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DETECTION AND IDENTIFICATION OF BACTERIAL CONTAMINANTS FROM STRAWBERRY RUNNER EXPLANTS[1]

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SUMMARY

Microbial contaminants were detected in surface-sterilized strawberry runner explants partially submerged in half-strength liquid Murashige and Skoog medium. From June to August, 1994, 45 of 70 strawberry genotypes were found to be contaminated, whereas from June to August, 1995, 53 of 72 contained contaminants. There were more bacterial than fungal contaminants. Bacterial contaminants from 22 strawberry genotypes were isolated, purified, and identified to genus by standard biochemical tests such as Gram's stain, motility, oxidase, and gelatinase, and carbon source utilization (Biolog Microplates, Biolog, Inc., Hayward, CA). Among the 30 isolates identified with the Biolog system, the majority were fluorescent pseudomonads including *Pseudomonas fluorescens* types A, F, and G. *P. corrugata, P. tolaasii, P. paucimobilis, Xanthomonas campestris, Xanthomonas* spp., and *Enterobacter cloacae* were also identified. Five Gram-negative and two Gram-positive contaminants could not be identified by the Biolog test. Biochemical tests were used to characterize the bacteria and to confirm Biolog test results. Bacteria identified in this study were soil, water, and plant related, indicating that efforts to reduce explant contaminant levels should be centered on the care of stock plants or the sterility of the watering system.

Key words: contaminant detection; endophytic bacteria; *Fragaria*; micropropagation; *Pseudomonas; Xanthomonas.*

INTRODUCTION

Bacterial contamination is one of the most crucial problems of plant tissue culture, both in research and commercial production (Cassells, 1991; Leifert and Waites, 1992). Often bacterial contamination is difficult to detect (Debergh and Vanderschaeghe, 1988; Viss et al., 1991). Even healthy plants can contain several bacteria (Debergh and Vanderschaeghe, 1988; Leggatt et al., 1988), and some plant exudates may look similar to bacterial growth (Bastiaens, 1983; Finer et al., 1991). Contaminated plants may lack symptoms, have reduced multiplication rates, reduced rooting rates, or may die (Leifert et al., 1989, 1992).

Contaminants in plant tissue cultures come from poor sterile technique (Cassells, 1991; Leifert and Waites, 1992), from bacteria that survive alcohol flaming, such as *Bacillus* spores (Boxus and Terzi, 1987; Singha et al., 1987), and from mites and thrips that contaminate subsequent to surface sterilization (Blake, 1988; Leifert et al., 1989). Contaminants also come from explants (Cassells, 1991; Kneifel and Leonhardt, 1992) such as epiphytic bacteria that are resistant to surface sterilization (Leggatt et al., 1988); and endophytic bacteria that are present in plants due to wounds and natural openings, or are present in root systems (Cassells, 1991). Seabrook and Farrell (1993) reported bacterial contamination of explants caused by watering stock plants with dirty water. Skirvin et al. (1993) found that woody plant sticks forced in water prior to explant collection were quickly contaminated with a marker bacterium added to the water. Bacteria may remain in plant tissues for a long time before bacterial growth or disease symptoms develop (Norman and Alvarez, 1994). Some contaminants are introduced during subculture and some are quiescent in the tissue for some time before beginning active growth; thus, plant cultures should be indexed at regular intervals and not only at the initiation of explants (Leifert et al., 1989; Kane, 1995). Bacterial outbreaks *in vitro* cause major losses of time and resources (Seabrook and Farrell, 1993); thus, rapid and dependable methods for early detection should be used to control and prevent bacterial spread in *in vitro* systems (Norman and Alvarez, 1994; Reed et al., 1995).

Several types of indexing media are used to detect bacteria: 523 medium (Viss et al., 1991; Reed et al., 1995); Murashige and Skoog (1962) medium alone or with additions such as yeast extract, peptone, or glucose (Boxus and Terzi, 1987; Leifert et al., 1989); and culture medium with the addition of coconut water (Norman and Alvarez, 1994).

