Population Dynamics of Erwinia carotovora and Pectolytic Clostridium spp. in Relation to Decay of Potatoes

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ABSTRACT

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Three pectolytic strains of *Clostridium* spp., in addition to *Erwinia carotovora* var. *carotovora* (Ecc) and *E. carotovora* var. *atroseptica* (Eca), were present in samples of potato stocks in Wisconsin. Type L (white) and LP (pink), were strongly pectolytic obligate anaerobes with subterminal oval spores; type S (white) was weakly pectolytic and micro-aerophilic with round terminal spores. Types L and LP were more pathogenic (based on the effective median dose [ED_{50}] for induction of visible decay in injected tubers) than type S. Under anaerobic conditions strains of *E. carotovora* grew faster in tubers and were more pathogenic at 16 C than were type L clostridia; however, at 20–22 C Eca and Ecc were as pathogenic as type L. Furthermore, Eca was more pathogenic than Ecc at 16 but not at 20–22 C. When Eca and type L clostridia were injected together in tubers and incubated under anaerobic conditions at 16 C, the former organism became the dominant component of the population in the decayed tissue; however, at 20 C the clostridia tended to predominate. Rotting was induced in tubers by clostridia alone; furthermore Erwinia-free tubers derived from stem cuttings could be induced to rot if clostridia were present. Onset of decay however, was accelerated especially at 16 C if strains of *E. carotovora* were present.

Decay of potatoes in storage or transit commonly is associated with conditions that cause water films to form on tubers. Anaerobic conditions cause an apparent decrease in tuber resistance to soft rot bacteria and tuber turgidity increases which ruptures the suberized layer of the lenticels. That combination of conditions is favorable for rapid growth of certain pectolytic bacteria present in lenticels and for invasion of cortical tissue (18).

Pectolytic bacteria present in potato tuber lenticels may include *Bacillus, Flavobacterium, Pseudomonas, Enterobacter,* and *Clostridium* spp. as well as *Erwinia carotovora* var. *carotovora* Dye and *E. carotovora* var. *atroseptica* (Hellm. and Dows.) Dye (18). *E. carotovora* long has been associated with soft rot in potatoes and can be detected in most stocks in the United Kingdom (14,15), in Germany (7), and in the United States (4,13). Similarly, contamination by pectolytic clostridia is likely to be widespread since these bacteria are ubiquitous in most soils (20). Prévot et al (20) listed 17 species of pectolytic obligate anaerobic bacteria commonly found in soils including six nonpigmented and eight pigmented (usually pink) *Clostridium* spp. and three *Plectridium* spp. Several of these bacteria long have been known to be widely present on potatoes and many have been investigated in connection with tuber rotting (20), although they generally have not been considered to be plant pathogens. Previously Rudd-Jones and Dowson (21) had isolated pectolytic clostridia from rotting tubers; however, only recently were clostridia definitely implicated in the rotting syndrome of potatoes in the United Kingdom (10,11,16,17) and in the United States (12). This has been based largely on the frequency of isolation of clostridia and their high pectolytic and tuber rotting capabilities.

The objectives of this study were to determine the relationships between soft-rotting *Erwinia* and certain *Clostridium* strains in the decay of potato tubers and also the environmental conditions that favor clostridia in the decay process.

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MATERIALS AND METHODS

Strains. *E. carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica* will be referred to as Ecc and Eca, respectively, and both varieties as *E. carotovora*.

Six strains of *E. carotovora* obtained from potatoes were used, three of Ecc, namely SR44 (California, 1946, Ark/Starr, ICPB-EC15), SR204 (Long Island, N.Y., 1953, Burkholder/Dickey E-72), and P2 (Wisconsin, 1975, Gullings-Handley); and three of Eca namely SR8 (Wisconsin, 1966, Kelman), SR247 (Arizona, 1974, Stanghellini), and SG2 (Wisconsin, 1975, Gullings-Handley).

Three strains of pectolytic clostridia from potatoes were used namely L3, LP1, and S4 (Wisconsin, 1975, Pérombelon).

