Emended Description of Enterobacter cancerogenus comb. nov.  
(Formerly Erwinia cancerogena)  
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The description for three strains of Erwinia cancerogena was expanded to include 105 phenotypic characters. These strains were compared with seven strains of Erwinia carotovora subsp. carotovora and the type strains of Enterobacter (Erwinia) nimpressuralis, Enterobacter cloacae, Enterobacter amnigenus, and Enterobacter intermediam. Our results support the proposal to transfer Erwinia cancerogena to the genus Enterobacter as Enterobacter cancerogenus comb. nov.

The bacteria isolated by Urošević from poplars (Populus species) affected by a canker disease were described and designated Erwinia cancerogena in 1966 (28). Although Lelliott (18) and Lelliott and Dickey (20) have indicated that Erwinia cancerogena probably is a species of Enterobacter because it produces positive reactions for arginine and ornithine decarboxylase, additional data have not been reported to substantiate the suggested change to Enterobacter.

A preliminary comparison of 48 characters showed that the level of similarity between Erwinia cancerogena and Erwinia amylovora is 54%, whereas the level of similarity between Erwinia cancerogena and Erwinia carotovora subsp. carotovora is 85%, which is greater than the levels of similarity for the other 14 species or subspecies of Erwinia (unpublished data). A close relationship between Erwinia cancerogena and Enterobacter (Erwinia) nimpressuralis, Enterobacter cloacae, Enterobacter amnigenus, and Enterobacter intermediam was indicated by a comparison with results reported previously for additional species of the family Enterobacteriaceae (3, 6, 8, 9, 15, 16, 21, 26, 29).

The purpose of this study was to expand the phenotypic characterization of Erwinia cancerogena and to determine its relationship to strains of Erwinia carotovora subsp. carotovora and to the type strains of Enterobacter nimpressuralis, Enterobacter cloacae, Enterobacter amnigenus, and Enterobacter intermediam.

MATERIALS AND METHODS

Bacterial strains. Three strains of Erwinia cancerogena Urošević 1966 (strains NCPPB 2176T [T = type strain], NCPPB 2177, and NCPPB 2178) were obtained from the National Collection of Plant Phytopathogenic Bacteria, Harpenden, England; these strains originally were designated by Urošević as strains A-1, A-201, and RA-SA. Type strain PDDCC 1577 (= ATCC 9912) of Enterobacter (Erwinia) nimpressuralis (Carter 1945) Dye 1969 was received from the Plant Disease Division Culture Collection, Auckland, New Zealand. Enterobacter cloacae (Jordan 1890) Hormaeche and Edwards 1960 strain ATCC 13047T, Enterobacter intermediam Izard, Gavini and Leclerc 1980 strain ATCC 33110T, and Enterobacter amnigenus Izard, Gavini, Trinel and Leclerc 1981 strain ATCC 33072T were obtained from the American Type Culture Collection, Rockville, Md. The following strains of Erwinia carotovora subsp. carotovora (Jones 1901) Bergey, Harrison, Breed, Hammes and Huntton 1923, which were obtained from six hosts and seven locations, also were tested: strain 14 (= E 34) from Iris siphoides Ehrh. (New York); strain 55 from Euphorbia pulcherrima Willd. (Missouri); strains 148 and 197 from Chrysanthemum morifolium Ramat. (Ohio and Florida); strain 57S from Lycopersicon esculentum Mill. (North Carolina); strain 579 (= PDDCC 1380) from Daucus carota L. var. sativus Hoffm. (Vermont); and strain 551 (= NCPPB 552) from Zea mays L. (Israel). Cultures were maintained on nutrient agar (Difco Laboratories, Detroit, Mich.).

Morphological, physiological, and biochemical tests. The strains were stained with the Hucker modification of the Gram staining procedure (10), and bacterial suspensions were examined by phase-contrast microscopy for cell morphology and motility. Motility test agar (Difco) and motility test medium (9) also were used. The bacteria were negatively stained for 5 to 10 min with a 1:1 mixture of 0.5% uranyl acetate and 0.025% barbitratin for examination of flagella by electron microscopy or were stained with Ryu flagellum stain (22) for light microscopy. Pigment production was determined on Difco nutrient agar and yeast extract-dextrose-calcium carbonate agar (7) at 27°C.

