Request for an Opinion

Rejection of the Names Erwinia carnegieana Standing 1942 and Pectobacterium carnegieana (Standing 1942) Brenner, Steigerwalt, Miklos and Fanning 1973†

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Two groups of bacteria with differing characteristics are currently referred to as Erwinia carnegieana Standing 1942. However, neither group fits the original description for this bacterium. Strain NCPPB 439T (= ATCC 13452 = ATCC 33259T = ICPB EC-1 = ICPB EC-251 = PDDCC 5701T) was designated as the cotype strain for E. carnegieana and as the type strain for Pectobacterium carnegieana (Standing 1942) Brenner, Steigerwalt, Miklos and Fanning 1973 in the 1980 Approved Lists. However, all of these isolates have the characteristics described for E. carnegieana. More recent strains isolated by us from rotting cacti are in various international collections (NCPPB 671, NCPPB 672, ICPB EC-186 [= PDDCC 1551], ICPB EC-187 [= PDDCC 1381], ICPB EC-188, ICPB EC-189, ICPB EC-190 [= PDDCC 1382], ICPB EC-191, ICPB EC-221, ICPB EC-222, ICPB EC-223). These isolates are similar to Erwinia carotovora (Jones 1901) Bergey, Harrison, Reed, Hammer and Huntoon 1923. Because of the lack of any culture of the originally described E. carnegieana, the fact that the cotype culture is a different organism, and the fact that repeated attempts to isolate the organism described as E. carnegieana from rots typical for the described disease have failed, we request an opinion that the name E. carnegieana be rejected as nomen dubium.

Erwinia carnegieana Standing 1942 is the name given to the bacterium causing a soft rot of saguaro cacti (Carnegiea gigantea (Engelm.) Britt. & Rose) (17). This organism was originally described as "actively motile, peritrichiate, Gram positive, . . ., with acid and gas produced from arabinose, dextranose, galactose, levulose, maltose, sucrose, raffinose, mannitol, and salicin," but not from lactose, inulin, glycerol, or inositol. The host range was stated as being strictly limited to saguaro cacti. This binomial has become a source of confusion in part because there are two very different bacterial isolates in international collections that have been labeled Erwinia carnegieana: there are nonpathogenic strains identical to the cotype strain, NCPPB 439T (National Collection of Plant Pathogenic Bacteria, Harpenden, England), and pathogenic strains similar to Erwinia carotovora (Jones 1901) Bergey, Harrison, Reed, Hammer and Huntoon 1923. Furthermore, both groups of bacteria are different from the culture described by Lightle et al. as Erwinia carnegieana (17). We know of no extant isolate, pathogenic or otherwise, that fits the original description of Standing.

NCPPB 439T was named the cotype for Erwinia carnegieana by Sneth and Skerman (19) and was listed as the type strain for Pectobacterium carnegieana, (Standing 1942) Brenner, Steigerwalt, Miklos and Fanning 1973 in the Approved Lists (18). Sneth and Skerman (19) indicated that NCPPB 439T was received at the National Collection of Plant Pathogenic Bacteria from D. C. Graham, who in turn (personal communication) had obtained it in 1957 as strain EC-1 from W. H. Burkholder. Burkholder logged the strain as being received on 25 January 1944 from J. G. Brown but made no other notation (R. S. Dickey, personal communication). American Type Culture Collection (ATCC) strain ATCC 13452 is a subculture of Burkholder strain EC-1 (2), as is International Collection of Phytopathogenic Bacteria (ICPB) strain ICPB EC-1 (M. P. Starr, personal communication). Brenner also sent a subculture of ATCC 13452 to Starr in May 1972; Starr labeled this subculture ICPB EC-251 (Starr, personal communication). PDDCC 5701T (Culture Collection of the Plant Diseases Division, New Zealand Department of Scientific and Industrial Research, Auckland) and ATCC 33259T are subcultures of NCPPB 439T, resulting from the designation of this organism as the type strain for Erwinia carnegieana and P. carnegieana in the Approved Lists. Currently, the following strains in international collections can be traced to Burkholder strain EC-1: ATCC 13452, ATCC 33259T, ICPB EC-1, ICPB EC-251, NCPPB 439T, and PDDCC 5701T.

