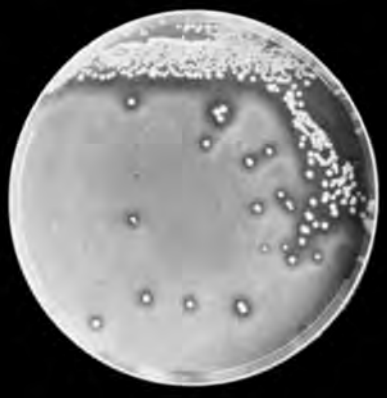
Survival of potato-blackleg and soft-rot bacteria

Thomas J. Burr

Milton N. Schroth

David N. Wright



A

B

Fig. 1. *A.* Colonies of *Erwinia* spp. growing on selective medium (PT) after 48-hour incubation at 28°C. Clear zones appear about colonies within 30 seconds after flooding with a 1 percent centrimide solution. *B.* Determinations of percentage of tubers in potato seedlots infested with *Erwinia* soft-rot species. Each set of 11 streaks represents one seed-tuber sample.

Potato blackleg and soft-rot of tubers, caused by *Erwinia carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica*, respectively, continue to cause mild to severe field, shipping, and storage losses of potatoes in California. An understanding of the survival capabilities of the bacteria and the factors that contribute to their spread is essential for the development of effective controls for these diseases. Previous studies have demonstrated that (1) the bacteria are seed-borne, and (2) they overwinter in the lenticels and stem-end portions of the seed tubers. Whether the bacteria can survive in soil, however, has been much more controversial, primarily because of the lack of sensitive techniques for detecting bacteria populations below 1000 cells/g of soil.

Selective medium

A highly selective medium for the isolation of soft-rot *Erwinia* spp. from soil and plant material was developed. The medium (PT) consists of (g/liter): polygalacturonic acid (Sunkist), 5.0; $NaNO_3$, 1.0; K_2HPO_4, 4.0; $MgSO_4 \cdot 6H_2O$, 0.2; ionagar (Wilson Diagnostics, Inc.), 9.0; Tergitol anionic 7 (sodium heptadecyl sufate), 0.1 ml; and 1N NaOH, 17 ml. The final pH of the medium was about 7.0. To differentiate *Erwinia* spp. from the few other bacterial species that may grow on the medium, we flooded plates with a 1 percent solution of cetyltrimethylammonium bromide (cetrimide) Sigma, and observed clear zones around the *Erwinia* colonies. The bacteria which are identical in appearance are further distinguished by their whitish, scalloped-edged colonies which are about 3 mm in diameter on PT after 48 hours incubation at 28°C (fig. 1A). Medium PT supports good growth of the *Erwinia* soft-rotters while eliminating 99.8 percent of all other soil microorganisms.

Detection of soft-rot

PT can be utilized for detecting the percentage of tubers in potato seedlots that are infested with soft-rot *Erwinia* spp. Ten lenticels and the stem end of each seed tuber are stabbed with a sterile wooden toothpick and streaks are made across the medium after each stab. Plates are incubated 48 hours at 28°C. Five tubers can be assayed on each petri plate and seedlot evaluations can be made in 48 hours (fig. 1B). Seedlot infestations ranged from 8 to 100 percent of the tubers in samples tested in 1976 (table 1). This technique is reliable and requires a minimum of time, space, and laboratory facilities.

PT, in conjunction with an anaerobic enrichment technique, was also used to detect *Erwinia* soft-rot spp. in the root zones of many cultivated and noncultivated plants and in 26 different field soils. Selective enrichment allows preferential multiplication of *Erwinia* soft-rot spp. in relation to other soil bacteria, thus making detection by standard plating methods possible. Fifteen ml of PT broth were added to 25 g soil in a petri dish when isolating from field soil, and 5.0 ml were added to 0.5 g root tissue with adhering soil when isolating from the root-zone soil. Enrichment samples were then incubated anaerobically at 28° C for 48 hours, at which time 10-fold serial dilutions with water were plated on PT. Plates were incubated in a GasPak System anaerobic jar; disposable hydrogen plus carbon dioxide envelopes were used to maintain an anaerobic environment.

