Erwinia carotovora subsp. *odorifera* subsp. nov., Associated with Odorous Soft Rot of Chicory (*Cichorium intybus* L.)

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Eleven strains of *Erwinia carotovora* that were isolated mainly, but not exclusively, from slimy rot of witloof chicory and were previously designated "atypical" *E. carotovora* subsp. *atroseptica* strains were characterized and compared with strains of *E. carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica*, and *Erwinia chrysanthemi* (including the type strains). The 11 atypical *E. carotovora* subsp. *atroseptica* strains produced a typical bananalike odor when they were inoculated onto witloof chicory leaves. DNA-DNA homology experiments, biochemical tests, tests to determine carbon utilization patterns, and tests to identify the volatile metabolites produced from rotting witloofs were performed. The volatile end products of witloof decay were analyzed by gas chromatography. Alcohols, methylketones, and ethylacetate were produced by all of the *Erwinia* strains which we studied, whereas propyl acetate, isobutyl acetate, isoamyl acetate, and 2-actamyl acetate were produced only by the flavoring witloof soft-rot strains. A DNA relatedness study was performed by hybridizing DNAs with a tritium-labeled DNA and estimating the ΔT_m values (ΔT_m is the difference between the thermal denaturation midpoint of a homoduplex and the thermal denaturation midpoint of a heteroduplex). The 11 flavoring strains constituted a tight DNA hybridization group (79 to 91% related to type strain CFBP 1878 isolated from witloof). Strains of *E. carotovora* subsp. *carotovora* were 59 to 88% related to strain CFBP 1878^T (T = type strain) (ΔT_m range, 3 to 4.5°C), indicating that they belonged to the same species but another subspecies. *E. carotovora* subsp. *atroseptica* and *E. carotovora* subsp. *betavascularum* appeared to be less closely related to strain CFBP 1878^T than *E. carotovora* subsp. *carotovora* was, exhibiting 53% homology (ΔT_m , 7°C) and 48 to 51% homology (ΔT_m , 8.5°C), respectively, with strain CFBP 1878^T. Therefore, we propose that the 11 flavoring strains are members of a new subspecies, *Erwinia carotovora* subsp. *odorifera*. We examined 95 biochemical characteristics, API strip tests, and assimilation tests in Biotype galleries and identified nine tests which can be used for phenotypic differentiation of the new subspecies.

Soft rot of witloof (broad-leaf chicory, *Cichorium intybus* L.) occurs indoors during forcing of the plants. First observed in France (16), this disease typically results in water-soaked, translucent, wet rot of the etiolated leaves and is called "slimy rot" (20). The bacterium that is responsible for this plant tissue maceration disease was designated "atypical" *Erwinia carotovora* subsp. *atroseptica* by Samson et al. (16) since a few of its characteristics did not exactly coincide with the characteristics of the potato blackleg pathogen.

The development of slimy rot in witloof chicories has been shown to be always associated with the production of a sweet, ripe, bananalike odor (2). Similar bacteria have been isolated from other soft-rotted plants, such as leeks (*Allium porrum*) and celery (*Apium graveolens*); these organisms also produce a bananalike odor when they are inoculated onto witloof leaves. Strains identified as *Erwinia chrysanthemi* have also been isolated from a soft rot of chicory roots, a disease which may occasionally develop in the field or before forcing of the cut roots.

The purposes of this work were (i) to examine the taxonomic position of strains associated with odorous soft rot of witloof by performing DNA hybridization experiments, (ii) to characterize by using phenotypic tests the odorous soft-rot organism, and (iii) to identify the volatile metabolites that are responsible for the bananalike odor. Our findings resulted in the description of a new subspecies, *Erwinia carotovora* subsp. *odorifera*.

MATERIALS AND METHODS

Bacterial strains. The strains which were used in this study are shown in Table 1. The bacteria were purified on YPD agar (yeast extract, 3 g/liter; peptone, 5 g/liter; dextrose, 5 g/liter; pH 7.2) and were stored during the study on yeast extract-peptone agar slants without dextrose.

