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# Properties of *Cytophaga johnsonae* Strains Causing Spoilage of Fresh Produce at Food Markets

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**Two strains of gliding, orange-pigmented bacteria, isolated from fresh bell pepper and watermelon, respectively, showing soft-rot lesions, were identified as *Cytophaga johnsonae*. They differed from seven type strains of *C. johnsonae* deposited at the American Type Culture Collection (ATCC) in some properties, such as the ability to utilize glucose, xylose, trehalose, rhamnose, and sucrose. Spherical bodies resembling microcysts of *Sporocytophaga* sp. in addition to short rods and long filaments were observed in two strains (ATCC 29583 and 29588) throughout the growth cycle and also in aged cultures of other strains. All strains examined were shown to degrade five natural or synthetic polymers (pectin, chitin, starch, protein, and carboxymethyl cellulose). Only six strains (including ATCC 17061, 29587, 29589, and 19366) were able to infect and macerate artificially wounded potato tubers and fruits of pepper, squash, and tomato. The pathogenic strains secreted more pectate lyase in broth medium than the nonpathogenic strains. *C. johnsonae*, generally known as a soil saprophyte, might occasionally act as an opportunistic pathogen, causing decay of fresh produce in storage or in transit.**

*Cytophaga johnsonae* is a gram-negative, nonflagellated, yellow (orange)-pigmented bacterium which exhibits spreading growth and gliding motility on agar media. Cells become filamentous as cultures age (12, 13, 17, 18). The organism is actively involved in biomass conversion because of its versatile ability to degrade various natural or synthetic polymers (chitin, carboxymethyl cellulose, pectin, protein, and starch) and is generally considered a soil saprophyte (16–18). *C. johnsonae* has never been shown to attack living plants or to cause decay of detached plant parts while they are in storage or transit despite its close ecological relationship to plant materials (16, 17).

The present report describes the isolation and characterization of two strains of *C. johnsonae* from decayed specimens of fresh produce. For comparison, seven type strains of *C. johnsonae*, previously examined by Christensen (3) and currently deposited at the American Type Culture Collection (ATCC) Rockville, Md., were included.

## MATERIALS AND METHODS

**Reference bacterial strains.** Seven strains of *C. johnsonae* (ATCC 17061, 19366, 29583, 29587, 29588, 29589, and 29590) previously characterized by Christensen (3) were obtained from the ATCC. Strain ATCC 19366, originally isolated from soil and identified as *Flavobacterium pectinovorum* (6), was recently reclassified as a member of *C. johnsonae* (3).

**Isolation.** Crystal violet-polypectate (CVP) medium (5) enriched with yeast extract (0.1%) was used for isolation. Fruits of bell pepper (*Capsicum annuum*) and watermelon (*Citrullus vulgaris*) showing soft-rot lesions were collected from local supermarkets in June 1984 and in October 1985. A loopful of macerated tissue was streaked onto the isolation medium, and plates were incubated at 26°C for 2 days. Colonies that showed orange pigmentation, spreading growth, and a deep depression indicative of pectolytic activity (5) were selected and purified by repeated streaking on CVP medium. One isolate from bell pepper (PF-062) and one from watermelon (WM-101) were maintained on NYM medium containing Difco nutrient agar (2.3%), yeast extract (0.1%) and Difco skim milk (1%). Viability of stock cultures could also be maintained in sterile distilled water at room temperature (20°C) for more than 4 months.

**Cellular characterization.** Strains PF-062 and WM-101 were characterized by the determinative scheme of the *Cytophaga* group described by Christensen (4). Absence of flagella was confirmed by two methods, silver nitrate impregnation (1) and tannic acid staining (7). Gliding motility was examined by the molten agar method of Reichenbach and Dworkin (16). Three agar media (Difco *Pseudomonas* agar F, NYM medium, and MM-9 medium) were used to detect spreading (or swarming) growth. The MM-9 medium (pH 7.2) containing glycerol (0.5%), yeast extract (0.1%), polygalacturonic acid (0.4%), $MgSO_4 \cdot 7H_2O$ (1 mM), $CaCl_2 \cdot 2H_2O$ (1 mM), and agar (1.5%) was formulated initially for the detection of pectic enzymes. Cellular morphology of each strain was monitored on NYM medium. Cells grown on NYM medium were suspended in saline and examined with a phase-contrast microscope (Nikon Optiphot; ×1,000).

