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Erwinia chrysanthemi: description of two new biovars (bv 8 and bv 9) isolated from kalanchoe and maize host plants

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Summary — The bacterial phytopathogen *Erwinia chrysanthemi* may attack a wide spectrum of host plants. The species is officially divided into 6 pathovars on the basis of their host range. An alternative biovar classification which was based on biochemical criteria was proposed independently of pathogenicity (table I). Two new biovars are described in the present study. Biovar 8 contains 4 maize strains originating from India, France and the USA. Biovar 9 contains 3 kalanchoe strains from Denmark, France and Switzerland (table II). The 2 new biovars were found to differ from bvs 3 and 7 by the arginin dihydrolase test (table III). The validity of using the biovar system instead of the official pathovar classification for *E chrysanthemi* is discussed. Particularly, it takes into account the diversity of the species that occurs in more than 50 host plants.

***Erwinia chrysanthemi* / maize / kalanchoe / biovar / identification**

Résumé — *Erwinia chrysanthemi*, description de deux nouveaux biovars (bv8 et bv9) isolés des plantes-hôtes maïs et kalanchoe. *La bactérie phytopathogène* Erwinia chrysanthemi *attaque une large gamme de plantes-hôtes. L'espèce est divisée officiellement en 6 pathovars sur la base de leur spectre d'hôtes. Une autre classification en biovars fut proposée, reposant sur des critères biochimiques (tableau I). Deux nouveaux biovars sont décrits dans cette étude. Le biovar 8 contient 4 souches de maïs provenant d'Inde, des Etats-Unis et de France, et le biovar 9 regroupe 3 souches de kalanchoe originaires du Danemark, de Suisse et de France (tableau II). Les 2 nouveaux biovars diffèrent des bvs 3 et 7 par le test arginine dihydrolase (tableau III). On y présente l'intérêt d'utiliser le système des biovars à la place de la classification actuelle d'* E chrysanthemi *en pathovars. En particulier, il permet de rendre compte de la diversité de cette espèce qui peut se trouver maintenant sur plus de cinquante plantes-hôtes.*

Erwinia chrysanthemi / *maïs* / *kalanchoe* / *biovar* / *identification*

INTRODUCTION

Erwinia chrysanthemi (Burkholder *et al,* 1953) is a phytopathogenic bacterium which induces soft-rot and wilting. The bacterium attacks a wide range of host-plants, and occurs in many areas of the world (Bradbury, 1984). In phytobacteriology, infra-subspecific epithets were chosen as "pathovars", terms currently used to designate organisms on the basis of their host range (Young *et al,* 1978). *E chrysanthemi* was first divided into 4 pathovars according to the host of origin: pv (pathovar) *chrysanthemi* from *Chrysanthemum morifolium,* pv *dieffenbachiae* from *Dieffenbachia* spp, pv *parthenii* from *Parthenium argentatum* and pv *zeae* from *Zea mays.* Then 2 more pathovars were added: pv *dianthicola* from *Dianthus* sp and pv *paradisiaca* from *Musa paradisiaca.* The 6 pathovars are listed in the last *Bergey's Manual of Systematic Bacteriology* (Lelliott and Dickey, 1984) with the mention that "the relationship between pathogenicity, phenotypic properties and serological reactions of strains of the pathovars" is "not entirely clear".

Since strains of *E chrysanthemi* have now been isolated from more than 50 plant species (Bradbury, 1984), it seems difficult to maintain the practice of naming the bacteria after the plant they come from. In the case of pathogenic differences between the isolates, host specificity seems difficult to prove (Dickey, 1981; Janse and Ruissen, 1988). However, physiological (biochemical) testing revealed differences between *E chrysanthemi* strains (Hildebrand *et al,* 1978; Samson and Nassan-Agha, 1978; Dickey, 1979; Dickey and Victoria, 1980; Thomson *et al,* 1981).

The biochemical differences have led to the classification into biovars (Samson *et al,* 1987) *ie* subdivisions of the bacterial species that could group all the strains showing the same biochemical profile. Seven biovars have been described using 10 biochemical tests (see table I). The present study adds 2 more biovars to the *Erwinia chrysanthemi* biovar system.

MATERIALS AND METHODS

Bacteria

The bacteria were isolated from 2 host-plants: *Kalanchoe blossfeldiana* and *Zea mays* (see table II). The strains were cultivated on LPA slants (yeast extract 3 g/l, peptone 5 g/l, agar 15 g/l, without glucose) that could be kept several months. Stocks of the strains were procured by freeze-drying.

Characterization

The identity of the strains was established by the criteria adopted in *Bergey's Manual* (Lelliott and Dickey, 1984). The tests, performed according to Lelliott and Stead (1987) unless otherwise specified were: Gram reaction by KOH solubilization (Suslow *et al,* 1982), pectate degradation (Sutton's medium; modified by Bonnet, 1973), oxidation/fermentation of glucose in Hugh and Leifson medium, gas production from *d*-glucose, starch hydrolysis, nitrate reduction, indole production from tryptophan, malonate utilization in ARJ medium (Ayers *et al,* 1919) by indicator shift (bromothymol blue), and lecithin hydrolysis on egg yolk medium.