The first study known to us indicating that Burkholder strain EC-1 is not an Erwinia is that of Sutton (D. D. Sutton, Ph.D. thesis, University of California, Davis, 1957), who concluded that EC-1 was indistinguishable from "Aerobacter aerogenes" (Enterobacter aerogenes Hornemaeche and Edwards 1960) and Klebsiella sp. In 1964, Graham (11) stated that NCPPB 439T was positive for lysine decarboxylase and oxidized gluconate to 2-ketogluconate and concluded that NCPPB 439T was similar to "Aerobacter (Enterobacter) cloacae." Brenner et al. (6) showed that only ca. 33% of the deoxyribonucleic acid from ATCC 13452 was homologous to deoxyribonucleic acid from pectolytic saguaro isolate ICPB EC-186 (originally isolated by us) at 60°C. These authors also cited personal communications from Dye, who noted the existence of two different strains labeled P. carnegieana, and from Ewing, who found that

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ATCC 13452 had the biochemical characteristics of *K. pneumoniae*. Edwards and Ewing (10) also have commented that two cultures of *Erwinia carnegieana* are typical strains of *K. pneumoniae*. Based on a numerical taxonomic study, Imbs et al. (13) considered NCPPB 439T to be *Enterobacter cloacaee*, whereas S. T. Bagley (in the laboratory of M. P. Starr) concluded that ICPB EC-1 and ICPB EC-251 were also *K. pneumoniae* (personal communication). ATCC 13452 and ATCC 33259T now are listed as *K. pneumoniae* by the American Type Culture Collection (3). Using API 20E tests (Analytab Products Inc, Plainview, N. Y.) in our laboratory, we found that strains ATCC 13452, ICPB EC-1, ICPB EC-251, NCPPB 439T, and PDDCC 5701T all gave test results identical to those for two *K. pneumoniae* reference strains (ICPB 3944 [= CDC 1736-71] and ICPB 3947 [= CDC 4240-72]).

Dye (8), Lelliott (15), and Lelliott and Dickey (16) have suggested the possibility that two very different pathogens cause symptomatically identical soft rots of cacti. We have made numerous isolations from a number of naturally infected, soft-rotting cacti. All of the pathogenic isolates that we have studied are motile, peptolytic erwiniaceae (Alcorn and Orum, manuscript in preparation); we have yet to isolate either a plant-pathogenic *Klebsiella* sp. or the bacterium described by Lightle et al. (17). Furthermore, we have tested EC-1 strains for pathogenicity on slices of carrot roots and potato tubers, prickly pear cactus (*Opuntia spp.*) pads and fruits, greenhouse-grown senita (*Lophocereus schottii* (Engelm.) Britt. & Rose), and saguaro seedlings. Carrot and potato slices were tested by placing drops of bacterial suspensions on the slices, whereas the cacti were tested by injecting bacterial suspensions into plant tissues. An isolate was considered pathogenic if a wet rot appeared and extended beyond the area of initial infiltration. There was no evidence of pathogenicity in any test with EC-1 strains. In contrast, peptolytic erwiniaceae isolated by us from rotting cacti were pathogenic (1; Alcorn and Orum, manuscript in preparation). We similarly tested *Klebsiella* reference strains ICPB 3866, ICPB 3372, ICPB 3739, ICPB 3740, ICPB 3832, ICPB 3836, ICPB 3944, and ICPB 3947, including three peptolytic isolates (not from cacti) provided by M. P. Starr from the International Collection of Phytopathogenic Bacteria; these organisms also did not cause soft rot. Additionally, Anson (4) reported that NCPPB 439T did not cause soft rot of cacti in greenhouse tests.

Lack of pathogenicity is not in itself sufficient for concluding that EC-1 is not the organism that Standring studied. However, other evidence also suggests that NCPPB 439T is probably a contaminant. Such information includes the important fact that the original isolate of Standring was motile with peritrichous flagella (17, note Fig. 5). In contrast, based on electron microscope observations, we conclude that NCPPB 439T has no flagella. Standring also observed that her isolate failed to produce acid from lactose and inositol. However, Burkholder (7) noted that EC-1 produced acid and gas from lactose. Our tests with NCPPB 439T and all of its various duplicates corroborate the observation of Burkholder concerning lactose and show acid production from inositol. The original *Erwinia carnegieana* isolate was described as being gram positive (17). Boyle (5) also indicated that young cultures of her isolate were gram positive but with age became gram negative. In contrast, Zalh et al. in 1944 (22) noted that EC-1 from Brown was gram negative and that it induced tumor hemorrhages, as did other gram-negative bacteria but not gram positive species. Burkholder (7) also found EC-1 to be gram negative, except that old cultures had gram-positive granules in cells. Thus, even though the original description of Standring is limited and possibly flawed, the lack of peritrichous flagella, the variations in Gram stain determinations, and the lactose and inositol results are strong evidence that NCPPB 439T is not the *Erwinia carnegieana* that was originally described.