**Polyacrylamide gel electrophoresis.** Cells were grown in nutrient broth (Difco) containing yeast extract (0.1%), harvested by centrifugation (17,000 × *g*, 10 min), and then disrupted by ultrasonification with a Branson sonifier cell disrupter microtip at 40 W for 3 min. Whole-cell proteins were solubilized in 2% sodium dodecyl sulfate. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed by methods described elsewhere (9). In each well, a sample (3 to 5 μl) containing 10 μg of protein was added.

**Physiological characterizations.** The following tests were performed by methods previously described (7): anaerobic growth, reactions of oxidase, phosphatase, catalase, $H_2S$, indole, urease, arginine dihydrolase, and sucrose reduction. A minimal salt solution (pH 7.0) containing $K_2HPO_4$ (0.7%), $KH_2PO_4$ (0.2%), $MgSO_4 \cdot 7H_2O$ (0.02%), and $(NH_4)_2SO_4$

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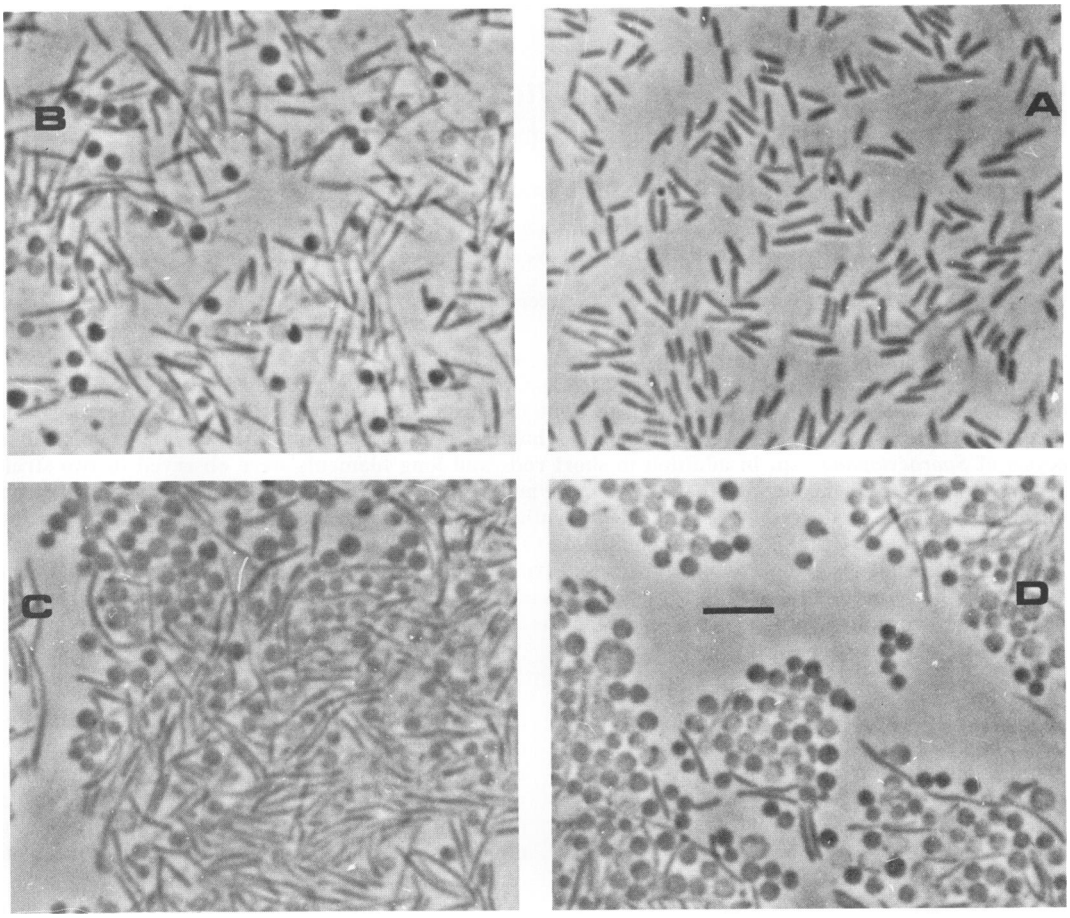


