SHORT COMMUNICATION

Reclassification of Corynebacterium flaccumfaciens, Corynebacterium betae, Corynebacterium oortii and Corynebacterium poinsettiae in the genus Curtobacterium, as Curtobacterium flaccumfaciens comb. nov.

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The phytopathogens Corynebacterium flaccumfaciens (Hedges), Corynebacterium betae (Keyworth, Howell & Dowson), Corynebacterium oortii (Saaltink & Maas Geesteranus) and Corynebacterium poinsettiae (Starr & Pirone) differ to such an extent from the type species of Corynebacterium, Corynebacterium diphtheriae (Lehmann & Neumann), that they cannot be retained in this genus. On the basis of biochemical, chemical and genetic data it is proposed that Cor. flaccumfaciens, Cor. betae, Cor. oortii and Cor. poinsettiae be reclassified in the genus Curtobacterium (Yamada & Komagata), as Curtobacterium flaccumfaciens (Hedges) comb. nov.

The generic assignment of the phytopathogens Corynebacterium flaccumfaciens, Cor. betae, Cor. oortii and Cor. poinsettiae has always been controversial (see Carlson & Vidaver, 1982). These species bear little similarity to the animal or human corynebacteria, and their retention within the genus Corynebacterium has been questioned by several workers (e.g. Yamada & Komagata, 1972; Schleifer & Kandler, 1972; Jones, 1975; Keddie & Cure, 1977, 1978; Minnikin et al., 1978; Collins et al., 1980; Dopfer et al., 1982).

Yamada & Komagata (1972) proposed the genus Curtobacterium for some motile brevibacteria (e.g. Brevibacterium albium, B. citreum, B. luteum, B. pusillum, etc.) and the phytopathogens Cor. flaccumfaciens and Cor. poinsettiae. Support for the close relationship between Cor. flaccumfaciens, Cor. poinsettiae and curtobacteria comes from the fact that Schleifer & Kandler (1972) independently grouped together the same species and also Cor. betae on the basis of their peptidoglycan structure. The peptidoglycan of these taxa is of the usual group B type (i.e. characterized by a cross-linkage between the α-carboxyl group of D-glutamic acid in position 2 of the peptide subunit and the C-terminal D-alanine of an adjacent subunit) based on D-ornithine (variation B2β) (Schleifer & Kandler, 1972). This peptidoglycan type is quite different from that of true corynebacteria, which possess a directly cross-linked peptidoglycan based upon meso-diaminopimelic acid (variation A1γ) (Schleifer & Kandler, 1972). Further support for the reclassification of the species Cor. flaccumfaciens, Cor. poinsettiae and Cor. betae in the genus Curtobacterium comes from the results of lipid investigations (Collins et al., 1980). It is now generally accepted that members of the genus Corynebacterium possess short-chain mycolic acids, predominantly straight-chain saturated and mono-unsaturated long-chain fatty acids, dihydrogenated menaquinones (MK-8(H2), MK-9(H2)), phosphatidylinositol and...
related phosphatidylinositol dimannoside(s) (see Minnikin et al., 1978; Collins et al., 1982a, b). In contrast, the species Cor. flaccumfaciens, Cor. poinsettae, Cor. betae and curtobacteria possess primarily iso- and anteiso-methyl branched fatty acids, and unsaturated menaquinones (MK-9), but lack mycolic acids and phosphatidylinositol and related dimannosides (Collins et al., 1980). The lipid investigations of Collins et al. (1980) indicated that the phytopathogenic species Cor. oortii was also a candidate for the genus Curtobacterium, a finding supported by the cell wall peptidoglycan studies of Döpfer et al. (1982). Recent genetic studies (Döpfer et al., 1982) have confirmed the close relationship between the ornithine-containing phytopathogens and curtobacteria. In the study of Döpfer et al. (1982), Cor. flaccumfaciens, Cor. betae, Cor. oortii and Cor. poinsettae shared relatively high DNA homologies (about 47–56%) with Curtobacterium citreum, the type species of the genus Curtobacterium, and with Curt. pusillum, indicating that these taxa are related at the generic level. Further, the very high DNA–DNA homology values (about 71–104%) between strains of Cor. flaccumfaciens, Cor. betae, Cor. oortii and Cor. poinsettae reported by Döpfer et al. (1982) are strong evidence that these taxa represent a single species.

Carlson & Vidaver (1982) have suggested that, on the basis of morphological similarity, Cor. flaccumfaciens, Cor. betae, Cor. oortii and Cor. poinsettae should be retained in the genus Corynebacterium. The same authors have also proposed the reclassification of Cor. betae, Cor. oortii and Cor. poinsettae as subspecies of Cor. flaccumfaciens (Carlson & Vidaver, 1982). These recommendations are however at variance with all recent phenetic and genetic data. As described above, there is now overwhelming evidence that these taxa should be classified in the genus Curtobacterium. Furthermore, the DNA–DNA homology studies of Döpfer et al. (1982) indicate that Cor. flaccumfaciens, Cor. betae, Cor. oortii and Cor. poinsettae are genetically identical and should be reduced to a single species. The above taxa are biochemically and physiologically closely related (Dye & Kemp, 1977), and differences in host specificity and bacteriocin production in our opinion are insufficient to justify differentiation at the subspecific level. We therefore formally propose that bacteria designated Cor. flaccumfaciens (Hedges), Cor. betae (Keyworth, Howell & Dowson), Cor. oortii (Saaltink & Maas Geesteranus) and Cor. poinsettae (Starr & Pirone) be reduced to synonymy, and reclassified in the genus Curtobacterium (Yamada & Komagata), as Curtobacterium flaccumfaciens comb. nov.

