Occurrence of Soft-rot Erwinia spp. in Soil and Plant Material

T. J. Burr and M. N. Schroth

Department of Plant Pathology, University of California, Berkeley, CA 94720.
Supported in part by the California Potato Research Advisory Board.
Accepted for publication 14 April 1977.

ABSTRACT

BURR, T. J., and M. N. SCHROTH. 1977. Occurrence of soft-rot Erwinia spp. in soil and plant material. Phytopathology 67:1382-1387.

A selective, differential agar medium (PT) was used to study the occurrence of the soft-rot bacteria, *Erwinia carotovora* var. *carotovora* and *Erwinia carotovora* var. *atroseptica* (ESR), in soil and plant materials. The efficiency of isolation was greatly enhanced by using an anaerobic enrichment technique prior to plating on the medium. *Erwinia* soft-rot spp. were isolated from rhizosphere soils of many weeds and crop plants, and from some field soils containing plant residues. Populations at some sites were large enough to be detected without the aid of enrichment. *Erwinia* soft-rot bacteria were not detected in fallow soils devoid of vegetation or recognizable plant residues. The ESR bacteria were not considered true soil inhabitants although they have a protracted soil phase because of their capacity to perpetuate themselves indefinitely when in association with roots of a diverse number of plants. A direct lenticel isolation technique was developed which enabled the determination of the percent ESR infestations in potato seedlots within a 48-hr period. Infestations ranged from eight to 100%. Many of the strains of ESR isolated in these studies could not be identified as either *Erwinia carotovora* var. *carotovora* or *E. carotovora* var. *atroseptica* with standard testing schemes.

Additional key words: potato blackleg, blackleg-free seed.

Recent studies on the ecology of the potato blackleg and soft-rot organisms, *Erwinia carotovora* var. *carotovora* Dye and *E. carotovora* var. *atroseptica*, (Hellmers & Dowson) Dye have resulted in contrary views concerning the ability of these bacteria to survive in soil (2, 11, 19, 25). Although it is generally agreed that potato seed-pieces frequently are infested with a substantial amount of blackleg inoculum (4, 11, 23, 24), both *E. carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica* (ESR) have been reported to survive in the rhizosphere or in association with plant material of cultivated and noncultivated crops (3, 8, 15, 16, 17, 26, 31, 32) and in some agricultural soils (19). In contrast, other researchers have claimed that neither *E. carotovora* var. *carotovora* nor var. *atroseptica* survive in field soils for an extended period of time (11, 21, 25). Kikumoto (15) and Meneley and Stanghellini (19) attribute these differences to the inefficiency of techniques for detection of small populations of the bacteria in soil.

An understanding of the occurrence and duration of a soil-borne phase of the bacteria is basic to developing controls for blackleg and soft-rot. The development of blackleg-free seed (10, 25) would be a logical method for control if the bacteria were not present in planting soils or if cultural methods could be used to reduce the amount of inoculum. A long series of selective media has been designed for the isolation of ESR, based primarily on the organism's ability to produce pectolytic enzymes (2, 20, 29). These media generally have become more selective and differential with each modification; however, many soil bacteria grow on these media and often interfere with recovery of ESR when isolating directly from plant material or from rhizosphere soils where microbial activity is great.

This paper presents the development of a highly selective, differential medium for the isolation of *E. carotovora* var. *carotovora* and var. *atroseptica* from soil and its employment in the evaluation of potato seedlots for detecting percent infestations of ESR. The controversy concerning the occurrence of ESR in soil also was examined by using an enrichment technique to detect the bacteria in the rhizospheres of cultivated and noncultivated crops, and in some field soils in California. An investigation also was made to determine which of the major ESR groups predominate in California.