DNA relatedness study. DNAs were extracted and purified as described elsewhere (4). The exact procedures used for in vitro labeling of DNA with tritium-labeled nucleotides and for hybridization experiments (S1 nuclease-trichloroacetic acid procedure) have been described previously (13). The temperature at which 50% of a reassociated DNA became hydrolyzable by S1 nuclease (T_m) was determined as described by Crosa et al. (7). ΔT_m (the difference between the T_m of a homoduplex and the T_m of a heteroduplex) is an estimate of the level of divergence between two DNAs (3).

Conventional identification tests. The techniques which we used for bacterial identification (Gram and flagellum staining, spore formation, oxidase, dextrose fermentation, nitrate reduction, Voges-Proskauer test, starch hydrolysis, lecithinase and phosphatase tests, indole production, Simmons citrate, esculin hydrolysis, growth at 37°C) were classical techniques (10, 15). Additional tests which we used included tests for hydrolysis of L-alanine-4-nitroanilide hydrochloride (6), liquefaction of calcium polygalacturonate in Sutton medium (1), liquefaction of carboxymethyl cellulose (14), and production of reducing substances from sucrose (9). Production of acid from carbohydrates was determined in a mineral medium (NH₄H₂PO₄, 0.1%; KCl, 0.02%; MgSO₄ ·

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TABLE 1. Sources of bacterial strains

Strain ^a	Host plant	Geographical origin	Source, year of isolation, other designation, and/or biovar ^a
<i>Erwinia carotovora</i> subsp. <i>odorifera</i> strains			
CFBP 1878 ^T	<i>Cichorium intybus</i>	Loiret, France	R. Samson, 1978
CFBP 1879	<i>Cichorium intybus</i>	Rhône, France	R. Samson, 1979
CFBP 1880	<i>Cichorium intybus</i>	Morbihan, France	R. Samson, 1979
CFBP 1892	<i>Cichorium intybus</i>	Oise, France	L. Gardan, 1978
CFBP 1893	<i>Apium graveolens</i>	Lot, France	R. Samson, 1976
CFBP 1959	<i>Cichorium intybus</i>	Pyrénées-Atlantiques, France	R. Samson, 1980
CFBP 2154	<i>Cichorium intybus</i>	Yvelines, France	R. Samson, 1982
CFBP 2155	<i>Cichorium intybus</i>	Côtes-d'Armor, France	L. Gardan, 1982
CFBP 3259	<i>Allium porrum</i>	Maine-et-Loire, France	R. Samson, 1980
CFBP 3260	<i>Allium porrum</i>	Loire-Atlantique, France	R. Samson, 1980
CFBP 3261	<i>Allium porrum</i>	Loire-Atlantique, France	R. Samson, 1982
<i>Erwinia carotovora</i> subsp. <i>carotovora</i> strains			
CFBP 798	<i>Daucus carota</i>	United States	L. R. Jones, ATCC 495
CFBP 1535	<i>Zea mays</i>	Israel	Z. Volcani, 1958, ICPB EA144
CFBP 2046 ^T	<i>Solanum tuberosum</i>	Denmark	E. Hellmers, NCPPB 312 ^T
CFBP 2141	<i>Solanum tuberosum</i>	France	R. Samson, 1978
<i>Erwinia carotovora</i> subsp. <i>atroseptica</i> strains			
CFBP 1330	<i>Solanum tuberosum</i>	United Kingdom	M. C. M. Perombelon, 1971
CFBP 1526 ^T	<i>Solanum tuberosum</i>	United Kingdom	D. C. Graham, NCPPB 549 ^T
CFBP 2391	<i>Solanum tuberosum</i>	Germany	B. Jouan (47), 1976
<i>Erwinia carotovora</i> subsp. <i>betavascularum</i> strains ^b			
CFBP 2121	<i>Beta vulgaris</i>	United States	S. V. Thomson, 1972, UCPB 162
CFBP 2122 ^T	<i>Beta vulgaris</i>	United States	S. V. Thomson, 1971, UCPB 193 ^T
<i>Erwinia chrysanthemi</i> strains			
CFBP 1528	<i>Zea mays</i>	United States	A. Kelman (SR80), 1966, biovar 3 ^c
CFBP 2048 ^T	<i>Chrysanthemum morifolium</i>	United States	W. H. Burkholder, NCPPB 402 ^T , biovar 5
CFBP 2052 ^d	<i>Zea mays</i>	United States	A. Kelman (SR58), 1970, biovar 3
CFBP 3262	<i>Cichorium intybus</i>	France	R. Samson (SA77-2), 1981, biovar 5
CFBP 3263	<i>Cichorium intybus</i>	France	R. Samson (SB50-18), 1982, biovar 5
CFBP 3264	<i>Cichorium intybus</i>	France	R. Samson (SC72-3), 1983, biovar 1
CFBP 3265	<i>Cichorium intybus</i>	France	R. Samson (SC72-4), 1983, biovar 1
CFBP 3266	<i>Cichorium intybus</i>	France	R. Samson (SC72-6), 1983, biovar 1
<i>Erwinia amylovora</i> ICPB EA178			
<i>Erwinia cypripedii</i> ATCC 29267 ^T	<i>Cypripedium</i> sp.	United States	ICPB EC155 ^T
<i>Erwinia herbicola</i> (<i>Enterobacter agglomerans</i>) NCTC 9381			
<i>Erwinia nigrifluens</i> ATCC 29275	<i>Juglans regia</i>	United States	E. Wilson, ICPB EN104
<i>Erwinia mallotivora</i> CFBP 2503 ^T	<i>Mallotus japonicus</i>	Japan	M. Goto, 1976, NCPPB 2851 ^T
<i>Erwinia rhapontici</i> NCPPB 1739	<i>Rheum rhaponticum</i>	United Kingdom	S. Masser
<i>Erwinia salicis</i> ICPB ES102			
<i>Enterobacter dissolvens</i> NCPPB 2209	<i>Sorghum vulgare</i>	United States	N. Zummo, 1969
<i>Enterobacter nimipressuralis</i> NCPPB 2045 ^T	<i>Ulmus</i> sp.	United States	J. C. Carter, 1968