Tests were performed at 27°C unless noted otherwise. The methods described by Dye (7) were used to test for catalase, gelatin hydrolysis, tolerance to KCN, and phenylalanine deaminase (method 2). Triple sugar iron agar (Difco) was used to determine production of H₂S. The medium of Hugh and Leifson (14) supplemented with 1% (wt/vol) α-glucose was used for the oxidation-fermentation test, and the tubes containing petrolatum were observed for 7 days for gas production. The nitrate reduction test and the potato soft rot test were done as described by Lelliott et al. (19). Pectate degradation was tested by the method of Beraha (2). Oxidase activity was determined 36 h after application of cells to Patho Tec-CO test papers (General Diagnostics Div., Warner-Chilcott, Morris Plains, N.J.). β-Galactosidase was detected by using o-nitrophenyl-β-D-galactopyranoside fermentation disks (Difco) after 20 min and 4 h of incubation at 37°C. MR/VP broth (Difco) was used for the Voges-Proskauer test after 48 h at 27°C and for the methyl red test after 5 days at 30°C (10). Indole production was determined after 3 and 5 days, and phoshatase production was determined after 48 h as described in Bergey's Manual (18, 20). Deoxyribonuclease activity was tested after 48 h on deoxyribonuclease test agar (Difco). Urease production was detected by a change in color of urea broth (Difco) after 3, 7, or 14 days. The agar diffusion method (nutrient agar containing 1% glucose) was used to test for susceptibility to Difco antibiotic disks containing erythromycin (15 μg) or penicillin G (2 U). Decarboxylase base Moeller medium (Difco) containing 1%
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RESULTS AND DISCUSSION

The phenotypic characteristics that were common to the three Erwinia cancerogena strains are given below and in Table 1. The type strain (strain NCPPB 2176) differed from the other strains (NCPPB 2177 and NCPPB 2178) by its production of acid from D-melibiose but not from quinate and by its inability to hydrolyze Tween 80.

Erwinia cancerogena definitely is not a member of the genus Erwinia. Our results support the previous suggestion (18, 20) that Erwinia cancerogena belongs in the genus Enterobacter. Enterobacter cancerogena conforms to the description for the family Enterobacteriaceae (3) and the genus Enterobacter (21). It can be distinguished from four genera (Citrobacter, Hafnia, Klebsiella, and Serratia) that are closely related to Enterobacter by two or more of the following characteristics: motility; not capsulated; positive for arginine dihydrolase, the Voges-Proskauer test, gelatin hydrolysis, and production of acid from D-sorbitol; negative for the methyl red test, production of lipase (corn oil), urease, deoxyribonuclease, ornithine and lysine decarboxylases, and acid production from myo-inositol and sucrose. Therefore, we propose that Erwinia cancerogena be transferred to the genus Enterobacter as Enterobacter cancerogena comb. nov.

Simple matching coefficients were calculated and used to prepare a shaded similarity matrix (Fig. 1) based on single-linkage analysis. The closest relationship for any strain of Erwinia carotovora subsp. carotovora and the other strains was a level of similarity of 75% between Erwinia carotovora subsp. carotovora strain 551 and Erwinia cancerogena NCPPB 2176T. Erwinia cancerogena NCPPB 2176T was more closely related (92%) to Enterobacter nimipressuralis than to the other strains of Enterobacter species included in this study.

The phenotypic characteristics that differentiate Enterobacter cancerogena from Enterobacter cloacae, Enterobacter nimipressuralis, Enterobacter amnigenus, Enterobacter intermedium, and seven strains of Erwinia carotovora subsp. carotovora are shown in Table 1.

The causative agent for the wetwood disease of elms (Ulmus species) originally was designated Erwinia nimipressuralis (6). This organism has been shown to be related phenotypically (8, 11, 26, 29) and on the basis of deoxyribonucleic acid characteristics (4, 25, 26) to Enterobacter species and to be unrelated serologically to Erwinia carotovora (13). Our results support the proposal of Brenner et al. (4) to