MATERIALS AND METHODS

The selective medium (PT) contains (g/liter): polygalacturonic acid (Sunkist, Corona, CA 91720), 5.0; $NaNO_3$, 1.0; K_2HPO_4, 4.0; $MgSO_4 \cdot 6H_2O$, 0.2; Ionagar® (Wilson Diagnostics, Inc., Glenwood, IL 60425), 9.0; Tergitol® anionic 7 (sodium heptadecyl sulfate, Sigma Chemical Co., St. Louis, MO 63178), 0.1 ml; and 1N NaOH, 17 ml. The final pH of the medium was about 7.0. Polygalacturonic acid lots from ICN Pharmaceuticals Inc. (Cleveland, OH 44128) and United States Biochemical Corp. (Cleveland, OH 44128) were satisfactory for use in PT. Difco Bacto-Agar (18 g/liter also could be substituted for Ionagar with no apparent loss in selectivity of the medium. All ingredients are added prior to autoclaving. The differentiation of ESR from other bacterial species that may grow on the medium is made by flooding plates with a 1% solution of cetyltrimethylammonium bromide (cetrimide) (Sigma Chemical Co., St. Louis, MO 63176) and observing clear zones about the *Erwinia* colonies. *Erwinia* colonies are further discerned on PT by their whitish, scallop-edged colonies which are about 3 mm in diameter after 48 hr of growth. Although cetrimide is toxic to ESR, isolations may be made within 5 min of flooding.

Criteria used to identify suspected ESR isolates were a negative Gram stain, glucose fermentation in 3 days (14), nonfluorescence in King's Medium B (KB), pectolytic activity, ability to produce acid from α-methyl-D-glucoside (5), production of reducing substances from sucrose (5), and growth at 37 C (5). The ability of isolates to produce blackleg symptoms at 18 C was determined by inserting a sterile toothpick smeared with inoculum from a 24-hr-old culture grown on KB into stems of 4-wk-old potato plants. Two plants were inoculated with each isolate and the experiment was repeated once. One set of inoculated plants were kept in a moist chamber to determine the effect of 100% RH on symptom development.

Medium PT was evaluated for its toxicity to ESR and common soil and rhizosphere microorganisms. Representative strains of *E. carotovora* var. *carotovora* (C-8, LW-1, SW-2, and C-2b) and *E. carotovora* var. *atroseptica* (SR-150, TLBL-1) were used in these tests. Serial dilutions of each strain were plated on PT, and on KB which was used as the control medium. Each dilution was plated three times and colony counts were made after 48 hr of incubation at 28 C. Isolates of ESR were obtained from ditch bank weeds (LW-1, SW-2), carrots (C-2b, C-8) collected in the Salinas Valley, California, and potato (TLBL-1) from Tulelake, California; isolate SR-150 was obtained from Arthur Kelman.

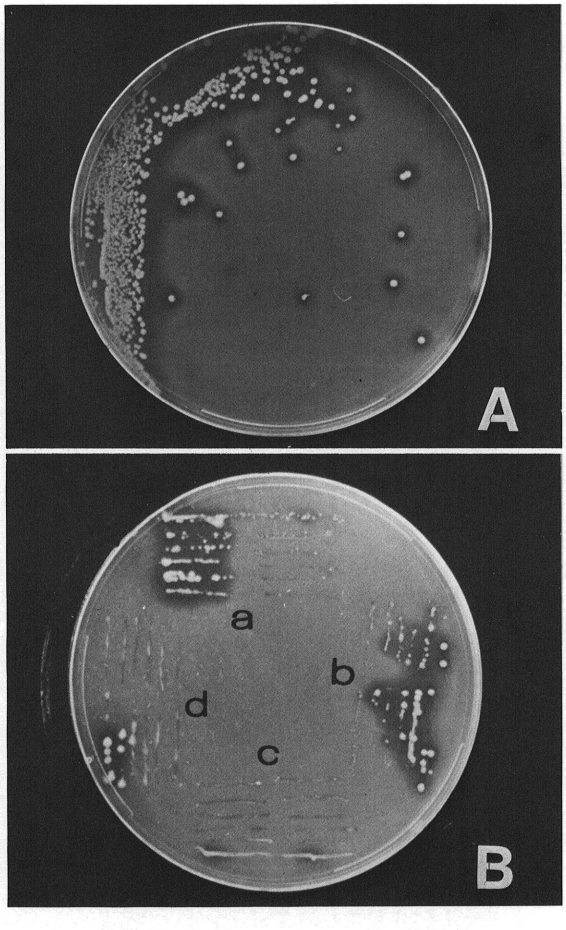

Fig. 1-(A, B). A) *Erwinia carotovora* var. *carotovora* colonies on selective medium (PT) after 48 hr of incubation at 28 C. Clear zones appear about colonies within 30 sec after flooding with a 1% solution of cetyltrimethylammonium bromide. **B)** Determinations of percent infestations of *Erwinia* soft-rot spp. of potato seedlots in PT. Each set (a, b, c, d) of 11 streaks represents one seed tuber sample.