Many different yeasts and bacteria are detected in plant cultures. Boxus and Terzi (1987) found the yeast *Torulopsis*, and bacteria *Pseudomonas, Acinetobacter, Flavobacterium*, and *Corynebacterium*. Leggatt et al. (1988) detected yeasts, *Corynebacterium* spp., and *Pseudomonas* spp. Leifert et al. (1989) isolated *Bacillus, Enterobacter, Staphylococcus, Pseudomonas*, and *Lactobacillus*. Bacterial contaminants found in plants cultured for 4 wk or less were motile Gram-

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negative bacteria from plants or soil, whereas contaminants from plants cultured for at least 12 mo. were more likely to be Gram-positive bacteria from human sources (Leifert et al., 1989). Kneifel and Leonhardt (1992) found that some plants contained mixtures of Gram-negative rods, Gram-positive rods, and cocci. Mint cultures were contaminated mostly with Gram-negative plant- and soil-associated bacteria such as *Agrobacterium* and *Xanthomonas* (Buckley et al., 1995). Different types of bacteria are found on various plant species and at specific stages of plant cultures. Leifert et al. (1989) suggests that by determining bacterial identity, it may be possible to determine the source of contaminants.

Bacteria are identified and characterized by standard biochemical tests such as Gram's stain, catalase activity, oxidase activity, and motility (Bradbury, 1988; Leifert et al., 1989); or by rapid identification methods such as the Biolog system (Jones, 1993; Lacroix et al., 1994) or API test strips (Leifert et al., 1989; Lacroix et al., 1994) for carbon utilization, fatty acid profiles (FAP) (Jones et al., 1993; Buckley et al., 1995); DNA techniques (Jones et al., 1993); and protein electrophoresis (Lacroix et al., 1994). Jones et al. (1993) and Verniere et al. (1993) recommend using more than one test for more accurate identification.

The goals of this study were to devise an effective method for detecting bacterial contaminants in strawberry runner explants, and to isolate, characterize, and identify bacterial contaminants.

Materials and Methods

Plant material used. In vitro cultures were initiated from runners taken from pot-grown strawberry plants (medium 2:1:1 bark: pumice: peat) in a screenhouse at the USDA-ARS National Clonal Germplasm Repository, Corvallis, OR. Runners were disinfested by immersion in a 10% household bleach (5.25% sodium hypochlorite; Clorox, Oakland, CA) solution containing 1% Tween-20 for 10 min, rinsed twice in sterile deionized water, and grown in individual 16- × 100-mm tubes containing half-strength liquid Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), pH 6.9 at 25° C for 10 d with 16 h of light (25 $mEm^2 s^{-1}$). After 10 d, runners were transferred to full strength MS medium with 1.4 μM NaH_2PO_4, 0.4 μM adenine sulfate, 4.4 μM benzyladenine, 0.3 μM gibberellic acid, 5.7 μM indoleacetic acid, 3 g l^{-1} agar, and 1.25 g l^{-1} gelrite at pH 5.7. Chemicals were obtained from Sigma Chemical Co., St. Louis, MO.

Plant response to submerged growth. Five runners each of six *Fragaria chiloensis* (L.) Duchesne genotypes (Lonquimay 02E, 04D, 04F, and 04G; Maullin; and Nashvelbuto National Park 01A) were submerged in half-strength liquid MS medium. Another five runners each of the same *Fragaria* genotypes were partially submerged (0.5–1.0 cm of the runner tips were not submerged). Cultures were grown for 10 d in the liquid medium and then transferred to solid medium to determine survival.

Detection of contaminants. Runners (2–12 from each genotype) from 70 genotypes of *Fragaria* species and cultivars were collected from June to August, 1994 and screened for contaminants by partial submersion in liquid medium as above. Runners (2–12 from each genotype) from 72 additional genotypes were collected from June to August, 1995 and screened as above but in medium with 265 mg peptone per liter and 88 mg yeast extract per liter. Bacterial growth produced turbidity within 10 d in liquid medium containing contaminated runners. Explants with no detectable contaminants after 10 d were transferred to solid medium and observed for visible contaminants

Isolation and purification of bacteria. Contaminants were transferred with a sterile cotton swab to 0.8% nutrient broth (Difco Laboratories, Detroit, MI; Sigma) with 1% glucose and 0.1% yeast extract at pH 6.9 and incubated at 25° C. Bacteria were purified by repetitive streaking on 1.2% nutrient agar plates until uniform colony types were observed.

