Leuconostoc mesenteroides subsp. *mesenteroides*, The First Report of a Coccoid Bacterium Causing a Plant Disease

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ABSTRACT

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A coccoid bacterium, *Leuconostoc mesenteroides* subsp. *mesenteroides*, was identified for the first time as the causal agent of a postharvest decay of fresh-market tomato fruit in California and Mexico. Symptoms included a sour odor and watery texture of firm and intact fruit. Our original strain (LMM 89-1) and an additional 10 strains isolated from *Leuconostoc*-decayed or fungal-decayed fruit were identified as the heterofermentative, lactic acid–producing bacterium *L. m.* subsp. *mesenteroides*. These strains were identical to the type strain, ATCC 8293. Bacterial strains were identified as members of the genus *Leuconostoc* primarily on the basis of Gram-positive stain, coccoid morphology, lack of catalase, inability to hydrolyze arginine, and production of CO_2 from glucose; strain LMM 89-1 was identified as *L. mesenteroides* based, in part, on a fatty acid methyl ester analysis. The strains were identified to the subspecies level based on production of acid from L-arabinose and dextran production on sucrose-amended media. Pathogenicity of strain LMM 89-1 was demonstrated on mature-green tomato fruit by fulfilling Koch's postulates. Symptom development was discernible within 24 h of inoculation as small, slightly sunken, firm, water-soaked lesions. Sequential sampling and culturing over 5 days indicated a logarithmic increase of bacterial populations in fruit tissues. The average doubling time over these periods was 3.6 h for fruit incubated at 20 C and 2.9 h for fruit incubated at 33 C. Using aqueous cell suspensions, strain LMM 89-1 was found to be more virulent (sensu aggressive) to tomato fruit than was the type strain. Strain LMM 89-1 was pathogenic to wound-inoculated fruit of pepper but not tomato leaflets, tomato stems, potato tubers, eggplant fruit, or other nonsolanaceous crops tested. In subsequent samplings of tomato fruit from packinghouses in California and Mexico, *L. m.* subsp. *mesenteroides* was isolated from 17 of 21 (81%) symptomatic fruit, as well as from 309 of 387 (80%) fungal-decayed fruit. From asymptomatic fruit, the bacterium was isolated from surface sections of tomato fruit but not from internal mesocarp tissue.

Decays of commercially harvested mature-green tomato (*Lycopersicon esculentum* Mill.) fruit have been attributed to either fungal pathogens or the soft-rot bacterium, *Erwinia carotovora* subsp. *carotovora* (5,13). In California, the primary postharvest fungal decays of fresh-market tomato fruit are caused by *Botrytis cinerea*, *Alternaria alternata*, *Rhizopus stolonifer*, and *Phytophthora capsici*, and less commonly *P. parasitica*, *P. infestans*, *Rhizoctonia solani*, *Geotrichum candidum*, and *Sclerotinia sclerotiorum*. Decay caused by the bacterium *E. c.* subsp. *carotovora* has been reported on fresh-market tomato in California (6) but soft rot was not observed during packinghouse decay evaluations by the authors in this study.

In 1987, J. M. Ogawa and B. T. Manji visited wholesale markets in New York City to evaluate decays of tomato fruit grown in California. Inspection of shipments that were rejected for sale in New York led to discovery of a firm, water-soaked decay that did not appear to be caused by either a fungal pathogen or the bacterial soft rot pathogen, *E. c.* subsp. *carotovora*. Isolation from surface-sterilized decayed fruit tissue onto a number of media consistently resulted in the growth of one bacterial type on medium 523 (14). This substrate is not commonly used for isolation of bacterial pathogens of plants but was developed for use as a general media for the culture of bacterial plant pathogens. An initial report on the pathogenicity of this bacterium has been published (2). The objectives of this study were to identify the bacterium, to test its pathogenicity, and to determine the extent of its role in postharvest decays of fresh-market tomato fruit.

MATERIALS AND METHODS

Morphological studies. Initial isolations of the bacterium from symptomatic, decayed fresh-market tomato fruit collected from a commercial field in Merced County, CA, were made on 523 agar (14). Subsequently, decayed fruit were collected from packinghouses in California and Sinaloa, Mexico, and isolations were made in MRS broth or on MRS agar (3), a medium developed specifically for the isolation and culture of lactic acid bacteria. Using micromanipulation techniques followed by microscopic confirmation, one strain, LMM 89-1, was single-celled to ensure a pure culture (4), and was used throughout the study.

Cell size and morphology were determined using bright-field and scanning electron microscopy. For bright-field microscopy, cells of 11 selected strains isolated from fresh-market tomato fruit were grown in litmus milk (1) for 7 days at 25 C, Gram-stained, and photographed using a Nikon camera mounted on a Leitz Wetzlar microscope (Metallux 2) (Ernst Leitz Ltd., Ontario, Canada). Cells were measured by projecting the images on a screen and measurements were made based on a photographed stage micrometer. For scanning electron microscopy, strain LMM 89-1 was prepared by suspending cells from 48-h cultures grown on 523 agar in aqueous suspension (approximately 1×10^8 cfu per milliliter) and placing the suspension onto a nucleopore filter membrane sealed within dialysis tubing. The tubing was placed in glutaraldehyde, fixed overnight in 1% OsO_4, and dehydrated through an ethanol series. The membrane was critical-point dried with CO_2 as a transitional fluid, placed on aluminum mounts, sputter-coated with 200 Å of gold, and examined using an ISI

DS-130 scanning electron microscope.