Isolation and characterization of bacteria. Isolations and presumptive identifications of *E. carotovora* and pectolytic clostridia were completed on Stewart's medium (22) and on Lund's medium (11), respectively, at 25 C. The varieties of *E. carotovora* were identified on the basis of acid production from α-methyl glucoside and production of reducing substances from sucrose (2). The diagnostic tests completed on the strains of clostridia (3,9) are listed in Table 1; a detailed taxonomic study of these strains was beyond the scope of this investigation. Representative cultures currently are being examined by L. V. Holdeman of the Anaerobe Laboratory, Virginia Polytechnic Institute, Blacksburg, Virginia, and B. Lund of the Food Research Institute, Norwich, England. Initial observations indicate that the strains under study differ in a number of characteristics from previously described *Clostridium* spp.

Potato stocks. Sound tubers of about 200 g were taken as required from three stocks grown in Wisconsin and harvested in September 1975 and stored at 5 C. When tested by the method described below, Stock 1 (cultivar Russet Burbank) was uniformly contaminated by Ecc only and Stock 2 (cultivar Sebago) by Eca. However, in Stock 3 (cultivar Russet Sebago), derived from stem cuttings, no *E. carotovora* was detected. Pectolytic clostridia were present in all three stocks.

Media. The following media were used in addition to Stewart's (22) and Lund's (11) media: Difco nutrient broth (NB); Difco

nutrient agar (NA); TGS broth which contained 25 g Difco tryptic soy broth, 10 g glucose, 0.05 g cysteine hydrochloride, and 1 L distilled water (pH 7); diluent PCW which contained 1 g Difco Bacto peptone, 0.05 g cysteine hydrochloride, and 1 L distilled water (pH 7); TSA medium which contained 25 g Difco tryptic soy broth, 0.05 g cysteine hydrochloride, 15 g agar, and 1 L distilled water (pH 7); PMG medium which contained 10 g Difco Bacto peptone, 10 g α-methyl glucoside, 0.05 g bromthymol blue, 15 g agar, and 1 L distilled water (pH 7).

Anaerobic conditions were obtained with Gas Pak anaerobic jars and hydrogen and carbon dioxide generators (Becton, Dickinson & Co., Cockeysville, MD 21030).

Lund's and TSA media were stored under anaerobic conditions overnight before use. All plates were inoculated with 0.1 ml of inoculum and incubated at 22 C for 2-3 days for Stewart's medium, 3-4 days for Lund's and TSA media, and 4-5 days for NA. Lund's and TSA media were incubated anaerobically.

Preparation of inocula. Strains of *E. carotovora* were grown at 28 C for 24 hr in shake culture in NB; strains of *Clostridium* were grown anaerobically in TSG broth for 5 days at 20 C. Serial dilutions of *Erwinia* and *Clostridium* cultures were prepared in saline and in diluent PCW, respectively.

Sensitivity of clostridia to O_2. (i) The three clostridia strains were streaked on Lund's and TSA media and incubated at 80% RH and 20 C in a N_2 and O_2 gas stream (6-8 liter/hr) containing 5% CO_2 and with different concentrations of O_2 : 0, 0.5, 2.5, and 7.0% as well as in air alone. Presence or absence of growth was recorded after appropriate growth periods. (ii) Decimal dilutions of suspensions of strains L3 and S4 and of samples of decayed tissue from lesions in a tuber were prepared and plated on Lund's medium under anaerobic conditions in a glove box (Coy Manufacturing, 1395 Harp St., Ann Arbor, MI 48104). Parallel dilution series were done in air with the same suspensions and tissue used in the glove box. In addition some of the suspensions of isolates L3 and S4 were treated with ethanol (50% final concentration) for 45 min to kill the vegetative cells (9) and another dilution series was prepared in air. All dilutions were plated on Lund's medium and viable counts were determined.