Inhibition of soil and rhizosphere bacteria by PT was determined using three peat soils from the Stockton delta and Tulelake areas, three clay loam soils from the Salinas Valley, and three loamy sand soils from the Bakersfield area, all in California. The Salinas Valley samples were potato rhizosphere soils whereas all others were taken randomly in potato fields. Serial dilutions were made of suspensions in water of each soil sample and plated on PT, and KB as the control medium. Each dilution was plated on three plates of each medium and incubated at 28 C for 48 hr and subsequently flooded with a 1% solution of cetrimide.

Percent ESR infestations of potato seedlots were determined using a direct lenticel isolation technique. Ten lenticels plus the stem end of each tuber were stabbed with a sterile wooden toothpick which was streaked on medium PT between each stab. Plates were incubated at 28 C for 48 hr, flooded with cetrimide, and ESR counts were made.

Efficiency of the direct lenticel assay was compared to the anaerobic wrap method of DeBoer and Kelman (4). Tubers from six seedlots were sampled on PT and the same tubers subsequently were sampled by the wrap method.

The occurrence of ESR in nonrhizosphere soils was investigated by making serial dilutions of suspensions in water of soil samples and plating on PT. Rhizosphere isolations were made by shaking soil loose from plant roots, adding 5.0 ml of sterile distilled H_2O to 0.5 g root tissue plus adhering soil in an Erlenmeyer flask and agitating for 1 min prior to making dilutions and plating on the medium. To increase efficiency of the assay, an enrichment technique with PT broth also was used. Fifteen ml of PT broth were added to 25 g of soil in a sterile petri dish when isolating from nonrhizosphere soil, and 5.0 ml of PT broth were added to 0.5 g of root tissue with adhering soil when isolating from the rhizosphere. Samples then were incubated at 28 C aerobically and/or anaerobically for 48 hr at which time serial dilutions were plated. A comparison of the efficiency of aerobic vs. anaerobic incubation was made with 12 soil samples. Anaerobic plates were incubated in a BBL (Division of BioQuest, Cockeysville, MD 21030) GasPak® System anaerobic jar. Disposable hydrogen plus carbon dioxide envelopes (BBL) were used to attain an anaerobic environment.

Eighteen nonrhizosphere soils from different fields at five different locations were sampled along with rhizosphere soils from nine cultivated crops and 10 different weed species. All samples were run with and without enrichment to determine if large populations of the bacteria were present at some locations.

Efficiency of the enrichment assay for detection of ESR in soil was determined by adding 1-ml aliquots of water dilutions of representative cultures of *E. carotovora* var. *carotovora* (CWR-1, Shr-2) and *E. carotovora* var. *atroseptica* (TLBL-1) to 25 g soil plus 15 ml PT broth. The

same dilutions were plated on KB to determine the *Erwinia* populations added to soil. After 48 hr of incubation at 28 C under anaerobic conditions, samples were serially diluted and plated on PT. Each dilution was replicated three times. The soil used in this experiment was from a loamy sand potato field near Bakersfield, California, which had undergone a 4-mo fallow and had no detectable *Erwinia* population.

RESULTS

The PT medium proved to be highly selective and differential for ESR bacteria from plant material and soil. The percentages of complete inhibition and suppression of soil bacteria when compared to KB averaged 99.8%.

The percentages of ESR colonies that grew on PT ranged from 68 to 100 percent of those which developed on KB with strains of *E. carotovora* var. *carotovora*, and 87 and 100 percent with strains of *E. carotovora* var. *atroseptica*. Although some isolates of each group grew faster than others, all grew well within 48 hr with about 3 mm average colony diameter. Clear zones were distinguishable around colonies in less than 30 sec after flooding with cetrimide (Fig. 1-A).