The consequences of such a union of the species would be that the traditional names of bacteria which cause specific and differentiable plant diseases would be lost. This, although taxonomically sound, would cause practical difficulties for plant pathologists especially with regard to quarantine regulations. To overcome these difficulties we propose that bacteria currently named Cor. betae, Cor. oortii and Cor. poinsettae be treated as pathovars (Lapage et al., 1975, Appendix 10B) of the species Curtobacterium flaccumfaciens comb. nov. and that they be designated Curtobacterium flaccumfaciens pv. betae, Curtobacterium flaccumfaciens pv. oortii and Curtobacterium flaccumfaciens pv. poinsettae (Lapage et al., 1975, Appendix 10C).

**Description of Curtobacterium flaccumfaciens**

This description is based on the studies of Yamada & Komagata (1970), Schleifer & Kandler (1972), Cummins et al. (1974), Jones (1975), Starr et al. (1975), Dye & Kemp (1977), Collins et al. (1980) and Döpfer et al. (1982).

Surface colonies of Curtobacterium flaccumfaciens on yeast extract/glucose agar after 3–4 d are 2–4 mm diam., smooth, entire, low convex, non-viscid and semi-fluidal. The colonies are pigmented in shades of yellow, orange and pink. Some variants may produce a blue to purple water-soluble pigment. Gram-positive, short rods (about 0.3–0.6 μm). The rods may be slightly curved or wedge shaped. Older cultures may show a proportion of coccolid cells, but a marked rod–coccus growth cycle characteristic of members of the genus Arthrobacter is not produced. Predominantly single cells but some V, Y and palisade arrangements are usually present. The organisms are non-acid fast and endospores are not formed. Some strains are motile. The organisms are nutritionally exacting: thiamin, biotin and pantethane are required for growth. The optimum temperature for growth is 24–27 °C and the maximum temperature 35–37 °C. Growth occurs in the presence of 7–9% NaCl.
Curtobacterium flaccumfaciens is obligately aerobic. Acid is produced oxidatively in medium C [NH₄H₂PO₄, 0.5 g; K₂HPO₄, 0.5 g; MgSO₄·7H₂O, 0.2 g; NaCl, 5 g; yeast extract (Difco), 1 g; bromocresol purple, 0.7 ml of 1-6% (w/v) alcoholic solution; agar, 12 g; H₂O, 1 L; pH 6-8; carbon sources (0.5%, w/v) are filter sterilized and added to the autoclaved basal medium] of Dye & Kemp (1977) from arabinose, cellobiose, fructose, galactose, glucose, glycerol, inositol, mannose, maltose, melibiose, raffinose, rhamnose, sucrose and xylose; most strains also produce acid from adonitol and melezitose. Acid is not produced from dextrin, inulin, glycogen or starch. Some organic acids are utilized (e.g. gluconate). It is catalase positive. Indole, oxidase, tyrosinase and urease are not produced. Aesculin and starch (potato) are hydrolysed. The organism is methyl red positive, and does not reduce nitrate or produce ammonia from peptone. Gelatin and pectate gel are not liquefied. Amino acid decarboxylases and phenylalanine deaminase are not produced. H₂S is produced from cysteine hydrochloride.

The cell wall peptidoglycan of Curtobacterium flaccumfaciens is based upon D-ornithine (variation B2β) (type: [L-Hsr]-D-Glu-D-Orn) (Schleifer & Kandler, 1972). Mycolic acids are not present. The long-chain fatty acids consist of predominantly anteiso- and iso-methyl branched types; straight-chain saturated acids are present in only small amounts. The major fatty acids are 12-methyltetradecanoic (anteiso-C₁₄:0), 14-methylhexadecanoic (anteiso-C₁₆:0) and 14-methylpentadecanoic (iso-C₁₆:0) acids. The principal isoprenoid quinones are unsaturated menaquinones with nine isoprene units (MK-9). The polar lipids comprise diphosphatidylglycerol, phosphatidylglycerol and two unidentified diglycosyl- and triglycosyldiacylglycerols. The guanine plus cytosine content of the DNA is within the approximate range 68.3-73.7 mol % (Tm, ρ). The type strain of Curtobacterium flaccumfaciens is NCPPB 1446.

The following pathovars are recognized for practical, quarantine purposes. Their description is the same as that for the species.

Curtobacterium flaccumfaciens pv. flaccumfaciens. This organism causes a vascular wilt of bean (Phaseolus vulgaris). The reference strain is NCPPB 1446.

Curtobacterium flaccumfaciens pv. betae. This organism causes a vascular wilt and leaf spot of red beet (Beta vulgaris). The reference strain is NCPPB 374.

Curtobacterium flaccumfaciens pv. oortii. This organism causes a vascular disease and leaf and bulb spot of tulips (Tulipa spp.). The reference strain is ATCC 25283.

Curtobacterium flaccumfaciens pv. poinsettiae. This organism causes a stem canker and leaf spot of the poinsettia (Euphorbia pulcherrima). The reference strain is ATCC 9682.

The work on Curtobacterium flaccumfaciens pv. flaccumfaciens and Curtobacterium flaccumfaciens pv. poinsettiae was carried out at the University of Leicester under MAFF licence no. PHF 157/22 issued under the Plant Pests (Great Britain) Order 1980.

REFERENCES


KEDDIE, R. M. & CURE, G. L. (1977). The cell wall...


