for each plant. Stems were cut further into 8- to 10-cm sections on aluminum foil and transported to the laboratory from the greenhouse in a plastic bag containing paper towels wetted with sterile distilled water. Explants, loosely wrapped in cheesecloth, were disinfested by submersing them in 10% Javex 5 for 20 min. Stem sections were transferred immediately to a laminar-flow transfer cabinet and rinsed twice for 2 min each time with sterile distilled water.

Culturing stem explants. Stem explants from greenhouse-grown stock plants were removed from sterile water, trimmed to 1.5 cm long, and cut in half longitudinally. The explants were placed in 125-ml Erlenmeyer flasks containing 10 ml of liquid medium with the following constituents per liter: MS salts (full strength), 0.3 µM thiamine-HCl, 26.6 µM glycine, 2.4 µM pyridoxine-HCl, 4.1 µM nicotinic acid, 14.6 mM sucrose, 54.9 mM mannitol, and 554.4 mM myo-inositol. Explants were pretreated for 48 h in the above medium with 1.0 g casein hydrolysate/liter. The purpose of this pretreatment medium was to promote the appearance of contaminating bacteria. No growth regulators were added to tissue culture media. Cultures were maintained in a growth room at 18C under cool-white fluorescent lights (photosynthetic photon flux 15 mol·m^{-2}·s^{-1}) with a 16-h photoperiod. Cultures were placed at 1 rpm on a Junior Orbit Shaker (Labline Instruments, Melrose Park, Ill.), a Rollerdrum (New Brunswick Scientific Co., Edison, N.J.), or a tilting machine (Harris and Mason, 1983). Experiments were assessed visually for contamination after 14 days.

In addition to the pretreatment medium noted above, stem explants also were placed directly in a nutrient broth (Difco Labs, Detroit). However, contaminating bacteria grew faster and were detected earlier by pretreating them with an MS medium containing casein hydrolysate. Stem explants also were placed directly in a nutrient broth (Difco Labs), but faster (2 to 3 days) initial bacterial growth was obtained by the above initial culturing on an MS medium containing casein hydrolysate. The experiment was conducted twice and the results averaged.

Isolating bacteria. Bacteria were isolated from contaminated cultures on yeast–glucose medium (YGM) (De Boer and Copeman, 1980) and nutrient medium (Oxoid, Hants, England). Cultures were selected randomly and purified by restreaking on the two media. Slants were prepared from single colonies and maintained on YGM and nutrient medium. Standard tests were carried out to identify isolates (Cowan and Steel, 1970).

We assessed the bacterial constituents of city water by filtering 20 liters of water through a 0.22-µm capsule. The filter membrane was cut into four pieces and placed on nutrient medium. Resulting bacterial colonies were isolated and maintained as outlined above. Bacteria were identified initially at the Fredericton Research Station and their identity was confirmed by B.N. Dhanvantari of the Agriculture Canada Research Station, Harrow, Ont., Canada.

Bacterial growth in stem explant cultures from greenhouse stock plants usually appeared as a cloudy, white, or yellow haze in the culture medium within 4 to 5 days of culture. In some cultures, contamination was slower to develop and was not evident until 7 or 8 days.

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Contaminated stem explants were observed in tissues from plants derived from surface-disinfested tubers and those from tissue culture plantlets.

Sterilizing city water affects contamination percentage of stem explants. Stem explant cultures established from greenhouse stock plants that were watered exclusively with filter-sterilized city water showed less contamination than those established from greenhouse stock plants that were watered with unfiltered city water (Table 1). Transporting stem explants in city water from the greenhouse to the laboratory also contaminated subsequent tissue cultures (Table 1).

Bacteria from randomly selected contaminated cultures were principally *Listeria* spp. and *Corynebacterium* spp. A third type was *Enterobacter* spp., *Pasteurella* spp., or *Actinobacillus* spp. (Table 2). Identifying isolates from a 0.22-µm filter capsule through which 20 liters of raw city water had passed indicated the possible presence of six genera (Table 2). The five principal bacterial genera found in contaminated cultures also were identified in the filter capsule. The staff of the local municipal water department reported that the presence of an "uncharacterized iron-manganes-bacterial complex" is common on the inside surface of the city's old iron water mains (Laurrie Corbett, Fredericton city water engineer, personal communication).

Contamination of potato stem explant cultures reported here probably resulted from contaminating greenhouse stock plants with bacterially infested city water. The similarity is striking between the bacterial flora found in stem explant cultures from greenhouse stock plants irrigated with city water and the flora grown from filter disks. The bacteria isolated from city water appear to be soil- and wind-borne (Cowan and Steel, 1970), and there is no evidence to suggest that they are pathogenic to potato (Hooker, 1983). Contamination by soil- and wind-borne bacteria has been reported previously in apple cultures (Zimmerman, 1984). We observed that the quantity of microorganisms isolated from raw city water varied considerably over time. This may have been caused by periodic changes in water pressure and velocity in the mains that removed varying amounts of the iron–manganes-ebacterial complex from the inside walls of the water mains. Watering greenhouse stock plants with, and transporting explants in, filter-sterilized city water effectively reduced contamination levels.

City water supplies are monitored routinely for bacterial contamination (Tobin et al., 1980). Fredericton's iron water pipes were installed in 1883, and the uncharacterized iron–manganese-bacterial complex on the inside surfaces forms a "slime" that is impervious to exposures of 0.3 ppm chlorine for <10 min. We are not aware of any previous reports linking contaminated water to bacterial infestation problems in tissue culture. Watering stock plants with filter-sterilized city water effectively provided propagules for tissue culture. However, this approach was too time-consuming for extended use. We have installed an ultraviolet disinfection assembly unit as an alternative to hand-filtering water, and this also has effectively reduced the contamination of stock plants used for tissue culture.

Table 1. Effect of transporting' Shepody' and 'Yukon Gold' potato stem explants in city water on percent contamination of tissue cultures. Stock greenhouse plants were grown from tissue-cultured potato plants grown in disinfested soil mix and watered with sterile or city water (20 stem explant cultures per treatment).

	Contamination (%)	
	Explants transported to laboratory	
Cultivar	In city water	In sterile water
Stock plants watered with flter-sterilized water		
Shepody	10	0
Yukon Gold	30	5
Stock plants watered with city water		
Shepody	40	5
Yukon Gold	55	**0**

Table 2. Bacterial isolates from contaminated potato stem explant cultures and cultured filter pieces of 0.22-pm filter capsule through which city water had been filtered.

Bacteria source	Isolates identified
Contaminated cultures	*Listerium* spp., *Corynebacterium* spp., *Enterobacter* spp., *Actinobacillus* spp. *Pasteurella* spp., *Bacillus* spp.
Filter capsule	*Listerium* spp., *Corynebacterium* spp., *Enterobacter* spp., *Actinobacillus* spp. *Pasteurella* spp., *Vibrio* **spp.**

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