Bacteriological assays. Strain LMM 89-1 and the 10 other strains isolated from symptomatic fresh-market tomato fruit were collected from four packinghouses and were characterized and identified using bacteriological tests (10) and specific assays for species of *Leuconostoc* (9,11,12,24). Comparisons with the type strain of *L. m.* subsp. *mesenteroides* (Tsenkovskii) van Tieghem strain ATCC 8293 (type culture) and two other species in the genus *Leuconostoc* (*L. m.* subsp. *dextranicum* (Beijerinck) Garvie strain ATCC 19255 [neotype culture] and *L. paramesenteroides* Garvie strain ATCC 33313 [type culture]) were made. Additionally, presumptive strains of *L. m.* subsp. *mesenteroides* isolated from symptomatic, *Leuconostoc*-decayed or from fungal-decayed fresh-market tomato fruit were characterized periodically as described previously. Bacteriological tests included Gram-stain, motility, spore production, oxidase reaction, and catalase production (10). Separation of the genus *Leuconostoc* from other lactic acid genera was based on cell morphology, predominant arrangement of cells, gas production from glucose, and hydrolysis of arginine (9,12,24). Fatty acid methyl ester analysis of strain LMM 89-1 was performed by Microbial ID (Newark, DE). Species of *Leuconostoc* were differentiated based on production of acid from L-arabinose, fructose, maltose, melibiose, salicin, sucrose, and trehalose, in addition to formation of dextran from sucrose. Production of gas from glucose was tested using tubes of MRS broth (5 ml per tube, ph 6.5) with meat extract omitted (11). Inoculated tubes were sealed with molten sterile vaspar (one part paraffin to six parts vaseline) and immediately placed in an aqueous ice slurry to harden the vaspar and make a seal on top of the liquid basal medium. Tubes were incubated in static culture up to 14 days at 25 C. Evidence for carbon dioxide production from glucose was shown by gas pushing the vaspar plugs above the basal medium. MRS broth prepared without triammonium citrate but with L-arginine monohydrochloride (0.3%) was used as a growth medium to test hydrolysis of arginine and Nessler's Reagent was used to test production of ammonia after 7 days growth (11). MRS broth without glucose and meat extract was used as a basal medium for carbohydrate fermentations. The carbon sources were prepared as 2% (w/v) solutions, except for esculin, which was prepared as a 1% (w/v) solution, and were filter sterilized and added (0.5 ml) to 5.0 ml of autoclaved basal medium. The pH of the broth was adjusted to 6.5 and 0.004% chlorphenol red (w/v) was added as an acid indicator (11). Production of acid from carbohydrate was recorded as a visual change in color of the broth medium after 14 days static culture at 25 C. For dextran production, the sugar agar of Garvie (9) was used initially but was replaced by trypticase soy agar (1) modified by the addition of 5% sucrose.

Pathogenicity studies on wounded tomato fruit. Mature-green fresh-market tomato fruit, cultivar P19, were used for all pathogenicity studies. Fruit were surface sterilized for 2 min in a 0.84% (v/v) solution of NaOCl (household bleach) and then air dried at 25 C before inoculation. Two inoculation methods were used to test pathogenicity of *L. m.* subsp. *mesenteroides*: fruit were wounded and inoculated with *Leuconostoc*-decayed tissue, and fruit were wounded and inoculated with aqueous suspensions of cells at decreasing concentrations.

For the first inoculation method, small pieces of tissue (0.1 g) from mature-green (harvest-ripe) tomato fruit were excised from the margin of decay caused by strain LMM 89-1. A piece of this excised tissue was inserted into a cross-cut injury (2 mm³) near the blossom end of 30 mature-green fruit. Incisions were covered with cellophane tape and half the total number of fruit were incubated at 20 C, >95% relative humidity (RH) and the other half at 33 C, >95% RH. At 24-h intervals, three tomatoes at each temperature were removed, 0.1 g of tissue was excised from the advancing margin of decay of each fruit, and populations of bacteria were determined by macerating the tissue and dilution plating in

triplicate onto MRS agar. An additional three tomato fruit were inoculated with strain LMM 89-1 as described previously and lesion diameter was measured daily for the duration of the experiment. Populations of bacteria in the inoculum were determined by dilution plating in triplicate from three additional 0.1-g pieces of decayed tissue onto MRS agar. For controls, five fruit were wounded as described above, inoculated with apparently healthy tomato fruit tissue, and were incubated at each temperature. The experiment was repeated once.

For the second inoculation method, strains LMM 89-1 or ATCC 8293 were grown for 48 h at 25 C on MRS agar and suspended in sterile, distilled water. Cell concentrations were adjusted to 70% transmittance using a spectrophotometer with wavelength set at 600 nm. This corresponded to approximately 1 × 10⁸ cfu per milliliter based on 10-fold dilutions. The blossom ends of 140 surface-sterilized, mature-green fruits were punctured to a uniform depth of 2 mm with a 23-gauge needle and 10-µl aliquots of inoculum of strains LMM 89-1 or ATCC 8293, ranging in concentration from approximately 10⁰ to 10⁶ cfu, were placed in the puncture wounds. Ten fruit per concentration per bacterial strain were inoculated. An additional 10 fruit were wounded and inoculated with sterile, distilled water. The wounds were covered with cellophane tape, fruit were incubated at 20 C, >95% RH, and the incidence of diseased fruit was determined daily for 22 days. This experiment was repeated once.