Sampling of lenticels. Tuber sampling and preparation of dilution series in this and subsequent experiments were completed in the air. (i) Ten tubers from stocks 1 and 2 were individually washed under running water and 10 lenticels per tuber were ringed with a marking pen. Then the lenticels were punctured to a depth about 3 mm with sterile toothpicks. The tubers were wrapped in wet paper towels and a double layer of Saran Wrap (polyvinilidene film–Dow Chemical Co., Indianapolis, IN 46268) to obtain anaerobic conditions (4) and groups of them were incubated at 16 C and 22 C for 8 and 4 days, respectively. The number of lenticels surrounded by decaying tissue was counted. A small segment of rotted tissue from each lenticel was placed in a drop of sterile water and the resulting suspensions were streaked on Stewart's and Lund's media. Presumptive presence of *E. carotovora* and *Clostridium* spp. was based on characteristic growth and colony morphology on these media. (ii) Groups of tubers from stocks 1, 2, and 3 were washed, wounded, wrapped, and incubated at 16 C and 22 C as described above. In addition, a second group of tubers from stock 1 was washed, wounded, and incubated in moist peat (aerobic conditions) at 16 and 22 C. Also a second group from stock 3 was vacuum infiltrated in a suspension of SR247 (Eca–10^5 cells/ml) prior to washing, wounding, and wrapping. Groups of these tubers were incubated at 16 C and 22 C. Periodically three tubers from the different treatments of each stock were examined and the number of lenticels with decay noted. When the tubers had an average of two or less infected lenticels per tuber, five sound lenticels per tuber were excised, pooled and ground in 2 ml saline. When more than two infected lenticels were present per tuber, two rotting lesions from each of two tubers were sampled. Sampling was done by suspending about 0.2 g of rotted tissue from the edge of the rot at each infected lenticel in 2 ml saline. Decimal dilutions were prepared and plated on Stewart's and Lund's media (stocks 1, 2 and 3) and on NA (stock 1). Colony counts of the different bacteria were expressed as $\log_{10}$ (x + 0.1) per lenticel or 0.2 g rotted tissue (equivalent to the weight of tissue for five lenticels). The mean of the transformed colony numbers was plotted against time to determine growth patterns. Clostridia colonies forming large (type L) and small (type S) pits on Lund's medium were enumerated separately.

Pathogenicity of strains. A method based on quantal response (5) was used to determine pathogenicity of strains. Tubers were surface-disinfested by immersion for 10 min in 10% Clorox (final concentration NaOCl 0.5%). Stock 1 tubers (seven per strain) were inoculated by injecting 0.01 ml of dilutions (1:10, or 1:4) to a depth of about 2.5 cm. Six dilutions were used per tuber and care was taken not to inject lenticels. The tubers were incubated anaerobically in jars at 16 C and 20 C for 8 and 4 days, respectively. The inoculated sites were then cut and the presence or absence of decay was noted. The effective medium dose (ED_{50} values) was calculated with a Wang 2200 computer.

Inoculation with mixtures of strains. (i) Dilutions containing known numbers of Ecc (P2) and Eca (SG2) mixed in different ratios were inoculated as above in triplicate in three tubers (stock 1) and incubated anaerobically in jars at 16 C and 20 C for 7 and 3 days respectively. Serial dilutions of the rotted tissue taken from a lesion were prepared as described above, and plated on PMG medium and incubated at 22 C for 48 hr. The different growth characteristics of Ecc (P2) and Eca (SG2) on this medium (Eca utilizes α-methyl glucoside with acid production and Ecc does not) allow differentiation of the two bacteria when these are plated together. The ratio of the two bacteria in the different lesions could therefore be determined from viability counts on PMG. (ii) Similar inoculations were carried out with mixtures of Eca strains (SG2 or SR8) and the L3 strain of *Clostridium*. Smears from the rotted tissue were prepared and stained with a fluorescent-labelled antiserum specific for Eca (1). A Zeiss Universal photomicroscope was used at a magnification of ×625, and the ratios of clostridia (L3) to Eca (SG2 or SR8) were determined from the number of organisms under phase microscopy (total count) and the number of fluorescing organisms (Eca) under U V epi-illumination in identical fields (1). (iii) Three replicated tubers (Stock 1) were injected as described above with dilutions of Eca (SR8) and *Clostridium* (L3) and mixtures of these strains and incubated anaerobically as described above. The diameter of the decayed tissue at injection points was determined after 7 and 3 days incubation at 16 and 20 C, respectively.

RESULTS

Characteristics of strains of Clostridia tested. Preliminary investigations showed that samples of eight different potato stocks in Wisconsin were extensively contaminated by pectolytic clostridia. Strains obtained from initially sound tubers which had been induced to rot at 22 C could be grouped into three main types (Table 1). Type LP represented by strains LP1 was obtained from two of eight stocks examined whereas group L (strain L3) was present in the remaining six stocks, and type S (strain S4) was present in all stocks. The three types of bacteria also were detected in soil adhering to the tubers.

The three clostridia strains (LP1, L3, and S4) grew well at 0% O_2 but at 0.5% O_2 growth of isolates L3 and LP1 was inhibited and isolate S4 grew poorly. At 2.5% O_2 or above, none of the strains grew. However, exposure of the plated cultures to air for up to 1 hr did not markedly affect growth of the bacteria when these were subsequently incubated anaerobically.