Biovar criteria

The criteria for biovar definition (Samson *et al,* 1987) were as follows: growth at 39 °C, anaerobic degradation of arginine (ADH) according to Moeller (1955), inulin assimilation in phenol red peptone water; other carbon sources were tested by acidification/alkalinisation of ARJ liquid medium (bromothymol blue) mixed with 0.3% of the carbohydrate: *d*(–)arabinose, 5-ketogluconate, mannitol, melibiose, raffinose and *d*(–) tartrate. *Cis*-aconitate was discarded because of the variability of the result for the same strain.

API galleries

Some criteria can be obtained by using API galleries (La Balme-les-Grottes, 38390 Montalieu Vercieu, France) such as arginine dihydrolase, indole, mannitol and melibiose from the API 20E, and *d*(–) arabinose, 5-ketogluconate, inulin, mannitol, melibiose and raffinose from API 50CHE, provided a longer period of time is used than specified (4 to 6 days).

RESULTS

The strains tested were identified as *E chrysanthemi* based on the negative Gram reaction, glu-

Table I. The 7 biovars of *Erwinia chrysanthemi.* According to Samson *et al* (1987): growth at 39 °C; arginin dihydrolase activity in Moeller's medium; acidificaton or alkalinisation of *d*(–)arabinose, 5-ketogluconate, inulin, mannitol, melibiose, raffinose and *d*(–)tartrate in mineral basal medium.

Biovars	*39 °C*	*ADH*	d-*arabinose*	*5 ketogluconate*	*inulin*	*mannitol*	*melibose*	*raffinose*	d-*tartrate*
bv1	–	+	–	–	+	+	+	+	+
bv2	+	–	+	–	–	+	–	–	–
bv3	+	–	+	–	–	+	+	+	–
bv4	+	–	+	+	–	–	+	+	+
bv5	+	+	–	–	+	+	+	+	–
bv6	+	–	–	–	–	+	+	+	–
bv7	–	+	–	–	+	+	–	–	+

Table II. Origin of the bacteria. a: CFBP (French Collection of Phytopathogenic Bacteria), INRA Angers, France; b: National Collection of Plant Pathogenic Bacteria, ADAS Harpenden, Great Britain.

Strain No[a]	*Host plant*	*Yr of isolation*	*Country*	*Author's No*	*NCPPB equivalent*[b]
1447	*Zea mays*	1969	India	Payak (17)	2546
1528	*Zea mays*	1966	USA	Kelman (W3.20/SR80)	2541
1531	*Zea mays*	1966	USA	Kelman (W1.1/SR62)	–
1596	*Zea mays*	1974	France	Samson (237-3)	–
1805	*Kalanchoe blossfeldiana*	1977	Denmark	Dinesen (EK II)	–
2598	*Kalanchoe blossfeldiana*	1985	Switzerland	Grimm (531)	–
2982	*Kalanchoe blossfeldiana*	1988	France	Samson (SH 170-1)	–

cose fermentation, nitrate reduction, Sutton polypectate acidification and liquefaction, no starch hydrolysis, gas production from *d*-glucose, indole production from tryptophan, malonate alkalinization and lecithin hydrolysis.

When the biovar criteria were examined, none of the strains studied corresponded to any of the 7 previously described biovars. The 4 *Zea mays* strains, which show the same new profile, are grouped in the biovar 8 category, while the Kalanchoe strains, displaying other common characteristics, are contained in the biovar 9 (see table III).

DISCUSSION

Two new biovars that differ from the 7 previously described biovars were found. They differed only in the ADH test. Biovar 8 is a biovar 3 that could be ADH positive, and biovar 9 is a biovar 7 that appears to react as ADH negative. However, the ADH test must not be considered as a variable test. When it is performed according to Moeller (1955), *ie* in the presence of 0.05% glucose (which allows better growth) and pyridoxal phosphate (which promotes the decarboxylase activity), the results are consistent for a given strain. This method was successfully applied to some *Erwinia* by Zherebilo and Gvozdyak (1976). It proved to be more sensitive in our laboratory for fermentative bacteria than Thornley's test (1960).

To allow comparisons between laboratories, we would recommend the use of previously described methods. We also designate reference strains for the new biovars: CFBP 1447 (NCPPB 2546) for bv8, and CFBP 1805 (Dinesen EKI) for bv9.

Table III. Description of biovars 8 and 9. T: the strains 1447 and 1805 are chosen as references for the new biovars, bv8 and bv9, respectively.

Biovars	*Strain No*	*39 °C*	*ADH*	d-*Arabinose*	*5 ketogluconate*	*Inulin*	*Mannitol*	*Melibiose*	*Raffinose*	d-*Tartrate*
bv8	1447^T 1528 1531 1596	+	+	+	–	–	+	+	+	–
bv9	1805^T 2598 2982	–	–	–	–	+	+	–	–	+

The classification of the kalanchoe strains into a new biovar is consistent with the results of Dinesen (1979) who described his strains pathogenic to kalanchoe as defined by ADH, melibiose, raffinose and *d*(–)arabinose negative tests. He concluded that the strains he tested did not fit in the 3 biovars known at that time. Similarly, Janse and Ruissen (1988), applying the biovar system to classify the *E chrysanthemi* isolated from the Netherlands, found that their own kalanchoe isolates could belong to biovar 7, but with a negative ADH. It thus seems that the creation of biovar 9 is enhanced.