Pathogenicity studies on nonwounded tomato fruit. Mature-green fresh-market tomato fruit, cultivar P19, were surface sterilized for 2 min as described above. Two inoculation methods were used to test pathogenicity of *L. m.* subsp. *mesenteroides* to nonwounded fruit: aqueous suspensions of cells were inoculated onto nonwounded fruit surfaces, and *Leuconostoc*-decayed fruit were placed in contact with nonwounded fruit to determine if the putative pathogen could spread from decayed fruit to healthy fruit.

To test the pathogenicity of *L. m.* subsp. *mesenteroides* to nonwounded, mature-green fruit, 0.1 ml of cell suspensions in sterile, distilled water (approximately 1 × 10⁸ cfu per milliliter) of strains LMM 89-1 or ATCC 8293, grown on MRS agar for 48 h at 25 C, were placed on the cheek of each fruit. Sterile, distilled water served as a control. Forty fruit were inoculated with strain LMM 89-1, strain ATCC 8293, or with sterile, distilled water. Replications of 10 fruit were incubated in plastic containers (31 cm × 24 cm × 10 cm) for 14 days at 25 C, >95% RH. Drops of bacterial inoculum or water were maintained on the fruit surfaces for 48 h by draping the plastic containers with wet cheesecloth to prevent evaporation. This experiment was repeated once.

To determine if decay could spread from decayed fruit to healthy fruit, 30 mature-green fruit were wound inoculated with an aqueous suspension of strain LMM 89-1 (approximately 10⁶ cfu per 10-µl drop) and incubated at 30 C in plastic containers held at >95% RH for 7 days to allow decay to develop. All 30 decayed fruit, with bacteria exuding from their wounds, were then paired with apparently healthy mature-green fruit so that the lesions of half the decayed fruit were in contact with the stem end of 15 healthy fruit and half were in contact with the cheek of 15 healthy fruit. All fruit were incubated at 20 C, >95% RH in plastic containers (three pairs of fruit per plastic container) and were examined daily for 14 days for symptom development. This experiment was repeated once.

Pathogenicity studies on tomato plants. In greenhouse studies to test the pathogenicity of *L. m.* subsp. *mesenteroides* to potted tomato plants, strains LMM 89-1 and ATCC 8293 were inoculated on leaflets and stems. For leaflet inoculations, bacterial cells, grown on MRS agar for 48 h at 25 C, were suspended in sterile, distilled water and cell concentrations were adjusted to approximately 10⁸ cfu per milliliter. A syringe with the needle removed was used to infiltrate 0.1 ml of inoculum of LMM 89-1, ATCC 8293, or sterile, distilled water into leaflets of 30 4-wk-old tomato plants. The lower surface of three leaflets per plant was

inoculated with either strain or with sterile, distilled water. Additionally, using 48-h bacterial colonies, individual colonies on MRS agar plates were touched with autoclaved, wood toothpicks. Stems of the same plants used for leaflet inoculations were puncture inoculated in three locations to a depth of 3 mm with the toothpicks (one puncture per toothpick per bacterial strain or sterile agar). Ten plants were kept on the greenhouse bench, 10 were sealed in plastic bags for 2 wk to increase relative humidity, and 10 were kept under intermittent mist (5 s mist every 15 min) for 2 wk. The plants were examined periodically for symptom development over a 2-mo period. This experiment was repeated once.

Pathogenicity studies on other crops. In laboratory studies to test pathogenicity of strain LMM 89-1 to other crops, five fruits each of apple (*Malus domestica* Borkh.), squash cv. butter nut (*Cucurbita moschata* (Duchesne) Duchesne ex. Poir.), squash var. summer crook neck (*C. pepo* L.), cucumber (*Cucumis sativus* L.), eggplant (*Solanum melongena* L.), grape (*Vitis vinifera* L.), honey dew melon (*Cucumis melo* L. var. *inodorus* Naudin), nectarine (*Prunus persica* (L.) var. *nectarina* (Aiton) Maxim.), orange (*Citrus sinensis* (L.) Osbeck), peach (*P. persica* (L.) Batsch.), pear (*Pyrus communis* L.), pepper (*Capsicum annuum* L.), plum (*P. salicina* Lindl.), and squash var. zucchini (*C. pepo* L.) were wounded to a depth of 2 mm as described above and inoculated with an aqueous bacterial suspension (10 µl of 1×10^8 cfu per milliliter) or with sterile, distilled water. Similarly, carrot (*Daucus carota* L.) roots, onion (*Allium cepa* L.) bulbs, and potato (*Solanum tuberosum* L.) tubers were wounded and inoculated as described above. All were incubated at 20 C, >95% RH for 14 days for symptom development.