^a CFBP, French Collection of Phytopathogenic Bacteria, Angers, France; ICPB, International Collection of Phytopathogenic Bacteria, Davis, Calif.; ATCC, American Type Culture Collection, Rockville, Md; NCTC, National Collection of Type Cultures, London, United Kingdom; NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, United Kingdom; UCPB, University of California Phytopathogenic Bacteria, Berkeley, Calif.

^b See reference 18.

^c See reference 17.

^d Type strain of *E. chrysanthemi* pv. *zeae*.

7H₂O, 0.02%; bromothymol blue, 0.008%; carbohydrate, 0.3% [wt/vol]; pH 6.5) that was derived from ARJ base (15); the preparations were incubated at 25°C for 15 days. API 20E and API 50CHE strips (BioMérieux, La Balme-les-Grottes, France) were inoculated following the manufacturer's instructions. The strips were incubated at 25°C for 5 days.

Assimilation tests. Carbon source utilization tests were performed by using Biotype galleries (Biomerieux) containing 99 pure carbon sources (12). A minimal medium (60 ml) provided by BioMérieux was inoculated with 2 ml of a

bacterial suspension calibrated to 100 Klett units (McFarland opacity standard no. 3), and the galleries were filled with the seeded mixture. Tests were conducted at 30°C. The galleries were examined visually for growth daily for 4 days.

Analysis of volatile odorous compounds. The volatile compounds which we analyzed were produced during soft rot of witloof chicories that were inoculated with bacteria. Detached leaves of fresh witloof chicories were disinfected with calcium hypochlorite (7%, wt/vol) and rinsed twice with sterile water. A 1-ml portion of a bacterial suspension (5×10^8 cells from a 24-h-old culture per ml) was then injected

into the parenchyma of each leaf. Inoculated leaves (75 g) were kept in sterile humid chambers (500-ml Erlenmeyer flasks closed with cotton plugs) and incubated at 25°C for 24 or 48 h (until complete liquefaction occurred). Leaves inoculated under the same conditions with sterile water were used as controls.

After 0.4 mg of ethyl isobutanoate was added as an internal standard, the Erlenmeyer flasks containing witloof leaves were placed in a 25°C water bath and purged with oxygen-free nitrogen (flow rate, 15 ml/min) for 1 h. For headspace trapping, a porous polymer (100 mg of 80/100-mesh Porapak Q; Waters Division, St. Quentin-en-Yvelines, France) was packed into a glass tube (bore, 3 mm; length, 100 mm). By using two traps in series, we ascertained that the adsorption capacity of the first trap was not exceeded. The volatile compounds were then eluted from the trap with 300 µl of redistilled diethyl ether. A 1-µl portion of the solvent solution was directly injected into a gas chromatograph.