FIG. 1. Phase-contrast photographs of *C. johnsonae* ATCC 29589 grown on nutrient agar enriched with yeast extract (0.1%) and skim milk (1%). Bar, 5 μm. (A) 1-day-old culture, exclusively short rods; (B) 2-day-old culture, long filaments mingle with spherical bodies; (C) 3-day-old culture, filaments grow longer and number of spherical bodies increases; (D) 4-day-old culture, spherical bodies become predominant.

(0.1%) and sometimes enriched with yeast extract (0.02%) was used to study nutritional requirements. Each strain was tested for utilization of 26 carbohydrates as carbon or energy sources. Each carbohydrate, sterilized by filtration, was added into the minimal medium to a final concentration of 0.2%. Results were recorded after 7 days of incubation at 26°C. Growth at 5 and 37°C was determined by inoculating a loopful of young culture onto NYM medium and placing inoculated plates at the required temperature for 10 days. Degradation of natural or synthetic polymers was assayed as previously described (C.-H. Liao and J. M. Wells, Phytopathology, in press). The polymers tested were carboxymethyl cellulose (Hercules Powder Co., Wilmington, Del.), skim milk proteins (Difco), sodium polypectate (Sunkist Growers, Inc., Ontario, Calif.) and crab shell chitin (Sigma Chemical Co., St. Louis, Mo.).

**Assays for pectic enzymes.** Each strain was grown in minimal salt solution containing yeast extract (0.05%) and one of three carbon sources (0.4%). The three carbon sources tested were polygalacturonic acid (ICN Pharmaceuticals, Inc., Cleveland, Ohio), citrus pectin (Sunkist Growers, Inc.), and glucose (Sigma). Cultures were incubated with shaking at 26°C for 2 days. Cells were removed by centrifugation (17,000 × *g*, 10 min), and the supernatant fluids were assayed for pectate lyase, polygalacturonase, and pectin lyase activity by the method of Nasuno and Starr (15). One unit of pectate lyase activity was defined as a change of absorbance of 1.0 (at 235 nm) per min at 20°C. The specific activity was calculated by dividing the unit activity by the cell density measured as the $A_{600}$.

**Effects on plants.** Potato (*Solanum tuberosum*) tubers and fruits of bell pepper, zucchini squash (*Cucurbita pepo*), and tomato (*Lycopersicon esculentum*) were surface sterilized by soaking in 1% sodium hypochlorite solution (10 min) followed by rinsing twice in sterile water. Slices of potato tubers and zucchini squash were prepared as previously described (Liao and Wells, in press). Intact fruits of bell peppers and tomato were wounded with a sterile 25-gauge syringe needle. Bacteria grown in nutrient broth-yeast extract were harvested by centrifugation (17,000 × *g*, 10 min), washed once in sterile distilled water, and resuspended in sterile water to a final concentration of $10^7$ to $10^8$ CFU/ml. The bacterial suspension was smeared onto the surface of potato or squash slices or onto the punctured sites of tomatoes or bell peppers. Incubation was at room temperature (20°C) for 3 days. For potato or squash slices, the degree of maceration was recorded on an arbitrary scale of 0 (no maceration) to 5 (total disintegration of tissue). On fruits of bell pepper and tomato, the degree of maceration was measured as the diameter of soft-rot lesions. Tests were repeated at least five times, each with five replications per treatment.