Detection of *Leuconostoc* decay in packinghouse and field samples. In 1991, fruit in two packinghouses in California were sampled; in 1992, fruit in three packinghouses in California and two packinghouses in Mexico were sampled for the incidence of *L. m.* subsp. *mesenteroides*. Decayed and fungal-decayed fruit were recovered from gondolas, packing lines, cull piles, or tomato boxes in ripening rooms. Fruit were placed in plastic bags in ice chests and transported to the laboratory. Isolations for *L. m.* subsp. *mesenteroides* were made by excising pieces of fruit (5 mm³) from the margin of fungal decays, rinsing the pieces of tissue in autoclaved, distilled water, and then surface sterilizing the pieces in a 0.84% (w/v) solution of NaOCl for 2 min. Fruit pieces were rinsed, blotted dry, and placed in test tubes containing 10 ml of MRS broth. Tubes were incubated at 25 C in static culture for 2–4 days to enrich for the facultative anaerobe, *L. m.*

subsp. *mesenteroides*. Once bacterial growth was evident in the bottom of a test tube, a 10-µl aliquot from each tube showing growth was plated onto MRS agar, and two to four transfers were made on MRS agar before storing strains in litmus milk (1). A subset of representative strains was identified as described previously. The fungus that caused the fruit decay was determined by visual assessment and confirmed by fungal sporulation and by isolation procedures. For isolations, pieces of decayed fruit (5 mm³) were surface sterilized as described above and plated onto acidified potato dextrose agar (1) or onto pimaricin ampicillin rifampicin pentachloronitrobenzene (PARP) agar, a medium selective for pythiaceous fungi (17). Petri plates were incubated at 25 C and fungal cultures recovered were identified based on colony morphology and microscopic characteristics.

Additionally, in order to determine whether *L. m.* subsp. *mesenteroides* occurs on the surface or within fresh-market tomato fruit, 40 apparently healthy fruit each were collected from two commercial tomato fields in Fresno County, CA. A cork borer was used to remove a 13-mm-diameter core extending to the locule from each fruit. Using a sterilized scalpel, cores were cut into a 1-mm section containing the epidermis and a 5-mm section extending into the mesocarp. A 6-mm-diameter core was taken from each of these two sections, epidermal and mesocarp tissues were incubated separately in static culture in MRS broth for 2–4 days at 25 C, and strains recovered on MRS agar were identified as described previously. The cork borers and scalpels used to make cuts were rinsed and flamed with 95% ethanol after each cut to prevent cross-contamination.

RESULTS

Morphological studies and bacteriological assays. Based on morphological and physiological studies, as well as the fatty acid methyl ester analysis, the bacterium was identified as the heterofermentative, facultatively anaerobic, lactic acid–producing bacterium *L. m.* subsp. *mesenteroides* (8,9,12,24,25). In comparative studies with the type strain of *L. m.* subsp. *mesenteroides* (ATCC 8293), and two other taxa in the genus *Leuconostoc* (*L. m.* subsp. *dextranicum* ATCC 19255 [neotype] and *L. paramesenteroides* ATCC 33313 [type]), strain LMM 89-1 and others isolated from fresh-market tomato fruit were shown to be *Leuconostoc* spp. and to be identical to the type strain of *L. m.* subsp. *mesenteroides* (Table 1). Tomato fruit strains and strain ATCC 8293 stained Gram-positive, were nonmotile, did not form spores, and were oxidase and catalase negative. On MRS agar, colonies were smooth, round, gray-white, and generally less than 1 mm in diameter. Using light and scanning electron microscopy, the mor-

TABLE 1. Characteristics used to compare bacterial strains recovered from decayed fresh-market tomato (including LMM 89-1) with type strains of *Leuconostoc mesenteroides* subsp. *mesenteroides* (ATCC 8293), *L. mesenteroides* subsp. *dextranicum* (ATCC 19255-neotype), and *L. paramesenteroides* (ATCC 33313)

Tests	LMM 89-1/ fruit strains	Lmm[a] ATCC 8293	Lmd[b] ATCC 19255	Lp[c] ATCC 33313
		Reaction		
Acid				
L-Arabinose	+[d]	+	−	+
Fructose	+	+	+	+
Maltose	+	+	+	+
Melibiose	+	+	+	+
Salicin	+	+	+	−
Sucrose	+	+	+	+
Trehalose	+	+	+	+
Dextran from sucrose	+	+	+	−

[a] *Leuconostoc mesenteroides* subsp. *mesenteroides*.
[b] *Leuconostoc mesenteroides* subsp. *dextranicum*.
[c] *Leuconostoc paramesenteroides*.
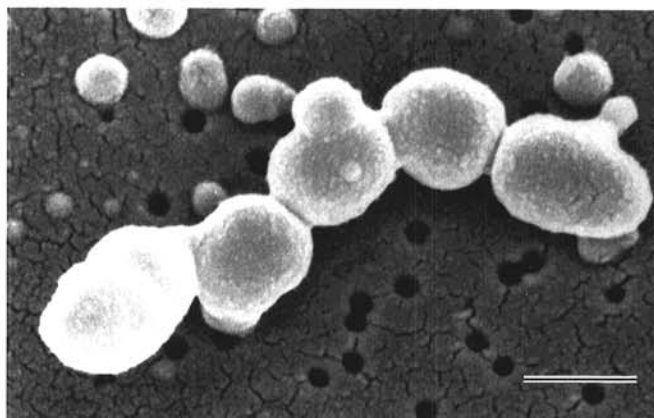[d] +, positive reaction; −, negative reaction.