The volatile compounds were separated by using a model DI 700 gas chromatograph (Delsi-Nermag Instruments, Argenteuil, France) equipped with an on-column injector (J. & W. Chromoptic, Montpellier, France) and a flame ionization detector (maintained at 240°C). A 0.90-m uncoated fused-silica capillary tube was fitted onto the beginning of the fused-silica capillary column (type DB5; 30 m by 0.32 mm [inside diameter]; J. & W. Chromoptic). The film thickness was 1 µm. The carrier gas was hydrogen (50 cm/s). The oven temperature was programmed to increase from 30 to 100°C at a rate of 2°C/min. Peak areas were measured with a model ENICA 21 integrator (Delsi-Nermag).

Spectra were recorded with a Delsi-Nermag model R10-10 mass spectrometer coupled to a Girdel model 31 gas chromatograph. The fused-silica type DB5 capillary column (60 m by 0.32 mm [inside diameter]) was connected directly to the ion source (temperature, 150°C). The operating gas chromatography conditions were the same as those described above. Mass spectra were recorded at 70 eV. The scanning rate was 0.8 s from 25 to 300 a.m.u. All identifications were confirmed by injecting reference compounds.

RESULTS

DNA-DNA hybridization. The relative levels of reassociation between unlabeled DNAs from *Erwinia* species and labeled DNA from witloof isolate CFBP 1878^T (T = type strain) are shown in Table 2. The atypical *E. carotovora* strains that caused flavoring soft rot of witloof were 79 to 91% related to strain CFBP 1878^T. The least closely related strain exhibited 0.5°C divergence (ΔT_m value) with strain CFBP 1878^T. Thus, the atypical *E. carotovora* strains that cause flavoring soft rot of witloof constituted a tight DNA hybridization group.

Strains of *E. carotovora* subsp. *carotovora* were 59 to 88% related to strain CFBP 1878^T, with ΔT_m values ranging from 3 to 4.5°C. *E. carotovora* subsp. *atroseptica* was 53% related to strain CFBP 1878^T, with a ΔT_m of 7°C. Strains of *E. carotovora* subsp. *betavascularum* were 48 to 51% related to strain CFBP 1878^T, with a ΔT_m of 8.5°C. All other *Erwinia* species (including *E. chrysanthemi* strains isolated from witloof chicory) and the two *Enterobacter* species which we tested were less than 16% related to strain CFBP 1878^T.

Phenotypic characterization of the flavoring witloof soft-rot organism. Conventional identification of the flavoring soft-rot strains resulted in identification as members of the species *Erwinia carotovora* (Table 3). The characteristics

TABLE 2. Levels of relatedness between *E. carotovora* subsp. *odorifera* CFBP 1878^T and *E. carotovora* subsp. *carotovora* and other *Erwinia* or *Enterobacter* species

Source of unlabeled DNA	% Relatedness with labeled DNA from strain CFBP 1878 ^T
<i>Erwinia carotovora</i> subsp. <i>odorifera</i> strains	
CFBP 1878 ^T	100 (0.0) ^a
CFBP 1879	80
CFBP 1880	90
CFBP 1892	91
CFBP 1893	83
CFBP 1959	85
CFBP 2154	81
CFBP 2155	88
CFBP 3259	79 (0.5)
CFBP 3260	89
CFBP 3261	81
<i>Erwinia carotovora</i> subsp. <i>carotovora</i> strains	
CFBP 798	88 (3.0)
CFBP 1535	58 (4.5)
CFBP 2046 ^T	68 (3.0)
<i>Erwinia carotovora</i> subsp. <i>atroseptica</i>	
CFBP 1526 ^T	53 (7.0)
<i>Erwinia carotovora</i> subsp. <i>betavascularum</i> strains	
CFBP 2121	51
CFBP 2122 ^T	51 (8.5)
<i>Erwinia chrysanthemi</i> strains	
CFBP 1528	14
CFBP 2048 ^T	13
CFBP 3262	15
CFBP 3263	12
CFBP 3365	15
CFBP 3266	16
<i>Erwinia amylovora</i> ICPB EA 178	5
<i>Erwinia cypripedii</i> ATCC 29267 ^T	10
<i>Erwinia herbicola</i> (<i>Enterobacter agglomerans</i>)	
NCTC 9381	6
<i>Erwinia nigrifluens</i> ATCC 29275	18
<i>Erwinia mallotivora</i> CFBP 2053 ^T	5
<i>Erwinia rhapontici</i> NCPPB 1739	9
<i>Erwinia salicis</i> ICPB ES 102	8
<i>Enterobacter dissolvens</i> NCPPB 2209	6
<i>Enterobacter nimipressuralis</i> NCPPB 2045 ^T	8