Diagnostic tests and standard cultures. Gram stain, KOH, motility, oxidase, starch hydrolysis, and gelatinase tests were performed according to the methods described by Klement et al. (1990). Cultures of standard bacterial strains were provided by Ms. Marilyn Miller, Department of Botany and Plant Pathology, Oregon State University: *Xanthomonas campestris, X. incanae,* and *X. vesicatoria*; Dr. Joyce Loper, USDA-ARS, Horticultural Crops Laboratory, Corvallis, OR: *Enterobacter cloacae* (EcCT-501), *Pseudomonas fluorescens* Pf-5, and *P. putida* (A 12); and Dr. Patricia Buckley, USDA-ARS National Clonal Germplasm Repository, Corvallis, OR: *Agrobacterium radiobacter*. These strains were used as reference cultures for comparison in the characterization and identification of bacterial strains isolated in this study.

Carbon substrate utilization. The ability of bacterial strains to oxidize 95 carbon substrates was tested on Biolog Gram-negative and Gram-positive Microplates (Biolog, Inc., Hayward, CA). Bacteria were grown on tryptic soy agar (TSA) for 4–18 h at 30° C, then diluted in 0.85% saline solution at pH 5.5–7.0 to a standard optical density. The turbidity of bacterial solutions was read at 600 nm (Spectronic 20, Bausch & Lomb Inc., Rochester, NY) to an absorbance of 0.21–0.26 for gram-negative and 0.34–0.43 for gram-positive bacteria. Bacterial solutions were aseptically transferred to Biolog Microplates (150 µl/well) with a multichannel micropipette. The results were read visually at 4 and 24 h. The data were analyzed with Biolog computer software, leading to placement of the isolates into genera, species, or strains.

TABLE 1

DETECTION OF CONTAMINANTS IN STRAWBERRY RUNNER EXPLANTS PARTIALLY SUBMERGED IN LIQUID MEDIUM FOR 10 DAYS

		Number of genotypes contaminated			
Year	Number tested	Only bacteria & yeasts	Only filamentous fungi	Mixed[a]	Total
1994[b]	70	23	11	11	45
1995[c]	72	32	12	9	53

[a]Some explants of these genotypes were contaminated with bacteria and others with fungi.

[b]1/2 MS liquid medium.

[c]1/2 MS liquid medium with 265 mg peptone per liter and 88 mg yeast extract per liter.

Results and Discussion

Plant response to submerged growth. Strawberry runners died when totally submerged in liquid medium, but all partially submerged plants survived and grew (data not shown). In contrast, mint explants survived total submersion in half-strength liquid MS for 6–10 d (Reed et al., 1995). These results suggest plant species respond differently to total submersion in liquid medium. Partial submersion provided high levels of contaminant detection and strawberry plant survival without agitation of the cultures.

Detection of contaminants. Contaminants were detected in 45 of 70 genotypes initiated in 1994 in basal medium (Table 1). Contaminants detected were 51% bacteria and yeasts, 24.5% filamentous fungi, and 24.5% mixed contaminants. Most genotypes had some uncontaminated explants and only in four genotypes were all explants contaminated. Similar results were seen in 1995 in enriched medium, with 53 of 72 genotypes contaminated; 60% were bacteria and yeasts, 23% filamentous fungi, and 17% mixed contaminants. Only in six genotypes were all explants contaminated. The 10% increase in contaminants detected in 1995 might be due to the peptone and yeast extract in the medium; however, this was only a screening process and these results need to be verified in a controlled experiment. This detection technique did not distinguish between endophytic and epiphytic contaminants.