Viable counts obtained from dilutions prepared in a glove box under anaerobic conditions were significantly higher than parallel ones from dilutions prepared in air with a pure culture of L3. The same was true for type L *Clostridium* when the intitial inoculum was taken directly from rotted tissue (Table 2). The counts were essentially similar with strain S4 and for type S *Clostridium* from rotted tissue. Treatment of the original broth suspensions with ethanol showed that the counts were similar to those of the air preparation in the case of L3 but considerably less for S4.

Isolation of pectolytic bacteria from lenticels. A limited survey for the presence of *E. carotovora* and clostridia (types L and S) in

TABLE 1. Characteristics of pectolytic clostridia from potatoes

Characteristic	Strains		
	L3	LP1	S4
Type of pit in pectate layer of Lund's medium	Large with vertical edges	Large with vertical edges	Small, crater like
Color of colony on Lund's and TSA media	White	Pink	White
Spores	Subterminal oval	Subterminal oval	Terminal, round
Growth			
at 16 C	Poor, slow	Poor, slow	Fair
at 20 C	Good	Good	Good
in 0% O_2	+	+	+
in 0.5% O_2	−	−	Poor
Gelatin hydrolysis	+	+	−
Catalase	−	−	−
Litmus milk	Acid, clotting	...	No change
NO_3 reduction to NO_2	−	...	−
SO_4 reduction	−	...	−
Indole	−	−	−
Motility	+	+	+

lenticels showed that most tubers in stocks 1 and 2 were contaminated by both organisms (Table 3). *E. carotovora* was obtained 5-14 times more frequently than clostridia at 16 C, whereas at 22 C both kinds of organisms were detected with equal frequency. The numbers of lenticels from which *E. carotovora* could be isolated was higher at 16 than at 22 C.

Dynamics of growth of pectolytic bacteria in lenticels. The results of the study on the dynamics of growth of *E. carotovora* and pectolytic clostridia in lenticels of tubers incubated under different conditions can be summarized as follows:

TABLE 2. Numbers of clostridia obtained in dilutions from broth cultures and from rotting potato tubers when plated anaerobically in a glove box or in the air

Treatments	Broth cultures (no. bacteria/ml)		Rotting tubers (no. bacteria/0.2 g)	
	L3	S4	type L	type S
Anaerobic (glove box)	4.8×10^7	1.7×10^7	9.6×10^5	2.3×10^6
Aerobic	5.9×10^4	1.3×10^7	6.7×10^4	4.5×10^6
After ethanol treatment	1.2×10^4	6.2×10^4	...	...

(i) Under aerobic conditions (stock 1 incubated in peat) no typical rotting lesions developed around the lenticels during the experiment at either 16 or 22 C. However, rotting occurred readily under anaerobic conditions. Lesions were present in 50% or more of the tested lenticels of tubers of stocks 1 and 2 after three days at 22 C and 5–6 days at 16 C. Rotting developed later at both temperatures in the noninoculated tubers of stock 3 than in stocks 1 and 2. As indicated previously, stock 3 tubers were *Erwinia*-free when tested by the Saran Wrap method. However, when tubers of stock 3 were inoculated, they rotted within 2–3 days at both temperatures.

(ii) Under aerobic conditions (incubated in peat) at 22 C little or no increase in the initial population of *E. carotovora* took place (Fig. 1). As expected, clostridia failed to grow under these conditions. Under anaerobic conditions the total bacterial population increased gradually, whereas under aerobic conditions an initial increase was followed by a decrease after 7 days. The bacterial growth patterns at 16 C were similar to those at 22 C.

(iii) Growth patterns of the different pectolytic bacteria under anaerobic conditions were similar in both stocks 1 and 2 tubers contaminated by Ecc and Eca respectively; only those in stock 1 are given (Fig. 2). Growth patterns of *E. carotovora* were different at 16 and 22 C (Fig. 2A, B). At 22 C population numbers

TABLE 3. Percentage of wounded lenticels[a] in Russet Burbank (stock 1) and Sebago (stock 2) potato tubers incubated under anaerobic conditions at 16 C for 8 days or at 22 C for 4 days from which *Erwinia carotovora* and clostridia (types L and S) were isolated

Temperature of Incubation (C)	Potato stock	Lenticels decayed[b] (%)	Lenticels	
			E. carotovora (%)	Clostridia (%)
16	Russet Burbank	75	68	13
	Sebago	72	70	5
22	Russet Burbank	73	56	44
	Sebago	83	52	58

[a]Based on 100 wounded lenticels (10 lenticels/tuber; a total of 10 tubers/stock for each incubation temperature).
[b]Both *E. carotovora* and clostridia were isolated from some lenticels.

peaked at 5×10^6 cells/0.2 g tissue, then decreased sharply. This peak coincided with the appearance of decay. Populations peaked at a higher level at 16 C but then remained relatively constant.