![FIG. 1. Similarity matrix showing relationships among seven strains of Erwinia carotovora subsp. carotovora (551, 148, 55, 197, 575, 579, and 14), three strains of Erwinia cancerogena (NCPPB 2176T, NCPPB 2177, and NCPPB 2178), Enterobacter amnigenus ATCC 33072T, Enterobacter cloacae ATCC 13047T, Enterobacter nimipressuralis PDDCC 1577T, and Enterobacter intermedium ATCC 33110T. S, Similarity.](image)
TABLE 1. Phenotypic characteristics that differentiate Enterobacter cancerogenus NCPPB 2176T, NCPPB 2177, and NCPPB 2178 from Enterobacter cloacae ATCC 13047T, Enterobacter nimipressuralis PDCC 1577T, Enterobacter amnigenus ATCC 33072T, Enterobacter intermedium ATCC 33110T, and seven strains of Erwinia carotovora subsp. carotovora

<table>
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<tr>
<th>Characteristic</th>
<th>NCPPB 2176T, NCPPB 2177, and NCPPB 2178</th>
<th>ATCC 13047T</th>
<th>PDCC 1577T</th>
<th>ATCC 33072T</th>
<th>ATCC 33110T</th>
<th>Erwinia carotovora subsp. carotovora (7 strains)</th>
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<tr>
<td>Arginine dihydrolase</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
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<td>+</td>
<td>-</td>
<td>+</td>
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<td>+</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>Pectate degradation</td>
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<td>+</td>
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<td>-</td>
<td>-</td>
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<td>Gas production from D-glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>- (14)</td>
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<td>β-Xylosidase</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
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<tr>
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<td>+D</td>
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<td>-D</td>
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<td>+</td>
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<tr>
<td>L-Arabinose, D-Xylose, D-Ribose, maltose, D-galactose, D-mannose, D-fructose, D-cellobiose, D-mannitol, and salicin</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>-</td>
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<td>+</td>
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<td>D-Raffinose</td>
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<td>+</td>
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<td>Sucrose</td>
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<tr>
<td>D-Sorbitol</td>
<td>-</td>
<td>+</td>
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<td>Dextrin</td>
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<td>Glycolen</td>
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<td>Pyruvate</td>
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<td>+</td>
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</tr>
</tbody>
</table>

α +, Positive within 7 days; −, negative after 7 days (14 days for starch); +D, positive between 7 and 14 days. The numbers in parentheses indicate the percentages of positive strains.

transfer Erwinia nimipressuralis to the genus Enterobacter as Enterobacter nimipressuralis comb. nov.

Emended description of Enterobacter cancerogenus (Urošević) comb. nov. Enterobacter cancerogenus (can. cer. o'ge. nus. L. n. cancer, crab, the disease cancer; L. v. gigno, to produce; L. masc. adj. cancerogenus, crab inducing). The description below is based on three strains. Gram-negative, straight rods that are motile with peritrichous flagella and are facultatively anaerobic. Positive for the following characteristics: catalase production; nitrate reduction; the Voges-Proskauer reaction; KCN tolerance; esculin hydrolysis; β-galactosidase production; utilization of acetate, citrate, glutamate, β-lactate, malate, succinate, L-alanine, D-α-alanine, and L-serine; production of acid from L-arabinose, D-xylose, D-ribose, D-glucose, D-lactose, D-galactose, L-rhamnose, D-mannose, D-fructose, D-trehalose, D-cellobiose, D-mannitol, glycerol, salicin, mucate, pyruvate, and α-D-galacturonate. Liquefaction of gelatin at 27°C is evident at 21 days. Negative for the following characteristics: pigment production; oxidase: the methyl red test; production of deoxyribonuclease, hydrogen sulfide, indole, lipase (corn oil), lysine decarboxylase, phosphatase, and urease; phenylalanine deamination; reducing substances produced from sucrose; utilization of alginate, benzoate, propionate, and sodium potassium tartrate; production of acid from L-sorbose, melezitose, ethanol, adonitol, i-erythritol, inulin, glycolen, chitin, and D-tartaric acid; production of gas from D-arabinose and myo-inositol and hypersensitive reaction on tobacco. Not susceptible to erythromycin (15 μg) or penicillin G (2 U). Additional characteristics are shown in Table 1. Isolated from poplars. The type strain is strain NCPPB 2176.

Emended description of the type strain. Strain NCPPB 2176T has all of the characteristics given above for the species. In addition, it produces acid from D-melibiose but not from quinate and does not hydrolyze Tween 80. Isolated from cankers on poplars (Populus species) in Czechoslovakia by Urošević.

LITERATURE CITED