Fig. 1. Scanning electron micrograph of critically dried, gold shadowed cells of *Leuconostoc mesenteroides* subsp. *mesenteroides* strain LMM 89-1 on a nucleopore filter membrane. The cells are 0.7–1.0 µm in diameter. Scale bar = 1.0 µm.

phology of strain LMM 89-1 was coccoid when grown in litmus milk but coccoid to slightly elongated when grown in MRS broth or on 523 agar (Fig. 1). Division of cells was in one plane only, and cells occurred in pairs or short chains. Gram-stained cells of 11 independently isolated strains from fresh-market tomato fruit (50 cells per strain) grown in litmus milk measured 0.7 to 1.0 μm in diameter. All tomato fruit strains and strain ATCC 8293 produced CO_2 from glucose, but none of the strains utilized arginine as a carbon source. The fatty acid analysis of strain LMM 89-1 was compared with known fatty acid profiles of bacteria in the Microbial ID database and was identified as *L. mesenteroides* (25). Determinative tests of particular importance in separating

some species and subspecies of *Leuconostoc* are shown in Table 1. All tomato fruit strains fermented L-arabinose and produced copious amounts of dextran from sucrose on sucrose-amended media, which resulted in distinctly convex, opaque colonies approaching 1 cm in diameter after 48 h at 25 C. Thus, all tomato fruit strains were identified as *L. m.* subsp. *mesenteroides*.

Pathogenicity studies on wounded tomato fruit. Inoculation with *Leuconostoc*-decayed tissue (strain LMM 89-1) resulted in 100% incidence of decay in two experiments. Results from one experiment are presented (Fig. 2A and B). Symptom development was discernible within 24 h of inoculation as small, slightly sunken, firm, water-soaked lesions. After 5 days, lesion diameters in fruit wound inoculated with decayed tissue increased to an average of 28 mm at 20 C and to 47.7 mm at 33 C (Fig. 2A and B). Sequential sampling and culturing showed a logarithmic increase of bacterial populations in fruit tissues from an initial population of 2.4×10^5 cfu per gram of tissue to a maximum of 2.6×10^9 cfu per gram of tissue after 48 h at 20 C and to 8.1×10^7 cfu per gram of tissue after 24 h at 33 C. The average doubling time over these periods was 3.6 h for fruit incubated at 20 C and 2.9 h for fruit incubated at 33 C. By day 5 viable populations had decreased to 7.3×10^7 and 1.8×10^5 cfu per gram of tissue for fruit incubated at 20 C and 33 C, respectively. No fruit wound inoculated with healthy tomato fruit tissue developed decay.

Strains LMM 89-1 or ATCC 8293 were both pathogenic to fresh-market tomato fruit when introduced as aqueous cell suspensions. Both experiments gave similar results and the data were combined. Strain LMM 89-1 caused 100% incidence of fruit infection with 10^4 cfu and caused a low incidence of fruit decay with as little as 10 cfu (Fig. 3). Strain ATCC 8293, however, caused 80% incidence of fruit infection at a cell concentration of 10^6 cfu and required 10^4 cfu to cause fruit decay (Fig. 3). Incidence of fruit decayed was greatest when fruit were inoculated with 10^4–10^6 cfu, whereas time to symptom expression decreased in all but one case with increasing inoculum concentration. Strain
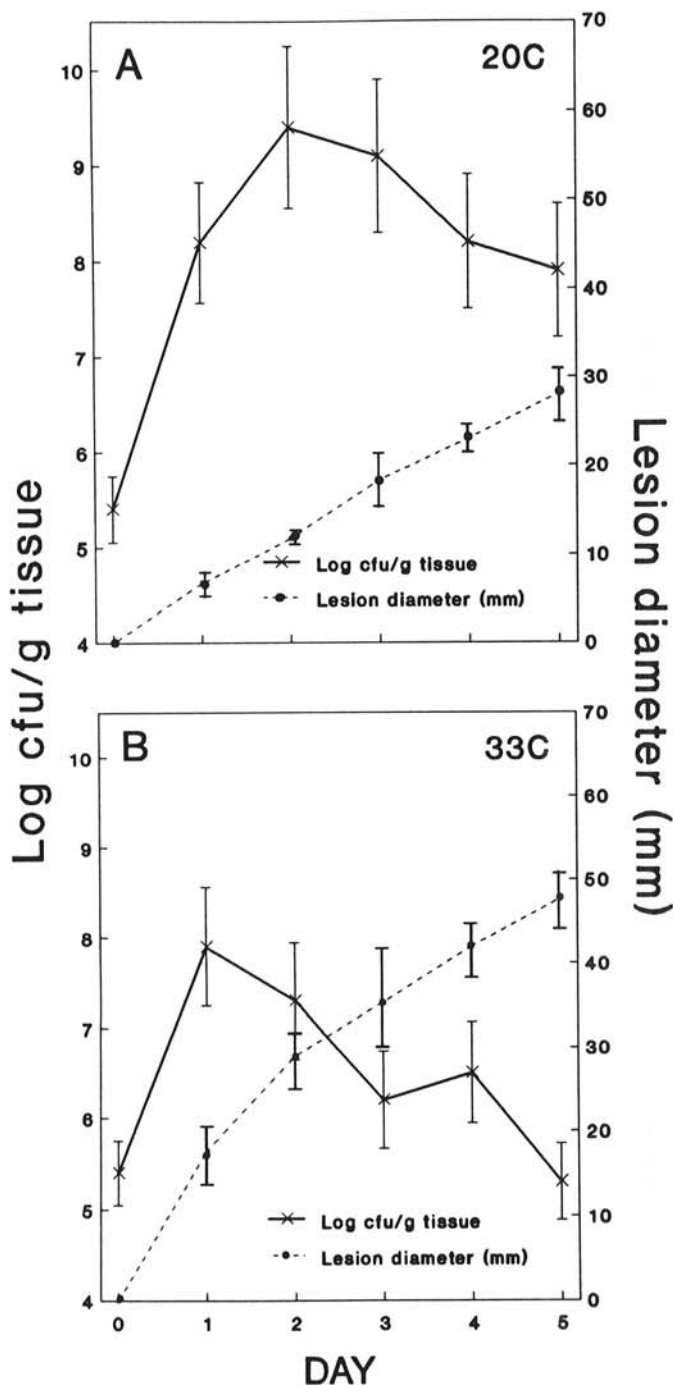