The bacteria were isolated from seven of the 26 soils, but only from those that either were being cropped at the time of sampling or had recently been cropped, with recognizable plant debris present. *Erwinia* soft-rot spp. were not isolated from soils that had been fallow for longer than six weeks. Many of the weeds sampled were obtained from along irrigation ditch banks several hundred yards from the nearest crop, and rhizosphere isolations demonstrated that the bacteria can survive in the root zones of many weeds and crop plants (table 2). As shown by other researchers, the bacteria may be disseminated by contaminated farm equipment, by insects, and possibly by aerosols.

Control of blackleg and soft-rot

Blackleg-free seed programs being established in some potato-growing regions should greatly reduce the incidence of disease, especially when compared to heavily infested seedlots. However, this practice alone would not entirely eliminate the disease in California since the bacteria survive readily in association with many crops and weed plants. Therefore, control of weeds in fields intended for planting is recommended. Also, previous crops may stimulate inoculum buildup in soils that leads to a high incidence of disease, especially if potatoes are planted within a few weeks after

TABLE 1. Soft-rot *Erwinia* spp. Detected in Potato Seed Tubers by the Direct Lenticel Isolation Method

Cultivar	Source	No. tubers sampled	Tubers infested with *Erwinia*
			percent
White Rose	California	24	88
White Rose	Oregon	24	100
White Rose	California	20	50
White Rose	Washington	25	12
Centennial	Colorado	24	46
Centennial	Colorado	24	88
Russet Burbank	Montana	25	24
Russet Burbank	Canada	25	24
Russet Burbank	Canada	24	8
Russet Burbank	California	25	9
Red Lasoda	California	25	9
Nooksack	Washington	17	12
Kennebec	North Dakota	12	17
Kennebec	California	12	82
Kennebec	California	12	75

TABLE 2. Soft-rot *Erwinia* spp. Isolated from Root Zone Soils of Various Crop and Weed Plants

Crop or weed	No. of plants sampled	No. of plants with *Erwinia*
Latuca sativa L. (lettuce)	15	8
Daucus carota L. var. *sativa*	10	4
Brassica oleracea L. var. *botrytis* L. (broccoli)	5	5
Medicago sativa L. (alfalfa)	8	2
Beta vulgaris L. (sugarbeet)	10	1
Sorghum vulgare Pers. (sorghum)	5	0
Solanum tuberosum L. (apparently healthy seedpiece)	20	11
Brassica oleracea L. var. *botrytis* L. (cauliflower)	6	0
Brassica oleracea var. *capitata* L. (cabbage) (seedlings)	5	0
Anagalis arvensis L. (scarlet pimpernel)	1	1
Sonchus asper (L.) Hill (spiny sowthistle)	2	1
Malva parviflora L. (little mallow)	9	6
Portulaca oleracea L. (common purslane)	8	1
Sisybrium irio L. (London rocket)	1	0
Polygonum argyrocoleon Steud. (silversheath knotweed)	1	0
Chenopodium murale L. (nettleleaf goosefoot)	1	0
Amaranthus palmeri Wats. (palmer amaranth)	1	0
Poa annua L. (annual bluegrass)	3	3
Chenopodium album L. (common lambsquarters)	7	0

crop harvest.

General sanitation should be practiced whenever possible since typical seedlots are infested with *Erwinia* spp. and contaminate any equipment they contact. Many chemicals such as chlorine will kill the bacteria on surfaces of tubers and equipment. However, the principal source of soft-rot and blackleg bacteria appears to be the potato lenticels. An effective control, therefore, appears to depend on the finding of a material that will penetrate and eradicate the bacteria in these sites without causing phytotoxicity.

Thomas J. Burr is Graduate Student and Milton N. Schroth is Professor, Department of Plant Pathology, University of California, Berkeley. David N. Wright is Farm Advisor, Kern County.

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