Boxus and Terzi (1987) found several bacterial and yeast contaminants could not be detected on proliferation or elongation medium

TABLE 2

CARBON UTILIZATION OF BACTERIAL TYPES ISOLATED FROM STRAWBERRY RUNNER EXPLANTS AND IDENTIFIED WITH BIOLOG CARBON SOURCE UTILIZATION TESTS

	Isolates									
	Enterobacterium								*Xanthomonas*	
			fluorescens							
Carbon source	*cloacae* A	*corrugata*	A	F	G	type	*paucimobilis* A	*tolaasii*	spp.	*campestris* pv. *vesicatoria*
water	−	−	−	−	−	−	−	−	−	
α-cyclodextrin	−	−	−	−	−	−	+	−	−	−
Dextrin	+	−	−	−	−	−	+	−	+	+
Glycogen	+	−	−	−	−	−	+	−	+	+
Tween 40	+	+	+	+	+	+	+	+	+	−
Tween 80	+	+	+	+	+	+	+	+	+	−
N-acetyl-D-galactosamine	+	−	−	−	−	−	−	−	−	−
N-acetyl-D-flucosamine	+	+/−	+/−	+	+	+	+	+	+	+
Adonitol	−	−	+/−	−	+	+/−	−	+	−	−
L-arabinose	+	+	+	+	+	+	+	+	+	−
D-arabitol	−	+	+	+	+	+	−	+	−	−
Cellobiose	+	+/−	−	−	−	+	+	−	+	+
i-Erythritol	−	+/−	−	−	−	+	−	−	−	−
D-fructose	+	+	+	+	+	+	+	+	+	+
L-fucose	−	−	+/−	−	+	+/−	+	−	+	+
D-galactose	+	+	+	+	+	+	+	+	+	+
Gentiobiose	+	−	−	−	−	−	+	−	+	+
α-D-glucose	+	+	+	+	+	+	+	+	+	+
m-Inositol	+	+	+	+	+	+	−	+	−	−
α-D-lactose	+	−	−	−	−	−	+	−	+	−
Lactulose	+	−	−	−	−	−	+	−	+	+
Maltose	+	−	+	+	−	−	+	−	+	+
Mannitol	+	+	+	+	+	+	−	+	−	−
Mannose	+	+	+	+	+	+	+	+	+	+
D-mellibiose	+	−	−	−	−	−	+	−	−	+
β-methyl-D-glucoside	+	−	−	−	−	−	+	−	−	−
D-psicose	+	+/−	+	+	+	+	+	+/−	+	+
D-raffinose	+	−	−	−	−	−	+	−	−	−
L-rhamnose	+	−	+/−	−	+	+/−	−	+	−	−
D-sorbitol	+	+	+	+	+	+	−	+	−	−
Sucrose	+	+	+/−	+	−	+/−	+	−	+	+
D-trehalose	+	+	+	+	+	+	+	+	+	+
Turanose	+	−	−	−	−	−	+	−	−	−
Xylitol	−	−	+/−	−	−	−	−	−	−	−
Methyl pyruvate	+	+	+	+	+	+	+	+	+	+
Mono-methyl succinate	+	+	+	+	+	+	+	+	+	+
Acetic acid	+	+	+	+	+	+	+	/	+	+
cis-Aconitic acid	+	+	+	+	+	+	+	+	−	+
Citric acid	+	+	+	+	+	+	−	+	+	−
Formic acid	+	+	+	+	+	+	+	+	−	−
D-galactonic acid lactone	+	+	+	+	+	+	−	+	−	−
D-galacturonic acid	+	+	+	+	+	+	−	+	−	−
D-gluconic acid	+	+	+	+	+	+	−	+	−	−
D-glucosaminic acid	−	+	+	+	+	+	−	+	−	−
D-glucuronic acid	+	+	+	+	+	+	−	+	−	−
α-hydroxybutyric acid	/	+/−	+	+	+	+	+	−	+	+
β-hydroxybutyric acid	+	+	+	+	+	+	+	+	+	+
γ-hydroxybutyric acid	−	−	−	−	+	−	−	−	−	−
p-hydroxyphenylacetic acid	+	+/−	−	−	−	−	−	−	−	−
Itaconic acid	−	+/−	+/−	−	+	+	+	+	−	−
α-keto butyric acid	−	+/−	+	+	+	+	+	+/−	−	+
α-keto glutamic acid	+	+	+	+	−	+	+	+	−	+
α-keto valeric acid	−	−	+	+	+	+	+	−	−	
D,L-lactic acid	+	+	+	+	+	+	+	+	+	
Malonic acid	+	+/−	+	+	+	+	−	+	+	
Propionic acid	/	+/−	+	+	+	+	+	+/−	+	
Quinic acid	/	+	+	+	+	+	−	+	−	
D-saccharic acid	+	+	+	+	+	+	−	+		