Fig. 2. Multiplication of *Leuconostoc mesenteroides* subsp. *mesenteroides* strain LMM 89-1 and mean lesion diameter measured daily in tomato fruit wound inoculated with 0.1 g of decayed tomato fruit tissue containing 2.4×10^5 cfu per gram of tissue. Tomato fruit were incubated at **A,** 20 C, >95% RH and **B,** 33 C, >95% RH.
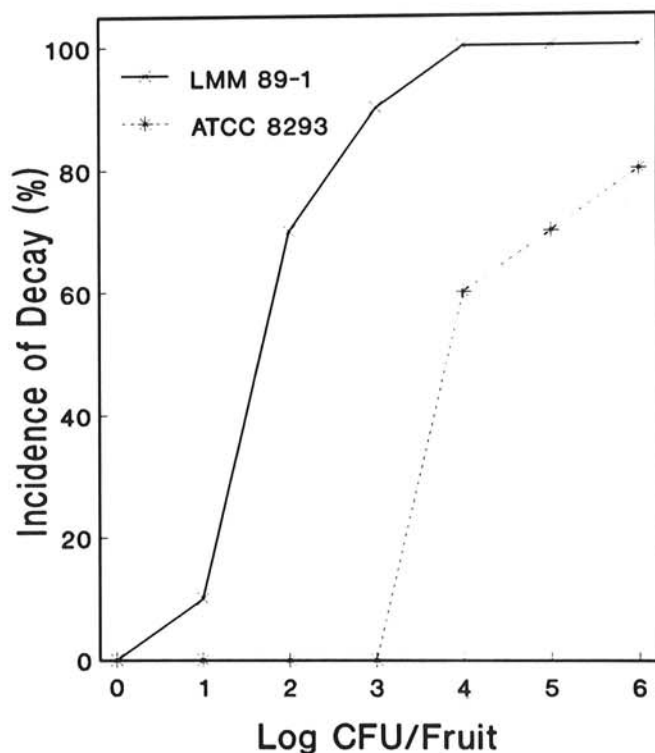

Fig. 3. Percent incidence of decay caused by *Leuconostoc mesenteroides* subsp. *mesenteroides* strains LMM 89-1 and ATCC 8293 in fresh-market tomato fruit after 22 days incubation at 20 C, >95% RH. Fruit were wound inoculated with aqueous suspensions of 0 to log 6 cfu. Data are the average of two experiments.

LMM 89-1 caused 100% incidence of decay within 4, 3, and 2 days when fruit were inoculated with 10^4, 10^5, and 10^6 cfu, respectively. At lower inoculum concentrations, incidence of decay did not reach 100%, even after 22 days. Fruit inoculated with 10^3 cfu reached a total incidence of 90% within 6 days; those inoculated with 10^2 cfu reached an incidence of 70% after 22 days; and those inoculated with 10 cfu reached an incidence of 10% after 6 days. Strain ATCC 8293 never caused 100% incidence of fruit decay, but did cause 80, 70, and 60% incidence of decay within 5, 7, and 9 days of inoculation when fruit were inoculated with 10^6, 10^5, and 10^4 cfu, respectively. No fruit inoculated with strain ATCC 8293 showed symptoms of decay at lower inoculum concentrations, even after 22 days. None of the 10 fruit wound inoculated with sterile, distilled water developed symptoms.

Pathogenicity studies on nonwounded tomato fruit and wounded plants. Bacterial strains LMM 89-1 or ATCC 8293 were not pathogenic to nonwounded fruit and did not spread from decayed fruit to the stem end or cheek of healthy fruit. Additionally, symptoms did not develop on leaflets or stems of 4-wk-old tomato plants that were wound inoculated with strain LMM 89-1 and incubated for 2 mo on greenhouse benches, enclosed in plastic bags, or under intermittent mist.

Pathogenicity studies on other crops. In laboratory studies to test pathogenicity of *L. m.* subsp. *mesenteroides* by wound inoculation of other crops, strain LMM 89-1 decayed fruit of pepper but not tubers of potato or eggplant fruit, or other wound-inoculated fruits and vegetables that were tested. Symptoms on pepper fruit were similar to those on tomato fruit. Small, slightly sunken, firm, water-soaked lesions were apparent within 24 h of inoculation and continued to expand, often decaying the entire pepper fruit. The bacterium was recovered readily from inoculated, decayed pepper fruit by isolation into static MRS broth.