(iv) Growth of the clostridia also responded differently to temperature. At 22 C populations of both the L and S followed a pattern similar to that of *E. carotovora*, except that the numbers of type L were significantly lower (Fig. 2A). At 16 C, however, only type S numbers increased initially to a level slightly below that of *E. carotovora* then decreased gradually. Type L grew poorly and the numbers never exceeded 10^3 cells/0.2 g tissue (Fig. 2B).

(v) Growth of the clostridia in lenticels of noninoculated tubers stock 3 at both temperatures (Fig. 3A) was similar to that observed in stocks 1 and 2. The patterns were similar when inoculated tubers were used; population numbers of Eca (strain SR247) tended to increase more rapidly than those of the clostridia (Fig. 3B, C).

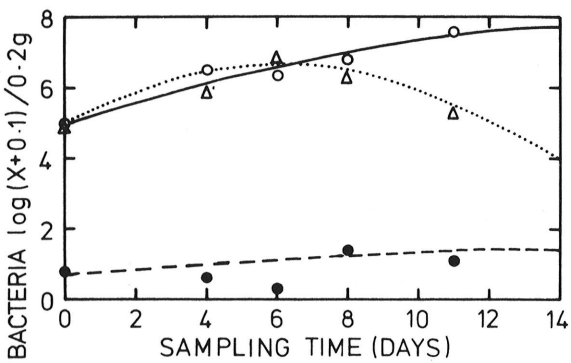

Fig. 1. Growth patterns of total bacteria under aerobic (Δ), (·····), and anaerobic conditions (○) (———), and *Erwinia carotovora* under aerobic conditions (●), (- - - -) in wounded lenticels of cultivar Russet Burbank tubers (stock 1) at 22 C.

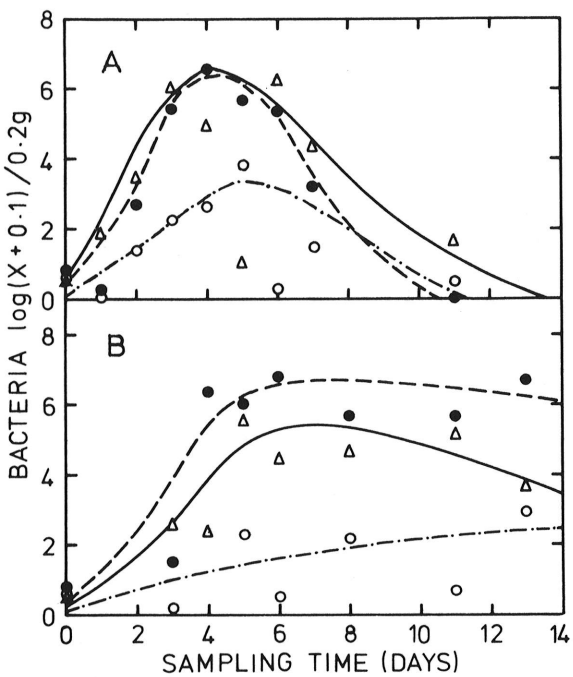

Fig. 2. Growth patterns of *Erwinia carotovora* (●), (- - - -); type L *Clostridium* (○), (-·-·-) and type S *Clostridium* (Δ) (———) in wounded lenticels of cultivar Russet Burbank tubers (stock 1) at **A)** 22 C and **B)** 16 C.

Relative pathogenicity of E. carotovora and clostridia. The relative pathogenicity (based on effective median dose) or rotting ability of isolates of Ecc (based on decay diameters in injected tubers) was equivalent to that of Eca at 22 C with an Ed_{50} about 10^2 (Table 4). Both L3 and LP1 tended to be equally or slightly more pathogenic than S4 at 22 C. At 16 C Ecc was less pathogenic than Eca and the clostridia were even less pathogenic than Ecc.

The differing incubation periods allowed a meaningful comparison of the relative pathogenicity of the organisms at the two temperatures. With equal incubation periods rotting was considerably greater at 22 C than at 16 C; thus, the loss of comparisons of pathogenicity in relation to temperature was considered not to be a serious drawback.

