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A strain of Erwinia herbicola pathogenic on Gypsophila paniculata

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Abstract

A yellow pigmented, rod-shaped, Gram negative, peritrichously flagellated, fermentative bacterium was isolated from galls on the stems of the ornamental plant, *Gypsophila paniculata*. The galls appeared to be similar to those described on the plant by Brown (1934). The isolate proved pathogenic when inoculated into healthy stems; it was identified as a strain of *Erwinia herbicola* (Lohnis) Dye. It is suggested that the organism should be named *E. herbicola* f. sp. *gypsophilae*.

Introduction

Galls occurring at unions of stock and scion of grafted plants of *Gypsophila paniculata* were described by Brown (1932). They were physically unlike those of crown gall and leafy gall of many plants, olive gall, or pocket disease of sugar beets. The causal organism of the disease on *G. paniculata* was described by Brown in 1934, and named *Bacterium gypsophilae*. Since then numerous other names of this organism have appeared but the binominal *Agrobacterium gypsophilae,* introduced by Starr and Weiss (1943), has been in common use for more than 30 years. The decision by Starr and Weiss to place the organism in *Agrobacterium* was partly based on the view that all gall inducing organisms should be placed in that genus, and partly to avoid using the illegitimate genus *Phytomonas,* which embraced a large heterogenous collection of plant pathogenic bacteria, a fact which Burkholder (1939) had already underlined. The general tendency to consider that all gall inducing bacteria belong to the same genus has persisted for many years and accounts for the retention of the name *A. gypsophilae* in phytopathological literature for so long.

De Ley (1968) and White (1972) suggested that certain cultures said to be *A. gypsophilae* might belong to the family Enterobacteriaceae, and possibly be related to the genus *Erwinia* (Winslow et al., 1920). Subsequently Graham and Quinn (1974) identified the cultures known as *A. gypsophilae* NCPPB 179 (previous to 1980 the type strain) and NCPPB 1948 (isolated from roses by Maas Geesteranus and Barendsen, 1966) as strains of *Erwinia herbicola* (Lohnis) Dye, but pathogenicity of these strains could not be demonstrated in the tests carried out on healthy plants of *G. paniculata*, other species of *Gypsophila,* and *Lychnis*

chalcedonica. However, a bacteriologically similar bacterium was isolated in the Netherlands from galls of *G. paniculata* (originating from the USA). These galls appeared similar to those described by Brown (1932, 1934). As this organism produced galls when inoculated into healthy plants, it was decided to determine its correct identity, thereby confirming the findings of Graham and Quinn (1974).

Materials and methods

Bacterial isolates. PD 128 was isolated from *G. paniculata* galls by one of us (H.J.M.). 35-A is a saprophytic bacterium isolated from dormant apple buds and identified as *E. herbicola* by R.N. Goodman, University of Missouri, USA.

Pathogenicity tests. Healthy plants of *G. paniculata* cv. Bristol Fairy were inoculated with either 35-A or PD 128. Other plants were left uninoculated to serve as controls. The methods used were those described by Graham and Quinn (1974).

Bacteriological tests. After growth and maintenance on Difco nutrient agar, the two isolates were characterized by the methods described by Graham and Hodgkiss (1967). The following substances were incorporated at 1.0% (w/v) in 1.0% (w/v) peptone water (Difco) with bromothymol-blue indicator: glucose, lactose, maltose, sucrose, melibiose, melezitose, glycerol, inositol, salicin and α-methyl-D-glucoside. These substances were sterilized separately by filtration before adding to the heat sterilized peptone water. Flagella staining for light microscopy was carried out according to the method of Rhodes (1958).

DNA base composition. DNA base compositions, expressed as moles percent G + C were determined with a recording thermal spectrophotometer according to the methods of Mendel and Marmur (1968). DNA preparation was done as follows. Cells in the late logarithmic phase of growth were harvested by centrifugation; the pellet was resuspended in 10 ml of a solution containing 8M urea, 0.25M phosphate buffer and 0.8% w/v sodium dodecyl sulphate at pH 6.8, and passed through a French pressure cell at 6000Pa. The DNA was then purified from the cell lysate by hydroxylapatite chromatography as described by Britten et al. (1970) and dialysed against 0.1 × SSC (standard saline citrate buffer).