The direct lenticel isolation method used to detect ESR in various potato seedlots in 1976 showed tuber infestations ranging from eight to 100 percent (Table 1). Determinations of percentage infestations of seedlots were made in 48 hr. *Erwinia* colonies were easily distinguished from the few other bacteria that grew on PT (Fig. 1-B).

The characterization of 26 randomly selected isolates from potato seedlots revealed that both *E. carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica* were present. Six typical "atroseptica" and nine typical "carotovora" isolates were obtained along with 11 isolates with taxonomic characters that did not adhere strictly to the standard schemes (6). The 11 isolates differed in one or more characters that are used in defining the "atroseptica" and "carotovora" groups. The sensitivity of our technique to detect ESR in seed pieces was as good or better than that of DeBoer and Kelman, except in one case (Table 2). The correlation coefficient for these data is 0.946 and is significant, $P = 0.01$.

TABLE 1. Detection of pectolytic *Erwinia* spp. in potato seed tubers by the direct lenticel isolation method

Cultivar	Source	Tubers sampled (no.)	Tubers infested with *Erwinia* sp. (%)
White Rose	California	24	88
	Oregon	24	100
	California	20	50
	Washington	25	12
Centennial	Colorado	24	46
	Colorado	24	88
Russet Burbank	Montana	25	24
	Canada	25	24
	Canada	24	8
	California	25	9
Red LaSoda	California	25	9
Nooksack	Washington	17	12
Kennebec	North Dakota	12	17
	California	12	82
	California	12	75

TABLE 2. Comparison of the direct lenticel and tuber incubation methods for isolation of pectolytic *Erwinia* spp. from tubers of different potato seedlots

Cultivar	Tubers sampled (no.)	Tubers infested with *Erwinia* (%)[a]	
		Tuber incubation[b]	Direct lenticel[c]
Kennebec	25	58	75
White Rose	10	0	0
Russet Burbank	25	12	12
Russet Burbank	25	12	24
Nooksack	17	24	12
Kennebec	25	4	4

[a]Correlation coefficient = 0.946, $P = 0.01$.
[b]Tubers assayed by the method of DeBoer and Kelman (4).
[c]Sterile toothpick stabs of 10 lenticels and the stem end of each seed tuber were streaked on selective medium, PT.

Known populations of several strains of ESR were added to soil to evaluate the efficiency of the PT broth enrichment technique. The bacteria were detected when introduced into soil at populations of 6.3, 3.0, 82.0, and 4.0 colony forming units/g of soil from strains CWR-1, Shr-2, TLBL-1, and Sol-6, respectively. Detection was accomplished (plating 0.1 ml/plate) at dilutions of 10^{-2} for CWR-1 and Shr-2 and at 10^{-4} for Sol-6 and TLBL-1. The detection of ESR at those dilutions indicated a 150 to 25,000-fold increase in the populations of the introduced bacteria during the enrichment period.

Erwinia spp. were readily recovered from artificially and naturally infested soil with both the aerobic and anaerobic enrichment techniques. However, anaerobic incubation prior to plating improved the selectivity of the process and made the recognition of ESR easier. These bacteria were detected in six of 18 soils (Table 3); they were not detected in loamy sand soils from the Bakersfield area that had undergone a 4-mo summer fallow. They were detected in some soils where there was vegetation or where a crop had recently been harvested and plant debris was visible.

Erwinia soft-rot bacteria were common inhabitants of the rhizosphere of cultivated crops and weeds (Table 4). Isolations of ESR were made without the aid of enrichment from six of 14 rhizosphere soil samples indicating that large natural populations were present at some sites.

The characterization of 37 randomly selected isolates from rhizosphere and field soils showed 27 typical "carotovora" types, one typical "atroseptica" type and 10 isolates which differed from either group. Isolates which could not be characterized by the tests used were put into this "atypical" group.

Greenhouse stem pathogenicity tests were unreliable for separating the "carotovora" from "atroseptica" types at 18 C. Only one of 30 isolates produced typical blackleg symptoms when plants were stem inoculated but not placed in moisture chambers. Eighteen of the same 30, however, were positive under the moist conditions. Thirteen of these 18 were not typical "atroseptica" types according to other test results used in this study. Pathogenicity tests therefore were unreliable for taxonomic determinations and were not used in this study.