^a The values in parentheses are ΔT_m values (in degrees Celsius).

common to all of the strains associated with flavoring soft rot of witloof, including the reactions on Biotype strips, are given below in the subspecies description. Some variability was observed (Table 3).

Table 4 shows the conventional tests that are useful for phytopathologists and allow differentiation among the *E. carotovora* subspecies, including the odorous soft-rot organism.

Volatile compounds from inoculated witloof chicories. Compounds belonging to three different chemical classes (esters, alcohols, and ketones) were found in the volatile compound profiles of all of the strains (Table 5). None of these compounds was present in the headspace of healthy-leaf preparations. Alcohols, methylketones, and ethylacetate were produced by all of the strains, whereas propyl acetate, isobutyl acetate, isoamyl acetate, and 2-methyl-butyl-(act-amyl)-acetate were produced only by the 11 flavoring witloof soft-rot strains.

Isoamyl acetate is well known as the odorous impact compound in bananas and, together with isobutyl acetate

TABLE 3. Phenotypic characteristics of the erwinias tested^a

Characteristic	% of strains positive				
	<i>E. carotovora</i> subsp. <i>odorifera</i> (n = 11) ^b	<i>E. carotovora</i> subsp. <i>carotovora</i> (n = 4)	<i>E. carotovora</i> subsp. <i>atroseptica</i> (n = 3)	<i>E. carotovora</i> subsp. <i>betavasculorum</i> (n = 2)	<i>E. chrysanthemi</i> (n = 8)
Lecithinase	0	0	0	0	100
Phosphatase	0	0	0	0	100
Indole production	0	0	0	0	100
Reducing sugars produced from sucrose	100	0	100	100	50(w) ^c
Growth at 37°C	100	100	0	100	100
Acidification of ^d :					
Sorbitol	100	0	0	0	0
α-Methylglucoside	100	0	100	100	0
Palatinose	100	0	100	100	0
Melibiose	100	100	100	0	100
D-Arabitol	100	0	0	0	0
Alkalinization of malonate ^d	0	0	0	0	100
Acid produced from inulin ^e	0	0	0	100	75
Citrate (Simmons)	100	100	100	0	100
API 20E tests					
Arginine dihydrolase	0	0	0	0	75
Citrate	100	100	100	0	100
Indole	0	0	0	0	100
Gelatin	100	100	0	100	100
Sorbitol	100	0	0	0	0
Melibiose	100	100	100	0	100
API 50 CHE tests					
D-Arabinose	0	0	0	0	25
Sorbitol	100	0	0	0	0
α-Methyl-D-glucoside	100	0	100	100	0
Amygdalin	9	50	67	100	0
Maltose	45	0	67	100	0
Lactose	100	100	100	100	25
Melibiose	100	100	100	0	100
Trehalose	100	100	100	100	0
Inulin	0	0	0	100	75
β-Gentiobiose	100	100	100	100	12
D-Turanose	27	0	67	100	0
D-Arabitol	100	0	0	0	0
Gluconate	0	0	100	100	0

^a The following tests were positive for all of the strains: L-alanine aminopeptidase; peritrichous; dextrose fermentation; nitrate reduction; pectate liquefaction; β-galactosidase; acetoin production; acid production from glucose, mannitol, inositol, rhamnose, sucrose, amygdalin, and L-arabinose in API 20E tests; and acid production from glycerol, L-arabinose, ribose, D-xylose, galactose, D-fructose, N-acetyl-glucosamine, arbutin, esculin, salicin, cellobiose, and D-raffinose in API 20E or API 50CHE tests. The following tests were negative for all of the strains: Gram reaction; spore formation; oxidase; starch hydrolysis; lysine and ornithine decarboxylases; H₂S; urease; tryptophan desaminase in API 20E tests; acid production from erythritol, L-xylose, adonitol, β-methylxyloside, L-sorbose, dulcitol, α-methyl-D-mannoside, melezitose, starch, glycogen, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, L-arabitol, 2-ketogluconate, and 5-ketogluconate in API 50CHE tests.