TABLE 2 (Continued)

CARBON UTILIZATION OF BACTERIAL TYPES ISOLATED FROM STRAWBERRY RUNNER EXPLANTS AND IDENTIFIED WITH BIOLOG CARBON SOURCE UTILIZATION TESTS

	Isolates									
	Enterobacterium	*Pseudomonas*							*Xanthomonas*	
			fluorescens							
Carbon source	*cloacae* A	*corrugata*	A	F	G	type	*paucimobilis* A	*tolaasii*	spp.	*campestris* pv *vesicatoria*
Sebacic acid	−	−	−	−	−	−	−	−	−	−
Succinamic acid	+	+	+	+	+	+	+	+	+	+
Bromo succinic acid	+	+	+	+	+	+	+	+	+	+
Succinamic acid	/	+	+	+	+	+	+	+	+	+
Glucuronamide	+	+	+	+	+	+	−	+	−	−
Alaninamide	+	+	+	+	+	+	+	+/−	+	+
D-alanine	+	+	+/−	+	+	+	−	+	+	+
L-alanine	+	+	+	+	+	+	+	+	+	+
L-alaryl-glucine	+	+	+	+	+	+	+	+	+	+
L-asparagine	+	+	+	+	+	+	+	+	+	−
L-aspartic acid	+	+	+	+	+	+	+	+	+	+
L-glutamic acid	+	+	+	+	+	+	+	+	+	+
Glycyl-L-aspartic acid	+	−	−	−	−	−	+	−	+	−
Glycyl-L-glutamic acid	+	+/−	+	+	+	+	+	+	+	+
L-histidine	+	+	+	+	+	+	−	+	−	−
Hydroxy L-proline	−	+	+	+	+	+	+	+	+	−
L-leucine	−	+/−	+	+	+	+	−	+	−	−
L-ornithine	+	+/−	+	+	+	+	+	+/−	−	−
L-phenylalanine	−	−	+/−	−	−	+/−	−	−	−	−
L-proline	+	+	+	+	+	+	+	+	+	+
L-pyroglutamic acid	−	+	+	+/−	+	+	−	+	−	−
D-serine	+	+/−	+	+	+	+	−	−	−	−
L-serine	+	+	+	+	+	+	+	+	+	+
L-threonine	+	+/−	+	+	+	+	+	+/−	−	−
D,L-camitine	−	+/−	+	+	+	+	−	+	−	−
γ-amino butyric acid	/	+	+	+	+	+	−	+	−	−
Urocanic acid	+	+	+	+	+	+	−	+	−	−
Inosine	+	+	+	+	+	+	−	+	−	−
Uridine	+	+/−	+	+	+	+	−	+	−	−
Thymidine	+	−	−	−	−	−	−	−	−	−
Phenyl ethylamine	−	−	−	−	−	−	−	−	−	−
Putrescine	+	+/−	+/−	+/−	+	+	−	+/−	−	−
2-Amino ethanol	−	+	+	+	+	+	−	+	−	−
2,3-Butanediol	−	−	−	−	−	+/−	−	−	−	−
Glycerol	+	+	+	+	+	+	−	+	+	+
D,L-α-glycerol phosphate	+	+/−	+/−	+/−	+	+	−	+/−	−	−
Glucose-1-phosphate	+	−	−	−	−	−	+	−	−	−
Glucose-6-phosphate	+	−	−	−	+	+/−	+	−	−	−

+ Metabolizes the carbon source.
+/− Several strains identified; some strains are +, other strains are −.
− Does not metabolize the carbon source.
/ Borderline reading; faint positive.

but were apparent and often lethal on rooting medium. Peptone and yeast extract added to the proliferation and elongation medium allowed detection of bacterial contaminants within two subcultures. Reed et al. (1995) found that liquid MS medium alone detected most contaminants in mint explants with few additional contaminants detected later. Additional experiments with contaminated mint explants showed that medium with peptone and yeast extract produced more obvious and faster bacterial growth providing better detection without causing toxicity to the plants (Tanprasert, unpublished data).