Tuber inoculation with paired mixtures of strains. In stock 1 tubers inoculated with both Eca (SG2) and Ecc (P2), SG2 tended to outgrow P2 at 16 C; the opposite was true at 20 C (Table 5). When mixed inocula of Eca (SG2 or SR8) and of clostridia (L3) were injected in tubers held at 16 C, the Eca strains became the dominant component of the population. Few cells of clostridia were present. In contrast growth of L3 was comparable to that of Eca at 20 C (Table 5).

The lesions in tubers inoculated with mixtures of L3 and SR8 were about the same size as those formed when the tubers were inoculated with either L3 alone at 20 C or SR8 alone at 16 C (Table 6). No lesions were formed at 16 C with L3 alone.

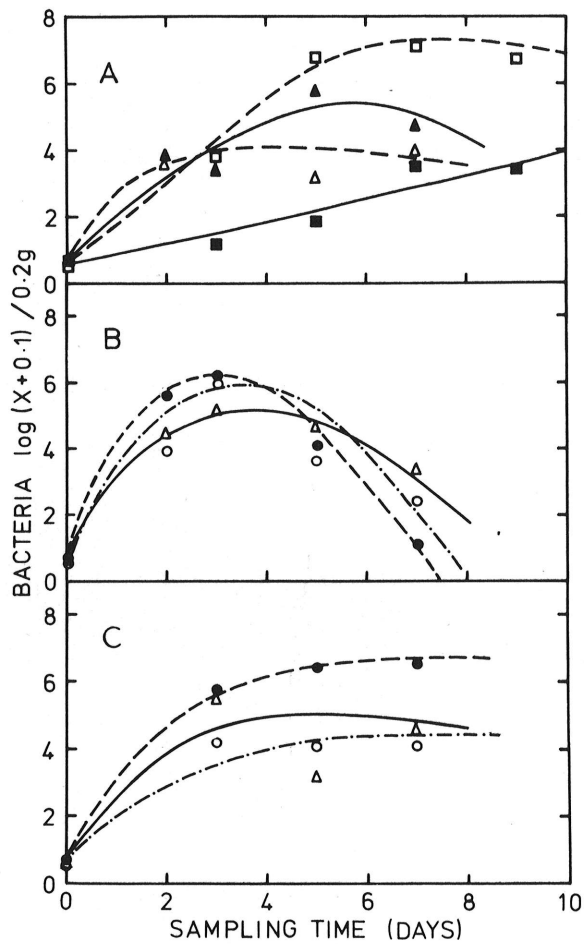

Fig. 3. A) Growth patterns of type S *Clostridium* at 16 C (□), (- - - -) and at 22 C (Δ), (- - -) and of type L *Clostridium* at 16 C (■) (———) and at 22 C (▲), (———) in wounded lenticels of noninoculated cultivar Russet Sebago potato tubers incubated anaerobically. Growth patterns of *Erwinia carotovora* (●), (- - - -), type L *Clostridium* (○), (-·-·-) and type S *Clostridium* (Δ), (———) in wounded lenticels of cultivar Russet Sebago tubers (stock 3) inoculated with *Erwinia carotovora* var. *atroseptica* (strain SR247) and incubated anaerobically **B)** at 22 C and **C)** 16 C.

DISCUSSION

Clostridia: assessment of methods. Three different types of pectolytic clostridia were present on tubers obtained in Wisconsin. Previously, morphologically similar organisms had been isolated from rotting potatoes and soil in Scotland (16,17) and from tubers in England (11). These bacteria have not yet been described sufficiently for species identification. However, on the basis of spore position and hydrolysis of gelatin, types L and LP would fall in Group II and type S in Group III according to the subdivision of the genus *Clostridium* in Bergey's Manual of Determinative Bacteriology (2). Of the three types, type S was found most frequently. On Lund's medium type S produces pits similar to but smaller than those produced by *E. carotovora* on Stewart's medium. Nevertheless, because of its weak pectolytic activity and low pathogenicity, its role in rotting may be marginal. Types L and LP, however, are strongly pectolytic and they produce distinctive cavities up to 2-3 cm in diameter with sharp vertical edges in the pectate over-layer of Lund's medium. Clostridial rots are strikingly different from those caused by *E. carotovora* and produce considerable slime with many gas bubbles and a distinctive odor. (21).