^b n is the number of strains tested.

^c w, weak reaction.

^d In ARJ basal medium.

^e In peptone medium.

and to a lesser extent actamyl acetate, was responsible for the bananalike odor in our study.

DISCUSSION

Patterns of volatile products are commonly used in taxonomy, especially in the identification of *Clostridium* spp. (5). In our laboratory, the bananalike odor of infected witloof chicories is a quick test for identification of the slimy rot produced by *E. carotovora* subsp. *odorifera*. The monitoring of volatile compounds has been proposed as a technique for detecting soft rot in potato tubers (21). The production of volatile metabolites has been studied in potatoes infected with both *E. carotovora* subsp. *carotovora* and *E. carotovora* subsp. *atroseptica* (23). Short-chain alcohols and car-

bonyl compounds were identified. Most of these compounds were found in our study. Ethyl acetate, a common microbial metabolite which was the most abundant metabolite compound in our study, has also been found in the volatile compound profile of potato tubers inoculated with *E. carotovora* subsp. *atroseptica* (22). However, none of these metabolites could impart to the diseased plants a bananalike odor. The concentrations of the volatile compounds shown in Table 5 are underestimated since the headspace did not allow accurate measurements. Nevertheless, the three acetates responsible for the bananalike odor (isobutyl acetate, isoamyl acetate, and actamyl acetate) were produced only by the 11 flavoring soft-rot strains when they were grown on witloof chicory leaves. These three acetates could be used to characterize the new subspecies.

TABLE 4. Phenotypic differentiation of *E. carotovora* subsp. *odorifera* from other *E. carotovora* subspecies

Characteristic	Reaction of:			
	<i>E. carotovora</i> subsp. <i>odorifera</i>	<i>E. carotovora</i> subsp. <i>carotovora</i>	<i>E. carotovora</i> subsp. <i>atroseptica</i>	<i>E. carotovora</i> subsp. <i>betavascularum</i>
α -Methylglucoside	+	—	+	+
Palatinose	+	—	+	+
Melibiose	+	+	+	—
D-Arabitol	+	—	—	—
Sorbitol	+	—	—	—
Citrate	+	+	+	—
Inulin	—	—	—	+
Reducing sugars produced from sucrose	+	—	+	+
Growth at 37°C	+	+	—	+

^a +, all strains are positive; —, all strains are negative.

The 11 strains associated with flavoring soft-rot of witloof constitute a tight genomic group. This group belongs to the *E. carotovora* genomic species since the type strain of *E. carotovora* was 68 to 69% related to strain CFBP 1878^T, with a ΔT_m of 3°C. It is now accepted that strains of a species are about 70% related, with ΔT_m values that do not exceed 5°C (24). The S1 nuclease method gives lower reassociation values than the hydroxyapatite method (12). Thus, the odorous soft-rot organism and *E. carotovora* subsp. *carotovora* may be members of one species. It should be noted that the same criteria would exclude *E. carotovora* subsp. *atroseptica* and *E. carotovora* subsp. *betavascularum* (ΔT_m , more than 7°C) from *E. carotovora* (ΔT_m , less than 5°C). The delineation of these groups is being studied by workers in our laboratories.

ΔT_m values between 3 and 5°C are often observed between different subspecies of a species (11). Thus, the flavoring soft-rot organism qualifies as a subspecies of *E. carotovora*.

Witloof pathogens have been included in two numerical taxonomy studies. The first study was based on 421 *Erwinia* strains (among them were seven witloof pathogens, including three of our present collection) that were described by using 72 API tests (22). The witloof pathogen strains constituted subphenon 1E, which was closely related to but distinct from subphena 1C (*E. carotovora* subsp. *carotovora*) and 1A (*E. carotovora* subsp. *atroseptica*). The second study was based on 60 witloof *Erwinia* strains, and subphenon 1E contained 28 isolates (19).