DISCUSSION

The PT medium was highly selective, generally nontoxic and enabled an ecological study of ESR in soil and in association with plant roots. Employment of PT for direct lenticel isolations of *Erwinia* from potato seedlots proved less time consuming and required less space than previous techniques (4, 22), especially when assaying many seedlots simultaneously. The direct lenticel assay was comparable in efficiency to the technique of DeBoer and Kelman (4). Although eight to 100% of tubers in different seedlots were infested, the significance of the degree of infestation on subsequent disease development has not been established. It seems logical that the high level of infestation would provide a blackleg inoculum source, as suggested by other researchers (4, 11, 23, 24).

Soil enrichment recently has been used for isolation of soft-rotting *Erwinia* spp. from soil (19). Our method also demonstrates the advantages of enrichment and of anaerobic incubation in inhibiting the growth of other pectolytic organisms while allowing *Erwinia* to multiply. Soil and rhizosphere isolations showed that ESR occur in the rhizospheres of a diverse number of plants present in agricultural soils. Populaltions sometimes were large enough to allow detection of ESR by direct plating on PT, without the use of enrichment techniques. We have confirmed that the bacteria occur in the rhizospheres of various plants, but this does not necessarily invalidate planting clean seed potatoes to provide effective control of blackleg and potato soft-rot (10, 25). Of particular significance was our inability to detect ESR in fallow field soils from potato-growing areas. This suggests the importance of ensuring that weeds do not grow on land intended for planting of potatoes. These data also indicate that the crop preceding potatoes could be an

TABLE 3. Isolation of soft-rot *Erwinia* spp. from various field soils

Soil location[a]	Present or previous crop	Individual fields sampled (no.)	Fields with soft-rot *Erwinia* spp. (no.)
Bakersfield, Calif.	potato - fallow (4 mo)[b]	5	0
	cotton	1	0
	alfalfa	3	1
Salinas Valley, Calif.	potato	1	1
	cauliflower–fallow (2 wk)[c]	1	1
Tulelake, Calif.	potato	2	2
	barley	2	0
Minidoha, Idaho	potato	2	1
Stockton, Calif.	potato	1	0

[a]Two to 3 kg of soil were collected from five sites throughout separate fields, mixed thoroughly and a 25-g sample removed for making determinations.

[b]Denotes length of the fallow since previous crop harvest.

[c]Recognizable plant debris was present.

TABLE 4. Isolation of soft-rot *Erwinia* spp. from rhizosphere soils of various crop and weed plants

Crop or weed[a]	Plants sampled[b] (no.)	Plants harboring *Erwinia* (no.)
Latuca sativa L. (lettuce)	15	8
Daucus carota L., var. *sativa* (carrot)	10	4
Brassica oleracea L., var. *botrytis* L. (broccoli)	5	5
Medicago sativa L. (alfalfa)	8	2
Beta vulgaris L. (sugarbeet)	10	1
Sorghum valgare Pers. (sorghum)	5	0
Solanum tuberosum L. (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

[a]Plants were collected, stored in polyethylene bags and all were assayed within a 24-hr period.
[b]Root tissue with adhering soil (0.5 g) was added to 5.0 ml PT broth which was then anaerobically incubated 48 hr prior to plating on PT.

important factor in contributing to soft-rot and blackleg diseases especially if potatoes are planted within weeks after the crop's harvest. It seems unlikely that ESR would overwinter in fallow soils of potato-growing regions such as Bakersfield, California, where fields are often left fallow between potato crops. Adjacent fields, however, which are frequently planted to alfalfa or other crops may harbor the pathogen which conceivably could be disseminated to the host crops by contaminated farm equipment (10), insects (13, 18, 21) or possibly aerosols (12). Although only one typical *E. carotovora* var. *atroseptica* isolate was obtained during our rhizosphere investigations, we believe that a more intensive survey would have revealed a greater occurrence of this bacterium.