Since the clostridia essentially are strict anaerobes, an assessment of their sensitivity to O_2 was necessary to determine the consequences of exposing the rotting material to air when determining numbers of bacteria. The vegetative cells of L3 (and probably also of LP1) were very sensitive to exposure to air even for the short time (ie, 5 min) needed to prepare a dilution series. However, the spores remained viable even after 1 hr of exposure in air and growth of type L clostridia in this study probably originated from spores. Both the vegetative cells and spores of S4 were sufficiently resistant to O_2 to survive during the time required to prepare and plate the dilutions.

Thus, it can be assumed that the population numbers of type L clostridia were underestimated. The ratio of vegetative cells to spores in the decay tissue of a tuber was about 100:1 as indicated in Table 2. Type L population numbers obtained under standard laboratory conditions should therefore be corrected by a factor of 100 to approximate the actual numbers present in rots. At the start of decay in tubers at 22 C the corrected numbers of type L clostridia then would be equivalent to those of *E. carotovora*; ie, about 10^6 cells/ 0.2 g tissue (Fig. 2A). Lesions in many other bacterial host-pathogen relationships become visible when the population approximates this level.

In the interpretation of the data on growth patterns of the bacteria consideration should be given to the likelihood that the numbers of type L bacteria were underestimated in the decayed lenticel tissue. Therefore, it was necessary to verify the findings indirectly by a method independent of the sensitivity of the vegetative cells to O_2.

Bacterial growth in lenticels. Both *E. carotovora* and clostridia (types L and S) were present initially in a high percentage of the lenticels of stocks 1 and 2 tubers. The numbers of the bacteria originally present in the lenticels were small and often could not be determined by plating ground lenticels; however, under the test conditions tubers become an efficient enrichment medium for both anaerobic and facultative anaerobic pectolytic bacteria.

Although wounding of the lenticels accelerated the onset of rotting in a uniform way, variability of the numbers of bacteria in the decayed tissue was great, probably because the numbers initially present in the lenticels were different. Nevertheless, the general growth pattern (tendency for population numbers to increase or decrease) of the bacteria could still be followed.

The numbers of pectolytic bacteria did not increase in lenticels of tubers held under aerobic conditions although the lenticels proliferated. The basis for failure to grow and invade the wounded tissue is not known. However, the total bacterial flora initially increased, but soon decreased. This probably occurred when nutrients in the lenticels were depleted. Under anaerobic conditions, tuber resistance is apparently so low that most anaerobic bacteria can grow readily, including pectolytic types (18).

Clostridia, especially types L and LP, can induce rotting in the absence of *E. carotovora* in tubers. This became evident when stock

TABLE 4. Relative pathogenicity[a] of different strains of *Erwinia carotovora* and *Clostridium* spp. injected in potato tubers of cv. Russet Burbank and incubated under anaerobic conditions at 16 or 22 C for 8 or 4 days, respectively

Strains	ED_{50} values at incubation temperatures:	
	16 C	22 C
Erwinia carotovora		
var. *carotovora*		
SR204	5.3×10^4	1.5×10^2
SR44	2.5×10^3	5.0×10^1
var. *atroseptica*		
SR8	7.5×10^1	1.5×10^2
SR247	7.5×10^2	5.5×10^1
Clostridium spp.		
L3	c. 10^5	3.6
LP1	Not sampled	2.5×10^1
S4	$> 10^6$	1.5×10^3

[a]Expressed as ED_{50} (viable cells in the inoculum injected) requisite for induction of visible decay symptoms.

TABLE 5. Percentage of *Erwinia carotovora* var. *atroseptica* (SR8 or SG2)[a] in decayed tissue after anaerobic incubation following injection in mixture with *Erwinia carotovora* var. *carotovora* (P2) or in mixture with *Clostridium* sp. (L3)

Inoculum mixtures	Incubation temperature/time					
	16 C/7 days			20 C/3 days		
	Total bacteria injected (no.)	var. *atroseptica* in inoculum (%)	var. *atroseptica* in decayed tissue (%)	Total bacteria injected	var. *atroseptica* in inoculum (%)	var. *atroseptica* in decayed tissue (%)
SG2 + P2	2.5×10^5	4	16	2.5×10^5	4	1
	3.0×10^5	19	37	3.0×10^5	19	10
	1.0×10^5	54	77	1.0×10^5	59	54
	1.6×10^6	86	92	1.6×10^6	86	77
SG2 + L3	3.0×10^4	16	100	2.6×10^3	6	7
	7.1×10^4	65	98	4.0×10^4	38	15
	4.8×10^5	95	100	1.5×10^5	98	21
SR8 + L3	5.3×10^4	53	90	5.7×10^3	4	6
	3.0×10^4	92	98	7.9×10^3	30	26
	2.8×10^5	99	94	3.0×10^3	81	34

[a]Percentages determined by counts made using fluorescent antibody stain specific for *E. carotovora* var. *atroseptica*.