Since the flavoring soft-rot witloof pathogen strains constitute a tight DNA subgroup which can be identified by using phenotypic tests, we propose that this organism should be recognized as a new subspecies, *Erwinia carotovora* subsp. *odorifera*. This subgroup is not suitable for pathovar classification since it contains isolates obtained from witloof chicory, celery and leeks (this study), and hyacinths (8).

Description of *Erwinia carotovora* subsp. *odorifera* subsp. nov. *Erwinia carotovora* subsp. *odorifera* (o.dor. if'er.a. L.fem.adj. *odorifera*, bringing odors, fragrant). Cells are gram-negative, nonsporeforming, peritrichous, straight rods. Facultatively anaerobic. Oxidase is not produced. Nitrates are reduced to nitrites. The species conforms to the definition of the family *Enterobacteriaceae* (10).

All strains grow on nutrient agar, producing colonies that are about 2.5 mm in diameter after 48 h at 25°C on YPDA medium. Colonies are circular, greyish (to yellowish on media containing sucrose or gelatin), slightly domed, and semitranslucent. All strains grow at 37°C. Aminopeptidase is produced. Dextrose is fermented without gas production.

Voges-Proskauer test positive. Starch is not hydrolyzed. Polypectate gel is acidified and liquefied (Sutton medium). Cellulase is produced (carboxymethyl cellulose). Lecithinase and phosphatase are not produced. Indole is not produced from tryptophan. Reducing substances are produced from sucrose (Benedict reagent). Acid is not produced from inulin in peptone-water. Acid is produced in minimal mineral medium from lactose (2 to 3 days), trehalose (5 to 10 days), α -methylglucoside (3 to 7 days), palatinose (2 to 3 days), melibiose (2 to 3 days), D-arabitol (2 to 4 days), and D-sorbitol (2 to 4 days). Alkalinization of mineral medium containing citrate occurs, but there is no alkalinization of mineral medium containing malonate. Esculin is hydrolyzed. β -Glucuronidase is not produced.

The following substrates are utilized as sole sources of carbon and energy on Biotype strips by at least 10 of the 11 strains: D-glucose, D-fructose, D-galactose, D-mannose, D-melibiose, sucrose, D-raffinose, lactose, lactulose, D-cellobiose, gentiobiose, 1-O-methyl- β -D-glucoside, D-ribose, L-arabinose, D-xylose, palatinose, L-rhamnose, D-arabitol, glycerol, myo-inositol, D-mannitol, D-sorbitol, 1-O-methyl- α -D-glucoside, D-saccharate, mucate, L-malate, citrate, D-galacturonate, N-acetyl-D-glucosamine, gluconate, DL-glycerate, D-glucosamine, L-aspartate, L-glutamate, and L-serine (in 3 or 4 days at 30°C). Maltose, 1-O-methyl- α -galactoside, maltitol, D-turanose, D-glucuronate, 2-ketoglucuronate, succinate, fumarate, L-aspartate, L-glutamate, L-proline, and L-serine are utilized by some strains but not by all strains. The following substrates are not utilized within 4 days: L-sorbose, maltotriose, L-fucose, D-melezitose, L-arabitol, xylitol, dulcitol, D-tagatose, adonitol, D-lyxose, i-erythritol, 3-O-methyl-D-glucose, L-tartrate, D-tartrate, meso-tartrate, D-malate, cis-aconitate, trans-aconitate, tri-carballylate, 5-ketogluconate, phenylacetate, protocatechuate, 4-hydroxybenzoate, quinate, gentisate, 3-hydroxybenzoate, benzoate, 3-phenylpropionate, m-coumarate, trigonelline, betaine, putrescine, 4-aminobutyrate, histamine, DL-lactate, caprate, caprylate, glutarate, 5-aminovalerate, ethanolamine, tryptamine, itaconate, 3-hydroxybutyrate, D-alanine, L-alanine, malonate, propionate, L-tyrosine, and 2-ketoglutarate.

The strains produce flavoring volatile compounds, such as propyl acetate, isobutyl acetate, isoamyl acetate, and actamyl acetate, when they are inoculated onto witloof leaves.