The number of ESR strains isolated from soil and plant rhizospheres that could cause blackleg and/or soft rot disease in the field needs further investigation. Although potato stem inoculations in the greenhouse are often used to determine pathogenicity (2, 27) and the varietal epithet (9, 28) of *E. carotovora*, we question the significance of a positive test. Our work, and that of others (7), has shown that stem inoculations were inadequate for differentiation of ESR even at 18 C, and that results are influenced greatly by moisture conditions. Although seedpiece inoculations were claimed to be more sensitive for differentiating among strains than stem inoculations, they also failed to distinguish "atroseptica" from "carotovora" in all cases (7).

Pathogenicity as measured in the laboratory and greenhouse may not be indicative of disease producing ability in the field. For example, *Bacillus subtilis*-like strains and *Pseudomonas* soft-rot spp. readily rot potatoes in greenhouse tests, are common in agricultural soils, but only rarely have been reported to cause disease in the field (1). Furthermore, the *Erwinia* sugarbeet pathogen (30) produces typical blackleg symptoms when stem-inoculated in the greenhouse, yet is rarely isolated from blackleg plants in the field even in the proximity of sugarbeet fields (Thomson, et al., *unpublished*).

Although ESR were not detected in field soils devoid of plant debris and weeds, the possibility exists that they may survive at populations below our detection level. However, we know of no conclusive data indicating that either "carotovora" or "atroseptica" will overwinter in fallow soil where weeds and plant residues are absent. Our data, and those of others, suggest that ESR are not true soil inhabitants, but may exhibit a protracted soil phase by their capacity to perpetuate themselves indefinitely when in association with plant roots.

LITERATURE CITED

1. CROSSE, J. E. 1968. Plant pathogenic bacteria in soil. Pages 552-572 *in* T. R. G. Gray and D. Parkinson, eds. The ecology of soil bacteria, an international symposium. Liverpool, U.K. 681 p.
2. CUPPELS, D., and A. KELMAN. 1974. Evaluation of selective media for isolation of soft-rot bacteria from soil and plant tissue. Phytopathology 64:468-475.
3. DEBOER, S. H., D. CUPPELS, and A. KELMAN. 1974. Populations of pectolytic Erwinia spp. in the rhizosphere of potato plants. Proc. Am. Phytopathol. Soc. 1:90.
4. DEBOER, S. H., and A. KELMAN. 1975. Evaluation of procedures for detection of pectolytic Erwinia spp. on potato tubers. Am. Potato J. 52:117-123.
5. DYE, D. W. 1968. A taxonomic study of the genus Erwinia. I. The "amylovora" group. N. Z. J. Sci. 11:590-607.
6. DYE, D. W. 1969. A taxonomic study of the genus Erwinia. II. The "carotovora" group. N. Z. J. Sci. 12:81-97.
7. ERINLE, I. D. 1975. Blackleg of potatoes: induction through tuber inoculation. Plant Pathol. 24:172-175.
8. GOTO, M. 1971. The significance of vegetation for the survival of plant pathogenic bacteria. Pages 39-53 *in* H. P. Maas-Geesteranus, ed. Proc. 3rd Int. Conf. on Plant

Pathogenic Bacteria, Wageningen, The Netherlands. 365 p.