Not only does the type strain differ from the original description, but so do all of the pathogenic bacteria isolated by us from saguaro and other cacti since 1957 (Alcorn and Orum, manuscript in preparation). In contrast to the observations of Lightle et al. (17), all of our isolates from naturally infected, soft-rotting cacti have been gram-negative, small rods (0.5 to 0.8 by 0.5 to 1.5 µm) which produce a distinct, sweetish odor on several media and which have an extended host range based on inoculations (1) and by virtue of the range of cactus species from which identical cultures have been isolated. Some of these cultures were from plants in the same area studied by Lightle et al. in the Saguaro National Monument east of Tucson, Ariz. Our failure to find pathogenic bacteria similar either to the type culture or to the original description for *Erwinia carnegieana* presents an enigma.

A number of our isolates which cause soft rot of cacti have been in international collections since 1959. These include NCPPB 671 (= Dye EH-4 [8]), NCPPB 672, ICPB EC-186 (= PDDCC 1551 = Dye EH-3 [8]), ICPB EC-187 (= PDDCC 1381 = Dye EH-1 [8]), ICPB EC-188, ICPB EC-189, ICPB EC-190 (= PDDCC 1382 = Dye EH-2 [8]), ICPB EC-191, ICPB EC-221, ICPB EC-222, and ICPB EC-223. With these isolates the confusion in nomenclature overlaps with indecision in taxonomy. Graham (12) originally classified NCPPB 671 and NCPPB 672 as *Erwinia carotovora*. However, based on more extensive evaluations, Graham now believes that NCPPB 671 and NCPPB 672 differ taxonomically from *Erwinia carotovora* subsp. *carotovora* (personal communication). Dye (8) classified PDDCC 1381, PDDCC 1382, PDDCC 1551, and NCPPB 671 as *Erwinia carotovora* but after a numerical taxonomic analysis of the same data reclassified them as *Erwinia carnegieana* (9). After specifically excluding ATCC 13452, Brenner et al. (6) classified ICPB EC-186 (= PDDCC 1551) and ICPB EC-223 as *P. carnegieana*. (It is ironic that a strain identical to ATCC 13452 became the type strain for *P. carnegieana* after Brenner et al. so carefully excluded it.) Starr and Mandel (20) also studied eight of our isolates from cacti; based on guanine-plus-cytosine ratios, they concluded that the isolates were close to, but distinct from, *Erwinia carotovora* reference strains. Although Graham (12), Lelliott (15), and Lelliott and Dickey (16) distinguished our isolates from the type strain of *Erwinia carnegieana* by naming them *Erwinia carotovora*, Starr and Mandel (20), Brenner et al. (6), and Dye (9) distinguished our isolates from *Erwinia carotovora* (*P. carotovorum*) by naming them *Erwinia carnegieana* (*P. carnegieana*). In a recent taxonomic study based on API 20E and API 50CHE tests Verdonck et al. (21) identified our strains NCPPB 671, NCPPB 672, and PDDCC 1382 as phenon 31, a group within *Erwinia* but distinct from *Erwinia carotovora* and *Erwinia chrysanthemi*. In contrast, strain NCPPB 439T was unclustered in their analysis.

Because of such uncertainties as whether to apply the binomial *Erwinia carnegieana* to the originally described bacterium (17), to the type culture (NCPPB 439T), or to the strains more recently isolated by us from cacti (NCPPB 671 et al.), and because cultures fitting the original description of *Erwinia carnegieana* are not available, we now request an opinion that the name *Erwinia carnegieana* be rejected as

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nomen dubium (International Code of Nomenclature of Bacteria Rule 56A [14]). We also request that P. carnegieana be rejected, because it is intended as a synonym for Erwinia carnegieana. The alternative of officially substituting an isolate such as ICPC EC-186 for NCPPB requires further study. If an isolate such as ICPC EC-186 is selected, the future a bacterium from saguaro cacti that matches the original description of Erwinia carnegieana. Furthermore, confusion would be compounded if in the future a bacterium from saguaro cacti that matches the original description of Erwinia carnegieana is isolated.

LITERATURE CITED