Isolated from soft rots of various plants, including *C. intybus* (cultivated for witloof production).

The type strain is strain CFBP 1878.

Description of the type strain. Strain CFBP 1878^T was

TABLE 5. Production of volatile compounds by *Erwinia* strains

Strain	Amt of the following volatile compounds (mg/kg of inoculated wilcoof)												
	Ethyl acetate	Propyl acetate	Isobutyl acetate	Isoamyl acetate	Actamyl acetate	2-Methyl-1-propanol	3-Methyl-1-propanol	2-Methyl-1-butanol	2-Butanone	3-Methyl-2-butanone	3-Hydroxy-2-butanone	2-Heptanone	2-Nonanone
<i>E. carotovora</i> subsp. <i>odorifera</i> strains													
CFBP 1878 ^T	57.0	0.9	19.7	11.7	8.6	1.5	0.7	Tr	0.2	1.3		0.03	
CFBP 1879	64.5	0.4	6.2	6.7	5.5	0.7	0.2	1.8	0.2	1.2		0.07	
CFBP 1880	56.4	0.06	1.9	2.0	3.2	0.2	0.4	1.3	0.2	1.0		0.07	
CFBP 1892	87.6	0.4	2.8	3.5	4.6	1.2	1.0	2.2	0.1	1.4		0.2	
CFBP 1959	78.6		5.6	2.4	3.9	0.7	0.9	2.0	0.06	0.6		0.1	0.1
CFBP 2154	45.1	0.06	6.2	4.9	6.9	0.8	0.8	1.4	Tr	0.7		0.4	0.04
CFBP 2155	62.1	0.3	6.2	4.5	4.5	1.0	0.7	1.4	0.02	0.4		0.2	
CFBP 1893	95.4	0.7	3.2	2.6	3.2	1.4	1.0	2.1	0.3	0.9		0.01	
CFBP 3259	40.2	0.6	5.1	2.8	3.4	0.5	1.1	2.1	0.04	0.3		0.4	
CFBP 3260	46.8	0.5	7.4	5.7	8.2	0.4	0.8	1.9	0.04	0.6		0.2	
CFBP 3261	46.7	0.3	2.4	2.0	1.3	0.3	0.8	0.9	1.0	2.0		1.4	
<i>E. carotovora</i> subsp. <i>carotovora</i> strains													
CFBP 2046 ^T	12.5					0.3	0.1	0.1	0.03	0.6		4.5	
CFBP 2141	2.4					1.7	1.0	1.2	0.05	1.3		0.5	
<i>E. carotovora</i> subsp. <i>atroseptica</i> strains													
CFBP 1330	1.3					0.2				0.01		1.0	
CFBP 1526 ^T	2.9					0.5				0.2		0.2	
<i>E. carotovora</i> subsp. <i>betavas-culorum</i> CFBP 2122 ^T													
	6.0					0.2	0.1	0.4	1.3	0.7		4.0	
<i>E. chrysanthemi</i> strains													
CFBP 2052	31.1					0.2			0.06	1.5		10.6	
CFBP 3262	4.1					0.4	0.2	Tr	Tr	0.2		1.0	
CFBP 3263	1.7					0.1	0.01		0.02	1.4		0.2	
CFBP 3264	1.6					0.2			0.01	0.2		0.02	
CFBP 3265	0.9					0.04			0.04	0.9		0.04	0.08
CFBP 3266	2.5					0.2			0.7	0.8		0.5	0.5

isolated from soft rot of *C. intybus* in Brittany (France) by R. Samson. This strain and five other strains (strains CFBP 1879, CFBP 1880, CFBP 1892, CFBP 1893, and CFBP 1959) have been deposited in the National Collection of Plant Pathogenic Bacteria, Harpenden, Great Britain, the International Collection of Microorganisms from Plants, Auckland, New Zealand, and the Laboratorium Microbiologie Rijksuniversiteit Culture Collection, Rijksuniversiteit, Gent, Belgium. The type strain has all of the properties given above for the subspecies. In addition, it utilizes as sole sources of carbon and energy succinate and fumarate in 2 days and 1-*O*-methyl- α -galactoside and proline in 4 days. It does not utilize trehalose, maltose, D-glucuronate, and 2-ketoglucuronate in 4 days.

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