TABLE 1. Comparison of properties of *C. johnsonae* strains deposited at the ATCC and strains isolated from decayed produce (PF-062 and WM-101)

| Property | PF-062 and WM-101[a] | ATCC no. | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 17061 | 29583 | 29587 | 29588 | 29589 | 29590 | 19366 |
| Utilization of: | | | | | | | | |
| Rhamnose | + | − | − | − | − | − | − | + |
| Trehalose | + | + | + | − | + | + | + | + |
| Xylose | + | + | − | + | − | + | + | + |
| Glucose | + | + | + | − | + | + | + | + |
| Sucrose reduction | − | − | + | − | + | + | + | + |
| Growth at: | | | | | | | | |
| 5°C | + | + | − | − | − | + | − | + |
| 37°C | − | − | − | − | − | − | − | − |
| Spreading growth[b] | + | + | + | − | + | − | − | − |
| Pigmentation on NYM[c] | O | PY | Y | O | Y | O | Y | O |

[a] +, Growth; −, no growth.
[b] Grown on MM-9 medium at 26°C; limited spreading observed with these negative strains at <15°C.
[c] NYM, Nutrient agar enriched with yeast extract (0.1%) and skim milk (1%); O, orange; PY, pale yellow; Y, yellow.

## RESULTS

**Culture characteristics.** Strains PF-062 and WM-101 isolated from naturally infected plant specimens were identified as members of *C. johnsonae* based on an array of characteristic properties. They were gram negative, orange pigmented, and nonflagellated but able to glide on the surface of the agar media. Spreading growth was detected on a number of culture media including *Pseudomonas* agar F and MM-9 medium. Plates (100 by 15 mm) of MM-9 medium inoculated with a streak of strain PF-062 were covered with a thin, translucent layer of cells, which appeared pale yellowish or colorless, within 4 days at 26°C. The size and shape of cells grown on NYM medium was monitored and is illustrated in Fig. 1. In young cultures (less than 18 h) a predominance of short rods was observed (Fig. 1A). The rods became thinner and longer (up to 20 μm) as the culture aged. At a later stage of development (2 days older), spherical bodies (2 to 3 μm in diameter) resembling microcysts (or myxospores) of *Sporocytophaga* sp. were found (Fig. 1B and C). Spherical bodies were present in some strains (ATCC 29583 and 29588) throughout the growth cycle and became predominant in cultures 4 days old (Fig. 1D). It was not determined whether the spherical bodies represent a resting stage or simply represent a degenerative form of aged cells. Formation of spherical bodies (on NYM medium) at some stages of growth and exhibition of spreading growth (on MM-9 medium at 15°C or below) were observed for all nine strains included in the study.

**Physiological characteristics.** All nine strains had the following properties in common: strictly aerobic, positive in tests of oxidase, phosphatase, catalase, and $H_2S$; negative in tests of urease, indole, arginine dihydrolase, and levan formation; and capable of degrading chitin, pectin, protein, cellulose, and starch. With the exception of two strains (ATCC 29583 and 29588), every strain was shown to grow in a minimal salt solution supplemented with 0.2% of an suitable carbohydrate. ATCC 29583 and 29588 were able to grow in the same minimal salt solution further enriched with yeast extract (0.05%). Strains PF-062 and WM-101 could be readily differentiated from other strains on the basis of their ability to utilize glucose, xylose, trehalose, sucrose, and rhamnose (Table 1) and appear to be identical to each other in terms of cultural and physiological properties. Both of them are closely related to ATCC 17061, 19366, and 29589 but are substantially different from the remaining strains.