Detection of *Leuconostoc* decay in packinghouse samples. Strains of bacteria isolated from symptomatic, *Leuconostoc*-decayed or from fungal-decayed fresh-market tomato fruit collected in packinghouses were characterized as described above and identified as *L. m.* subsp. *mesenteroides*. The bacterium was rarely the sole organism isolated from fresh-market tomato fruit but was associated commonly with decays caused by major fungal decay pathogens of tomato fruit. In three different packinghouses in California in 1991 and 1992, *L. m.* subsp. *mesenteroides* was isolated from seven of nine (78%) fruit collected with typical *Leuconostoc*-decay symptoms and from which no fungi were isolated. Overall, *L. m.* subsp. *mesenteroides* was isolated from 63% of 27 fungal-decayed fruit collected from two different packinghouses in 1991 and from 81% of 340 fungal-decayed fruit collected from three different packinghouses in 1992 (Table 2). Similarly, in two packinghouses in Mexico in 1992, *L. m.* subsp. *mesenteroides* was isolated from 10 of 12 (83%) fruit collected with typical *Leuconostoc*-decay symptoms and from which no

fungi were isolated, and from 15 of 20 (75%) fruit also colonized by *Geotrichum candidum*. When *L. m.* subsp. *mesenteroides* was the sole organism recovered from fruit, diseased portions of tomato fruit had a sour odor and were watery in texture but otherwise firm and intact. In general, fruit affected by both *L. m.* subsp. *mesenteroides* and various decay fungi showed fungal decay symptoms only, although the presence of *L. m.* subsp. *mesenteroides* was reliably predicted by the distinct sour odor of *Leuconostoc*-decayed fruit.

Additionally, *L. m.* subsp. *mesenteroides* was recovered from the surface sections of 9 of 40 (23%) and 4 of 40 (10%) healthy fruit collected from two fresh-market tomato fields in Fresno County, CA, but was not recovered from any of the mesocarp tissue sections.

DISCUSSION

Bacteriological tests and the fatty acid methyl ester analysis showed that the strains isolated from fresh-market tomato fruit were *L. mesenteroides* (8,9,12,24,25). Subspecies identification was based on utilization of L-arabinose, which differentiates *L. m.* subsp. *mesenteroides* from other subspecies of *L. mesenteroides*, including *L. m.* subsp. *dextranicum* (12). This is the only test in which >90% of *L. m.* subsp. *mesenteroides* strains are positive and > 90% of *L. m.* subsp. *dextranicum* strains are negative (7,9, 12). Thus, according to the current designation (12), the strains isolated from fresh-market tomato fruit were *L. m.* subsp. *mesenteroides*. Previously, *L. m.* subsp. *mesenteroides*, *L. m.* subsp. *dextranicum*, and *L. m.* subsp. *cremoris* (Knudsen and Sorensen) Garvie were considered distinct species of *Leuconostoc* (*L. mesenteroides*, *L. dextranicum*, and *L. cremoris*, respectively) (7), but were demoted to subspecies rank within *L. mesenteroides* because they belong to a single deoxyribonucleic acid homology group (8). Also, the lactate dehydrogenases and glucose-6-phosphate dehydrogenases of these three organisms are similar (8). No significant differences were found for the fatty acids of *L. m.* subsp. *mesenteroides* and *L. m.* subsp. *dextranicum*, although differences were found for *L. m.* subsp. *cremoris* (25). Strains of *L. m.* subsp. *cremoris* have been isolated only from milk or related habitats, and this subspecies is readily differentiated from the other two subspecies of *L. mesenteroides* due to its inability to produce acid from more than a few sugars (7,9,12,24). However, few traits differentiate *L. m.* subsp. *dextranicum* from *L. m.* subsp. *mesenteroides*, and in the future these subspecies designations may change due to this lack of diversity. Similarly, few characteristics differentiate *L. m.* subsp. *mesenteroides* from *L. paramesenteroides* (7,9,12,24). In this study, strains recovered from decayed fresh-market tomato fruit and *L. m.* subsp. *mesenteroides* type strain ATCC 8293 were differentiated from *L. paramesenteroides* type strain ATCC 33313 based on production of acid from salicin

TABLE 2. Detection of *Leuconostoc mesenteroides* subsp. *mesenteroides* from fungal-decayed fresh-market tomato fruit collected from packinghouses in California in 1991 and 1992

PH/year[a]	Fungal decay organism[b]								
	A. alt.	B. cin.	G. can.	P. cap.	P. inf.	P. par.	R. sol.	R. sto.	Total
PH 1-1991	ND[c]	1/1	ND	ND	ND	10/15	3/6	ND	14/22
PH 2-1991	1/1	1/1	ND	ND	1/1	ND	0/2	ND	3/5
PH 1-1992	3/5	31/45	ND	ND	ND	ND	3/3	37/44	74/97
PH 2-1992	8/10	30/31	13/14	13/19	3/3	34/37	17/19	ND	118/133
PH 3-1992	11/17	28/34	18/19	21/28	ND	1/1	2/5	4/6	85/110
Lmm Isolated/Total Decayed Fruit Sampled[d,e]	23/33	91/112	31/33	34/47	4/4	45/53	25/35	41/50	294/367

[a] Packinghouse and year sampled.
[b] A. alt. = *Alternaria alternata*; B. cin. = *Botrytis cinerea*; G. can. = *Geotrichum candidum*; P. cap. = *Phytophthora capsici*; P. inf. = *P. infestans*; P. par. = *P. parasitica*; R. sol. = *Rhizoctonia solani*; R. sto. = *Rhizopus stolonifer*.
[c] ND = not determined due to absence of decayed fruit.
[d] Lmm = *Leuconostoc mesenteroides* subsp. *mesenteroides*.
[e] Number of fruit from which *L. m.* subsp. *mesenteroides* was isolated per total number of fungal-decayed fruit sampled.

and dextran from sucrose. These two species have been separated into different groups based on fatty acid composition (25), immunological relationships of lactate dehydrogenases and glucose-6-phosphate dehydrogenases (9), and deoxyribonucleic acid homologies (9,24).