Electron microscopy. Flagella were observed on cells grown on nutrient agar at 20 °C for 48 h with a JOEL JEM 100B electron microscope using an accelerating voltage of 80kV and an initial magnification of 10000; the cells were negatively stained with a 1.0% w/v potassium phosphotungstic acid solution.

Results and discussion

Isolates 35-A and PD 128 were gram-negative rods. These isolates were examined by light and electron microscopy and no significant differences in morphology or flagellation could be found. Many cells were non-flagellated but some had a single lateral flagellum and a few had several (2-5) lateral flagella (Fig. 1). These findings correspond with those of Graham and Quinn (1974).

Fig. 1. Electron micrograph or *Erwinia herbicola* strain PD 128 showing peritrichous flagella. Bar represnts 1μm.

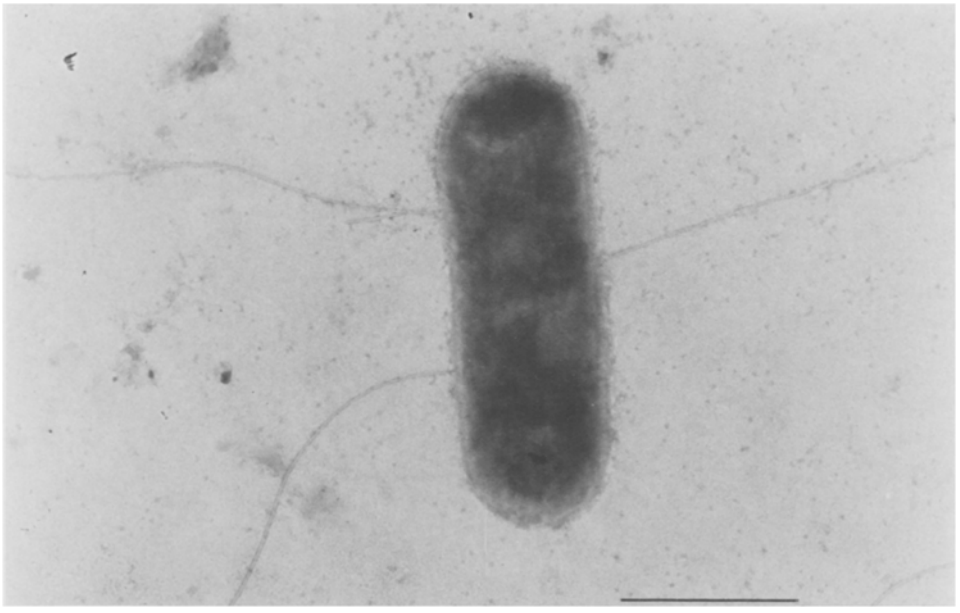

Fig. 1. Elektronenmicroscopische opname van Erwinia herbicola stam PD 128 met peritriche flagellen. Vergrotingsstaaf geeft 1 μm weer.

The results of the biochemical tests have been collected in Table 1 and compared with the results of strains NCPPB 179 and NCPPB 1948 which had already been tested by Graham and Quinn. The differences between 35-A and PD 128 are well within the range of strain variability accepted by Graham and Hodgkiss (1967). The phenotypic characters of 35-A and PD 128 described in Table 1 are not only very similar to those described by Graham and Quinn but are equally similar to those described for the bacteria identified by Graham and Hodgkiss as *E. herbicola*. The G+C contents, 56.8% and 55.6% respectively, also showed no appreciable difference and closely matched those found by De Ley et al. (1966) for *A. gypsophilae* NCPPB 179 (56.2%). De Smedt and De Ley (1979) gave the G+C content for *E. herbicola* NCTC 9381 as 55.9%.

Pathogenicity of isolate PD 128 was established both at the Dutch Plant Protection Service, Wageningen and the Agricultural Scientific Services, East Craigs. It produced visible galls about 1 month after inoculation, and these continued to develop over a further 2 months, when the experiments were concluded (Fig. 2). The bacterium was reisolated from the infected plants. 35-A was tested on *G. panuculata*, but no disease symptoms were obtained. Likewise Brown (1934) obtained symptoms on inoculation of *Gypsophila* plants. She was unable, however to find galls on many other plants which she tested, including carnations *(Dianthus caryophyllus* L.). Maas Geesteranus and Barendsen (1966) on the other hand record