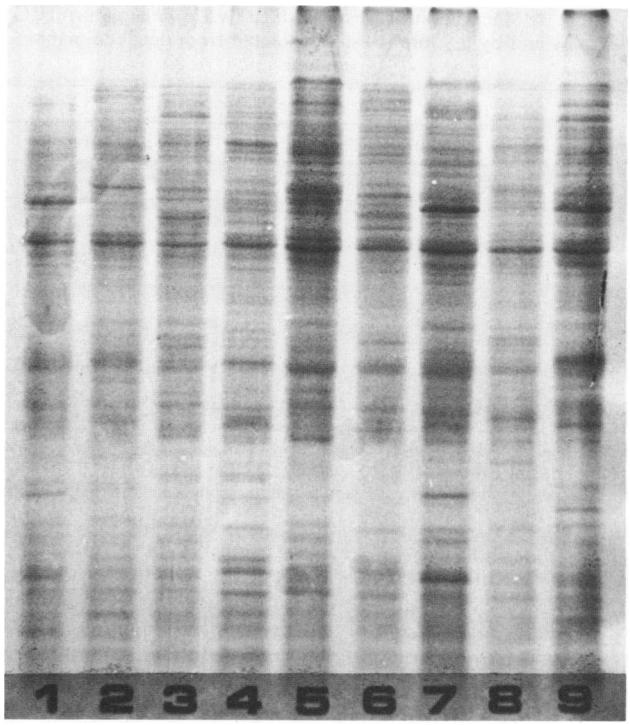


FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of cellular proteins extracted from *C. johnsonae* ATCC strains and strains isolated from decayed plants. Lanes 1 to 6, ATCC 17061, 29583, 29587, 29588, 29589, 29590, respectively; lane 7, PF-062; lane 8, ATCC 19366; lane 9, WM-101.

**Electrophoresis of cellular proteins.** In addition to their nutritional variability, *C. johnsonae* strains differed considerably in electrophoretic patterns of whole-cell proteins extracted with sodium dodecyl sulfate (Fig. 2). Even strains PF-062 and WM-101, which appear identical in nutritional and physiological properties, show some degree of variation in cellular proteins (Fig. 2, lanes 7 and 9).

**Secretion of pectate lyase.** The presence of pectolytic enzymes in each strain was initially detected on CVP me-

TABLE 2. Secretion of pectate lyase by *C. johnsonae* ATCC strains and by a strain isolated from decayed produce in medium containing glucose, polygalacturonate, or pectin

| Medium | Sp act (u $ml^{-1}$ $A_{600}^{-1}$)[a] with: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | PF-062 | ATCC strain | | | | | | |
| | | 17061 | 29583 | 29587 | 29588 | 29589 | 29590 | 19366 |
| Glucose | 0.52 | 0.31 | 0.00 | 0.01 | 0.00 | 0.02 | 0.00 | 0.03 |
| Polygalacturonate | 1.80 | 1.65 | 0.02 | 0.08 | 0.03 | 0.08 | 0.06 | 1.37 |
| Pectin | 1.84 | 1.71 | 0.01 | 0.05 | 0.01 | 0.06 | 0.02 | 0.68 |

[a] One unit of activity is defined as a change of absorbancy of 1.0 per min at 20°C. The specific activity wa calculated by dividing the activity by the $A_{600}$.

TABLE 3. Maceration of fresh produce by *C. johnsonae* ATCC strains and by a strain (PF-062) isolated from decayed plant specimens

| Strains | Degree of maceration | | | |
|---|---|---|---|---|
| | Avg scale[a] | | Avg diam (mm)[b] | |
| | Potato | Squash | Pepper | Tomato |
| PF-062 | 4.4 | 3.4 | 20.0 | 15.2 |
| ATCC | | | | |
| 17061 | 3.8 | 2.6 | 11.8 | 9.0 |
| 29583 | 0 | 0 | 0 | 0 |
| 29587 | 2.2 | 0.8 | 1.4 | 1.6 |
| 29588 | 0 | 0 | 0 | 0 |
| 29589 | 2.8 | 0.8 | 1.0 | 1.8 |
| 29590 | 0 | 0 | 0 | 0 |
| 19366 | 1.2 | 2.4 | 14.6 | 10.0 |

[a] Average scale based on a scale of 0 to 5: 0, no maceration; 5, 100% maceration; and 1 to 4, 20, 40, 60, 80% maceration, respectively.