Characterization and identification of bacteria. Bacterial contaminants isolated from 22 strawberry genotypes included approximately 16 bacterial strains as determined by the Biolog database (Table 2), standard cultures, and biochemical tests (Table 3). One to four bacterial strains were found per strawberry genotype. More than half of the genotypes tested contained more than one bacterial strain. The 30 bacterial isolates identified with the Biolog system of carbon utilization and confirmed with standard biochemical tests were predominantly fluorescent pseudomonads (18 isolates) or other pseudomonads (8 isolates), with three xanthomonads, and one enterobacterium. Seven isolates were not in the Biolog database and were not identified.

Pseudomonads are widely distributed in nature and some of them are plant pathogens. Saprophytic fluorescent pseudomonads are very common in soil and on plant surfaces. All *Pseudomonas* spp. isolated from strawberry runners were fawn (medium tan) colored except *P. paucimobilis* which was yellow. Many produced fluorescent pigment

TABLE 3

CHARACTERISTICS OF BACTERIAL TYPES ISOLATED FROM STRAWBERRY RUNNER EXPLANTS AND IDENTIFIED WITH BIOLOG CARBON SOURCE UTILIZATION TESTS

Bacterial species	No. isolates[a]	Motility	Gelatinase	Oxidase[b]	Kings's B[c]	Starch hydrolysis	SI[d]
Enterobacter cloacae A	1	+	+	−	−	−	0.960
Pseudomonas corrugata	5	+	+	+	−	−	0.507–0.906
P. fluorescens type A	2	+	+	+	+	−	0.611
P. fluorescens type F	13	+	+	+	+/−	−	0.501–0.942
P. fluorescens type G	1	+	+	+	+	−	0.532
P. fluorescens type	2	+	+	+	+	−	
P. paucimobilis A	1	+	+	+	−	−	0.747
P. tolaasil	2	+	+	+	−	−	0.690–0.708
Xanthomonas spp.	2	+	−	w	−	−	
X. campestris pv. *vesicatoria*	1	−	−	w	−	+	0.937

[a]Isolated from one or more strawberry genotypes.
[b]w = Weak reaction.
[c]Fluorescent on King's B medium under ultraviolet light.
[d]Similarity index of ≥ 0.5 is a significant match to known organisms on the Biolog database.

(fluoresced on King's B medium). *P. fluorescens* is separated into seven different biotypes (A–G) (Stanier et al., 1966) or into five biovars (I–V) (Krieg and Holt, 1984). Each type can be further separated into many strains. Differentiation between biotypes requires a number of diagnostic tests and results are variable among strains of the same biovar. In this study, the most common bacteria found were *P. fluorescens* type F (13 isolates); two strains could be distinguished based on the presence (10) or absence (3) of the fluorescent pigment.

P. corrugata (5 isolates) had fawn-colored, wrinkled colonies and could hydrolyze gelatin, but not starch, as indicated in descriptions in Bergey's manual (Krieg and Holt, 1984). *P. tolaasii* (2 isolates) has a translucent colony and is pathogenic for cultivated mushrooms (Wong and Preece, 1979). *P. paucimobilis* (1 isolate) was motile, did not produce fluorescent pigment, and did not hydrolyze gelatin or starch. *P. paucimobilis* can be isolated from many sources such as tap water and plant surfaces (Holmes et al., 1977).

Xanthomonad colonies are usually yellow, smooth, and butyrous or viscid. All except one have xanthomonadin pigment produced from brominated aryl polyenes. They do not use asparagine as a sole carbon and nitrogen source. All are considered to be plant pathogens (Krieg and Holt, 1984). *Xanthomonas* spp. found were yellow and oxidase negative or weakly positive. One, *X. campestris* pv. *vesicatoria*, is pathogenic to many plant species such as *Capsicum* spp. and *Datura stramonium*, but is not considered to be pathogenic on strawberries (Krieg and Holt, 1984). *X. campestris* pv. *vesicatoria* colonies were yellow and hydrolyzed starch. Two other *Xanthomonas* spp. had yellow colonies but did not hydrolyze starch and could not be identified to species level by Biolog tests.