Table 1. Comparison of physiological tests performed with isolates 35-A, PD 128, NCPPB 179 and NCPPB 1948;

	35-A	PD 128	NCPPB 179[1]	NCPPB 1948[1]
O & F	+/+	+/+	+/+	+/+
Glucose (gas)	−	−	−	−
Glucose (acid)	+	+	+	+
Maltose	+	+	+	+
Lactose	−	−	−	−
Melibiose	−	−	n.d.[2]	n.d.
Melezitose	−	−	n.d.	n.d.
Sucrose	+	+	+	+
Inositol	+	+	+	+
Glycerol	+	+	+	+
α-Methyl-D-glucoside	+	+	n.d.	n.d.
Gluconate test	+	−	−	+
Salicin	+	+	+	+
Gelatin liquefaction	+	±3	+	+
Nitrite from nitrate	+	+	+	+
Citrate utilization	+	+	+	+
Catalase production	+	+	+	+
H_2S production	+	+	+	−
Methyl red test	−	−	−	+
Voges-Proskauer test	+	+	−	+
Indole production	−	−	−	−
Oxidase production	−	−	−	−
Urease production	−	−	−	−
Starch hydrolysis	−	−	−	−
Pectate gel liquefaction	−	−	−	−
Lysine decarboxylase	−	−	−	−
Ornithine decarboxylase	−	−	−	−
Arginine dihydrolase	−	−	−	−
Non-diffusible pigment (yellow)	+	+	+	+
DNA − G+C content (in %)	56.8	55.6	56.2[4]	n.d.

[1] See results of Graham and Quinn (1974).
[2] n.d. not done.
[3] ± weak reaction.
[4] See De Ley et al. (1966).

Tabel 1. Vergelijking van fysiologische toetsen van isolaten 35-A, PD 128, NCPPB 179 en NCPPB 1948.

A. gypsophilae from roses, carnations and cacti. They were able to obtain symptoms on roses and carnations after artificial inoculation but were unable to reisolate the organism. Graham and Quinn could not demonstrate pathogenicity in strains NCPPB 179 or NCPPB 1948 when tested on *G. paniculata* and other plants. It is not known whether NCPPB 1948 had just lost its pathogenicity or whether it had been incorrectly identified by Maas Geesteranus and Barendsen who do not report having tested their isolates on *Gypsophila* species. It is possible that their isolates represent another pathogenic form.

Fig. 2. Stems of *Gypsophila paniculata 3* months after inoculation with *Erwinia herbicola* strain PD 128.

Fig. 2. Stengels van Gypsophila paniculata 3 maanden na inoculatie met Erwinia herbicola stam PD 128.

The results of this study support the conclusions drawn earlier by Graham and Quinn that the saprophyte *E. herbicola* and the organism referred to as *A. gypsophilae* must be considered as being the same species and that the former is the correct name (*A. gypsophilae* is therefore a subjective synonym and has also no official standing in nomenclature – see Skerman et al., 1980). The correct citation, *E. herbicola* (Lohnis 1911) Dye 1964, is given in the Bergey's Manual (Buchanan et al., 1974), and in the 'Approved lists of bacterial names' (Skerman et al., 1980). However, on account of its pathogenicity, we propose that strain PD 128 should be refered to as *E. herbicola* f.sp. *gypsophilae* as it is a plant pathogenic micro-organism characterised by gall formation in a particular host, *G. paniculata.* The taxonomic recognition of the separate identity of the gall-forming strain is particularly valuable for the plant pathologist.

The organism PD 128 has been deposited in the National Collection of Plant Pathogenic Bacteria (England) as NCPPB 3091.

Samenvatting

Een stam van Erwinia herbicola pathogeen voor Gypsophila paniculata

Een geel gepigmenteerde, staafvormige, Gram-negatieve, fermentatieve bacterie

met petritriche flagellen werd geïsoleerd uit gallen op de stengels van de sierplant *Gypsophila paniculata*. De ziektesymptomen kwamen overeen met de door Brown in 1934 van deze plant beschreven gallen. Het isolaat bleek na inoculatie in gezonde stengels pathogeen te zijn en werd geïdentificeerd als een stam van *Erwinia herbicola*. Voorgesteld wordt dit organism aan te duiden als *E. herbicola* f. sp. *gypsophilae*.

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