[b] Average diameter of the rot lesion measured from the site of inoculation.

dium supplemented with yeast extract and later confirmed on MM-9 medium. Strains varied in their ability to secrete pectic enzymes (Table 2). Pectate lyase was detected in broth media containing polygalacturonate or pectin, but in lesser amounts in media containing glucose. Under the conditions examined, very little or no polygalacturonase or pectin lyase activity was detected for all strains tested.

**Macerating ability.** Strain PF-062 and strain WM-101 were able to infect and induce soft rots of artificially wounded potato tubers and fruits of squash, pepper, and tomato, although the two strains were derived from two different plant materials. Three ATCC strains (17061, 29587, 19366), originally isolated from soil or other sources (3), were also shown to cause maceration of injured tissues of apparently healthy and vigorous plant parts. However, the degree of maceration incited by ATCC strains was in general less severe than that induced by strains of plant origin. Furthermore, the soft-rotting ability of six strains (Table 3) generally reflected the ability of each strain to produce pectate lyase in culture filtrates (Table 2).

## DISCUSSION

Pectolytic bacteria of several genera (*Erwinia*, *Pseudomonas*, *Clostridium*, *Bacillus*, and *Xanthomonas*) have been previously shown to be the cause of decay of fresh produce in storage or in transit (11, 12; Liao and Wells, in press). In our study, the aforementioned pectolytic bacteria were not detected or recovered from the decayed plant specimens examined. Two strains of *C. johnsonae* (PF-062 and WM-101) isolated as described in the text appear to be the primary or the principal cause of decay in those specimens examined. This notion is supported by the observation that pure cultures of PF-062 and WM-101 were able to infect and attack artificially wounded potato slices and fruits of pepper and tomato (Table 3). It was not determined, and seemed to be unlikely, that *C. johnsonae* would cause diseases of growing plants in the field. Like *Clostridium* and *Bacillus* species, *C. johnsonae* probably acts as an opportunistic, weak pathogen and attacks only detached plant parts that gradually lose their vigor and defense mechanisms.

Pectate lyase is believed to be the principal factor responsible for tissue maceration and development of soft-rot disease induced by *Erwinia* species and possibly *Pseudomonas* species (14). At present, very little is known about the pectic enzymes produced by *C. johnsonae* (6, 8, 20). Our results (Table 2) show that pectate lyase is produced by all strains of *C. johnsonae* examined. However, we found that the amounts of pectate lyase produced by strains PF-062 and WM-101 isolated from decayed plants are much greater than those produced by strains ATCC 29583, 29588, and 29590 isolated from other sources (soil or moose dung).

The nutritional requirements of *C. johnsonae* are relatively simple (16). A minimal salt solution supplemented with a suitable carbohydrate is sufficient to support the growth of the majority of strains we tested. Two strains (ATCC 29583 and 29588) which failed to grow in the minimal medium used in this study and in a complex minimal medium previously reported by Chang and Pate (2) apparently require growth factors present in yeast extract. Since all the strains examined in our study can be distinguished from each other on the basis of their different abilities to utilize five sugars (Table 1), *C. johnsonae* as a species seems to represent a heterogeneous group. The heterogeneity of the group is also indicated by the variation of electrophoretic patterns of cellular proteins extracted from each strain (Fig. 2).

*C. johnsonae* strains have never been shown to form microcysts (myxospores or fruiting bodies) (4, 17, 18), characteristic of the genus *Sporocytophaga* (12, 13). We found that in addition to short rods and long filaments, spherical bodies resembling microcysts were present in appreciable numbers in cultures of *C. johnsonae* ATCC 29583 and 29588 throughout the growth cycle and also in aged cultures of other strains. The spherical bodies of *C. johnsonae* found in our study are somewhat different in uniformity of size from those of *Cytophaga fermentans* and *Cytophaga salmonicolor* previously described by Veldkamp (19). It remains to be determined whether these spherical forms are viable and whether they represent a resting stage.

## ACKNOWLEDGMENTS

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