9. GRAHAM, D. C. 1972. Identification of soft-rot coliform bacteria. Pages 273-279 *in* H. P. Maas-Geesteranus, ed. Proc. 3rd Int. Conf. on Plant Pathogenic Bacteria, Wageningen, The Netherlands. 365 p.
10. GRAHAM, D. C., and J. L. HARDIE. 1971. Prospects for control of blackleg disease by the use of stem cuttings. Pages 219-224 *in* Proc. 6th Br. Insecticide Conf. (Vol. 1) 15-18 November, Brighton, England. 323 p.
11. GRAHAM, D. C., and HARPER, P. C. 1967. Potato blackleg and tuber soft rot. Scottish Agric. 46:68-74.
12. GRAHAM, D. C., and M. D. HARRISON. 1975. Potential spread of Erwinia spp. in aerosols. Phytopathology 65:739-741.
13. GRAHAM, D. C., C. E. QUINN, and M. D. HARRISON. 1976. Recurrence of soft-rot coliform bacterial infections in potato stem cuttings: an epidemiological study on the central nuclear stock production farm in Scotland, 1967-74. Potato Res. 19:3-20.
14. HUGH, R., and E. LEIFSON. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-bacteria. J. Bacteriol. 66:24-26.
15. KIKUMOTO, T. 1974. Ecological studies on the soft-rot bacteria of vegetables (13). The role of Chinese cabbage culture in the seasonal trends in the populations of soft-rot bacteria in soil. Bull. Inst. Agric. Res. Tohotu Univ. 25:125-137.
16. KIKUMOTO, T., and M. SAKAMOTO. 1969. Ecological studies on the soft-rot bacteria of vegetables. VII. The preferential stimulation of the soft-rot bacteria in the rhizosphere of crop plants and weeds. Ann. Phytopathol. Soc. Japn. 35:36-40.
17. KLINGER, A. E., D. C. HILDEBRAND, and S. WILHELM. 1971. Occurrence of Erwinia carotovora in the rhizosphere of cotton plants which escape Verticillium wilt. Plant Soil 35:215-218.
18. LEACH, J. G. 1926. The relation of the seed-corn maggot (Phorbia fusciceps Zett.) to the spread and development of potato blackleg. Phytopathology 16:149-177.
19. MENELEY, J. C., and M. E. STANGHELLINI. 1976. Isolation of soft-rot Erwinia spp. from agricultural soils using an enrichment technique. Phytopathology 66:367-370.
20. MILLER, T. D., and M. N. SCHROTH. 1970. Selective medium for the isolation of Erwinia carotovora and other Erwinia spp. Phytopathology 60:1304 (Abstr.).
21. MOLINA, J. J., M. D. HARRISON, and J. W. BREWER. 1975. Transmission of Erwinia carotovora var. atroseptica by Drosophila melanogaster Meig. I. Acquisition and transmission of the bacterium. Am. Potato J. 51:245-250.
22. PEROMBELON, M. C. M. 1972. A reliable and rapid method for detecting contamination of potato tubers by Erwinia carotovora. Plant Dis. Rep. 56:552-554.
23. PEROMBELON, M. C. M. 1973. The extent and survival of contamination of potato stocks in Scotland by Erwinia carotovora var. carotovora and E. carotovora var. atroseptica. Ann. Appl. Biol. 71:111-117.
24. PEROMBELON, M. C. M. 1973. Sites of contamination and numbers of Erwinia carotovora present in stored potato stocks in Scotland. Ann. Appl. Biol. 74:59-65.
25. PEROMBELON, M. C. M. 1974. The role of the seed tuber in the contamination by Erwinia carotovora of potato crops in Scotland. Potato Res. 17:187-199.
26. PIECZARKA, D. J., and J. W. LORBEER. 1975. Microorganisms associated with bottom rot of lettuce grown on organic soil in New York State. Phytopathology 65:6-21.
27. SMITH, W. L. 1950. Pathogenic differences manifested by Erwinia atroseptica and Erwinia carotovora. Phytopathology 40:1011-1017.
28. STANGHELLINI, M. E., and J. C. MENELEY. 1975. Identification of soft-rot Erwinia associated with blackleg of potato in Arizona. Phytopathology 65:86-87.
29. STEWART, D. J. 1962. A selective-diagnostic medium for the isolation of pectinolytic organisms in the Enterobacteriaceae. Nature (Lond.) 195:1023.
30. THOMSON, S. V., M. N. SCHROTH, E. D. WHITNEY, and F. J. HILLS. 1973. Bacterial rot–a new threat to sugarbeet. Pages 30-32 *in* The Calif. Sugar Beet, December issue. 52 p.
31. TOGASHI, J. 1972. Studies on the outbreak of the soft-rot disease of Chinese cabbage by Erwinia aroideae (Towns.) Holl. Rep. Inst. Agric., Res. Tohoku Univ. (Japn) 23:17-52.
32. TSUYAMA, H. 1962. Studies on the soft-rot disease of Chinease cabbage by Erwinia aroideae (Towns.) Holl. Rep. Inst. Agric. Res. Tohoku Univ. (Japn) 13:221-345.