Enterobacteria are found in soil, water, fruits, vegetables, grains, flowering plants, and trees, and can be plant pathogens (Krieg and Holt, 1984). *Enterobacter cloacae* A, found in strawberry runner explants in this study, was beige, moist, slimy, very motile, and very fast growing.

Five other Gram-negative bacteria, two Gram-positive bacteria, and two yeasts were not identified in this study. They were likely to be soil, water, or plant-related microorganisms since strawberry runners are closely associated with the soil and would be easily contaminated by soil-borne microorganisms.

Standard bacterial cultures. Standard cultures were used together with other identification and characterization methods such as biochemical and carbon source utilization (Biolog) tests. Standard cultures were very useful as controls in diagnostic tests and also for colony characterization. Two strawberry runner bacterial isolates were the same as the standard cultures of *Enterobacter cloacae* (Fra 655) and *X. campestris* (Fra 557b).

Carbon substrate utilization (Biolog) test. Biolog was a rapid (24 h) and dependable method for identifying most of the Gram-negative bacteria detected in this study because most bacterial strains found were *Pseudomonas* spp., which were included in the database (Table 2). Classification into strains was simplified with the Biolog tests. The main difficulty in reading Biolog plates was visually detecting color change. Color analysis requires careful observation because even a small misreading can result in a shift in identification from one bacterial strain to another. An optical scanner would provide more reliable data although machine misreading also may occur.

Because of the possibility of misleading results from test kits, Verniere et al. (1993) recommend using other biochemical tests to confirm results. Leifert et al. (1989) recommend using biochemical tests together with the API test for more reliable identification. API test strips were developed mainly for medical use and thus are less effective for plant and soil bacteria. The addition of carbon source utilization information to the database by an individual researcher can improve the usefulness of test kits. When Verniere et al. (1993) used Biolog's Microlog database, only 6.8% of their plant pathogenic bacteria were identified. When they did other biochemical tests and added their own database to Microlog, up to 70% of the bacteria were identified.

Standard biochemical tests. Standard biochemical tests were useful for identifying many of the isolates (Table 3). Gram staining was done immediately after purifying cultures in order to group bacteria and to better visualize bacterial shapes. King's B medium was used for identifying fluorescent pseudomonads; it was especially useful in this study because many fluorescent pseudomonads were found. Starch hydrolysis, together with xanthomonadins, distinguished some xan-

thomonads; however, two xanthomonad isolates produced xanthomonadins but did not hydrolyze starch.

After identification and characterization with Biolog and special biochemical tests, bacterial characteristics should also be confirmed with Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984). Some Gram-negative and Gram-positive isolates could not be identified by Biolog. Biolog testing would have been more useful in this study if we had built our own database and included more plant-related standard bacterial cultures.

Conclusions

Microbial contaminants were successfully detected in strawberry runners partially submerged in half-strength liquid MS medium, but mortality was high in totally submerged runners. We recommend partial submersion of runners for effective contaminant detection. Bacterial, yeast, and fungal contaminants were detected, but only bacteria were cultured for further identification. The 30 bacterial isolates identified with the Biolog system of carbon utilization and confirmed with standard biochemical tests were predominantly fluorescent pseudomonads (18 isolates) or other pseudomonads (8 isolates), with three xanthomonads, and one enterobacterium. Seven isolates were not in the Biolog database and were not identified. For these unidentified isolates, additional standard tests and comparison with descriptions in Bergey's Manual (Krieg and Holt, 1984) may provide identification.

Identification and characterization of bacterial contaminants provided information on the sources of contaminants. Bacteria found in this study were soil, water, and plant related, indicating that efforts to reduce explant contaminant levels should be centered on the care of stock plants, type of soil mix, and the sterility of the watering system.

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