TABLE 6. Diameter of decayed tissue in potato tubers injected with *Erwinia carotovora* var. *atroseptica* (SR8) and *Clostridium* sp. (L3) alone or mixed and incubated anaerobically at 16 or 20 C for 7 or 3 days, respectively

Inoculum	Bacterial inoculum	*E. carotovora* var. *atroseptica* in mixed inoculum	Diameter of tuber decay after incubation at:	
	(no.)	(%)	16 C (mm)	20 C (mm)
SR8	4.8×10^3		9.0[a]	4.0
L3	1.1×10^4		0	10.0
SR8 + L3	7.9×10^3	30	3.5	9.0
SR8	1.2×10^5		...[b]	5.7
L3	8.2×10^4		...	12.0
SR + L3	1.0×10^5	60	...	10.0
SR8	4.8×10^3		9.0	4.0
L3	1.1×10^3		0	7.0
SR8 + L3	3.0×10^3	81	7.0	7.0

[a]Mean of three injection points.
[b]Samples not tested.

3 tubers, ostensibly free of contamination by *E. carotovora*, decayed when subjected to the Saran Wrap treatment. Rotting was delayed especially at 16 C; however, many tubers in stock 3 failed to rot even after prolonged incubation at 16 C if the lenticels were not wounded. In contrast, similarly treated tubers of stocks 1 and 2 rotted as readily as those with wounded lenticels although this did not occur invariably.

The decrease in numbers of pectolytic bacteria at 22 but not at 16 C after extensive rotting may be attributed to the accumulation of self-limiting products such as gases, organic acids and higher alcohols (20), which are possible by-products of the rapid growth of clostridia at 22 C.

Effect of temperature on pathogenicity and growth of bacteria in host tissue. The growth patterns of *E. carotovora* and clostridia in lenticel rots were different at 16 and at 22 C. If the assumption is valid that the tuber tissue is equally susceptible at both temperatures under anaerobic conditions, then growth and pathogenicity of the bacteria must be affected mainly by the incubation temperature.

At 20-22 C type L clostridia grew as well as, if not slightly better than, strains of *E. carotovora* when inoculated together in tubers; also its pathogenicity (ED_{50}) was similar to that of the *E. carotovora* strains. Growth patterns of these bacteria in lenticel rots were similar. Growth and pathogenicity of the bacteria at 20 and 22 C were not significantly different (author's *unpublished*). In contrast to results at 20–22 C *E. carotovora* outgrew type L clostridia at 16 C when inoculated as a mixture in tubers. The relationship between size of the decayed area and incubation temperature following single and mixed inoculations with *E. carotovora* and type L clostridia indicate that rotting in the mixture at 20 C was caused mostly by clostridia, whereas at 16 C it was caused by *E. carotovora*. The effect of temperature on growth that differentiated *E. carotovora* from clostridia also was observed in the relationship of Eca to Ecc in mixed inoculations.

Although rotting at 16 C can be induced in tubers by clostridia alone, the presence of *E. carotovora* greatly accelerates the onset of rotting. Since the temperature within most bulk-stored potatoes rarely exceed 10 C and because most potato stocks are contaminated by *E. carotovora*, strains of *E. carotovora* probably are responsible for rotting which occurs in storage. The clostridia may become more important in transit when the temperature exceeds 20 C.

These findings have relevance to the production of stocks free from *E. carotovora* when stem cuttings are used as initial propagative material (8). In addition to the reduction of black leg (caused usually by Eca), a decrease in the risk of rotting in storage also would be achieved if the tubers were free from contamination by both varieties of *E. carotovora*. Furthermore, if tests of seed tubers for presence of *E. carotovora* are included in seed certification programs as has been suggested (6,19), it will be imperative to define carefully the incubation temperatures. Results obtained in this study show that even if *E. carotovora* is present it might not be isolated if temperatures and incubation periods greater than 20 C and 7 days, respectively, were used.

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