Pathogenicity of *L. m.* subsp. *mesenteroides* on plants had not been reported previous to this study. Strain LMM 89-1 was shown to be pathogenic to wound-inoculated fresh-market tomato fruit by fulfilling Koch's postulates. Bacterial population size in the advancing margins of decay reached a maximum of 2.6×10^9 cfu per gram of tissue after 2 days and 8.1×10^7 cfu per gram of tissue after 1 day in fruit incubated at 20 and 33 C, respectively (Fig. 2A and B). At these times lesion diameters were 11.7 and 17.7 mm, respectively. With further incubation, cell populations at the advancing margins of decay fluctuated but tended to decrease, whereas lesion diameters continued to increase. Possibly, bacterial populations in the entire area of decay could have influenced lesion expansion, and not just those bacteria present at the advancing margin of decay. For example, the production of lactic acid by the bacterium might reduce multiplication of the bacterium and increase host susceptibility. Additionally, strain LMM 89-1 was shown to be more virulent to tomato fruit than was strain ATCC 8293. Strain LMM 89-1 was originally isolated from fresh-market tomato fruit and strain ATCC 8293 was originally isolated from fermenting olives. Moreover, strain LMM 89-1 was isolated in 1989 and has been in culture for less than 5 yr, whereas type strain ATCC 8293 has been in culture for many years. Without knowing the mode of pathogenicity of *L. m.* subsp. *mesenteroides*, however, it is impossible to determine if long-term storage might affect the production of a virulence factor.

Species of *Leuconostoc* and other genera of lactic acid bacteria including *Lactobacillus*, *Pediococcus*, and *Lactococcus* (formerly lactic *Streptococcus* species), have been isolated from surface tissues of apparently healthy vegetable and forage grass species (16,17,19,20,22). Mundt et al found that *L. m.* subsp. *mesenteroides* was the most commonly isolated lactic acid bacterium on various vegetable crops (21), whereas Smith and Niven isolated the organism from decayed potatoes and garlic cloves (27). Additionally, both *L. m.* subsp. *mesenteroides* and *L. m.* subsp. *dextranicum* were associated with deterioration of sugar during processing of sugar beet and sugar cane tissues (28). In studies on the natural microflora occurring on and within apparently healthy tomato fruit tissues, however, members of the Pseudomonadaceae were predominant and no lactic acid bacteria were recovered (23, 24). In our studies *L. m.* subsp. *mesenteroides* was recovered from naturally occurring, symptomatic, fresh-market tomato fruit, and in one instance the bacterium was recovered from naturally occurring, symptomatic, sweet pepper fruit at a packinghouse in California in the summer of 1993. The host range included both tomato fruit and pepper fruit in our laboratory studies.

In identifying causal agents of postharvest fruit decays, isolation results often emphasize known decay agents when several organisms are obtained. This may explain, in part, why the presence and role of *L. m.* subsp. *mesenteroides* in the decay complex of tomato fruit was previously overlooked. Furthermore, media used in the isolation and identification of plant pathogenic bacteria are commonly not formulated for culturing facultative anaerobes or bacteria dependent on specific carbohydrates or other nutrients for growth. *Leuconostoc m.* subsp. *mesenteroides* grows poorly in or on standard bacteriological media such as nutrient or King's Medium B agar that are used for isolation of plant pathogenic bacteria (1). Using static enrichment, which optimizes recovery of these facultative anaerobes, and MRS broth, a medium developed for the isolation of lactic acid bacteria, *L. m.* subsp. *mesenteroides* was isolated consistently from surface-sterilized, *Leuconostoc*-decayed, and fungal-decayed fresh-market tomato fruit. Overall, the bacterium was recovered from 17 of 21 (81%)

fruit that showed symptoms of *Leuconostoc* decay only, but was also recovered from 309 of 387 (80%) fungal-decayed fruit sampled during this study. Although decay caused solely by *L. m.* subsp. *mesenteroides* was not observed frequently, the bacterium was present in 80% of the fungal-decayed fruit sampled. Thus, the bacterium could be of significance in the postharvest decay complex of fresh-market tomato fruit. The low incidence of *L. m.* subsp. *mesenteroides* causing decay by itself is probably indicative of injuries needed for infection and good sorting of injured fruit in the packing process. Because of the high incidence of *L. m.* subsp. *mesenteroides* in fungal-decayed fruit, and because we recovered *L. m.* subsp. *mesenteroides* from the surface but not from the mesocarp of fruit collected from two fresh market tomato fields, we suspect that this bacterium is epiphytic on tomato plants.

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