The question of relationships between host plants and biovars has again arisen. The majority of the maize strains studied belong to either biovar 3 (Samson and Nassan-Agha, 1978; Dickey, 1979 whose subdivision IV is equivalent to bv3), or to biovar 8 (this study). But biovar 3 is not restricted to maize strains, since it harbours isolates from many other plants: *Aechmea* sp, *Aglaonema, Ananas, Chrysanthemum morifolium, Cyclamen, Dieffenbachia, Dracoena, Euphorbia* sp, *Ipomae* sp, *Musa* sp, *Pelargonium, Phalaenopsis, Philodendron, Saintpaulia, Syngonium.* Biovar 9 was created for the 3 kalanchoe strains studied. The fact that these strains were from different origins, such as Denmark, Switzerland and France, leads us to hypothesize that biovar 9 could be linked to the host of origin. However, one *Dianthus* sp strain from the Netherlands may belong to the same biovar (Janse and Ruissen, 1988).

In fact, with 9 criteria that may be plus or minus, one could mathematically expect 2^9 = 512 combinations. Up to now, 9 biovars have been discovered. It seems evident that if other biovars were found in nature, they would probably be less numerous than 512. The biovar system is therefore proposed to classify *E chrysanthemi* isolates independently of their host. In the case of biochemical differences being detected between the strains, it only means that the strains are "biochemical variants". The taxonomic value of such variants must be estimated on wide collections of bacteria. Such a need was expressed during an EPPO conference held in 1985 in Wageningen, on "the new diagnostic technics in plant protection". The biovar distribution would give a biological structure to the diversity of this bacterial species. In the Netherlands, 41 strains were found to belong to 3 biovars (Janse and Ruissen, 1988). In our laboratory, a study of almost 200 strains is being carried out isolated from 27 host plants and originating from 5 continents (Samson *et al,* 1990). The aim is to confirm the respective weight of each biovar, in order to propose a true taxonomical subdivision of *E chrysanthemi* species instead of the confusing pathovar classification.

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REFERENCES

Ayers SH, Rupp P, Johnson WT (1919) A study of the alkali forming bacteria. *Milk USDA Bull* 782

Bonnet P (1973) Les *Erwinia* pectinolytiques. I. Diagnostic biochimique rapide. *Ann Phytopathol* 5, 355-376

Bradbury JF (1984) *Guide to Plant Pathogenic Bacteria.* CAB International Mycological Institute, Kew, 72-76

Burkholder WH, Mc Fadden LH, Dimock AW (1953) A bacterial blight of chrysanthemums. *Phytopathology* 43, 522-525

Dickey RS (1979) *Erwinia chrysanthemi:* a comparative study of phenotypic properties of strains from several hosts and other *Erwinia species. Phytopathology* 69, 324-329

Dickey RS (1981) *Erwinia chrysanthemi:* reaction of 8 plant to strains from several hosts and to strains of other *Erwinia* species. *Phytopathology* 71, 23-29

Dickey RS, Victoria JI (1980) Taxonomy and amended description of strains of *Erwinia* isolated from *Musa paradisiaca* Linnaeus. *Int J Syst Bacteriol* 30, 129-134

Dinesen IG (1979) A disease of *Kalanchoë blossfeldiana* caused by *Erwinia chrysanthemi. Phytopathology Z* 95, 59-64

Dye DW, Bradbury JF, Goto M, Hayward AC, Lelliott RA, Schroth MN (1980) International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. *Rev Plant Pathol* 59, 153-167

Hildebrand DC, Schroth MN, Thomson SV (1978) *Nutritional Properties Useful for Identification of Soft-Rotting* Erwinia *species.* Proc 4th Int Conf Plant Pathogenic Bacteria, Angers, 561-562

Janse JD, Ruissen MA (1988) Characterization and classification of *Erwinia chrysanthemi* strains from several hosts in the Netherlands. *Phytopathology* 78, 800-808

Lelliott RA, Dickey RS (1984) *Genus VII: Erwinia. Bergey's Manual of Systematic Bacteriology* (Krieg NR, ed) Williams and Wilkins, Baltimore, 471

Lelliott RA, Stead DE (1987) *Methods for the Diagnosis of Bacterial Diseases of Plants* (Preece TF, ed) Blackwell Scientific Publications, Oxford

Moeller V (1955) *Manuel des Techniques Bactériologiques* (Buttiaux R, Beerens H, Tacquet A, eds) Flammarion, Paris, 302 pp

Samson R, Nassan-Agha N (1978) *Biovars and Serovars Among 129* Strains of Erwinia chrysanthemi. Proc 4th Int Conf Plant Pathogenic Bacteria, Angers, 547-553

Samson R, Ngwira N, Rivera N (1990) Biochemical and Serological Diversity of *Erwinia chrysanthemi*. *Proc 7th Int Conf Plant Pathogenic Bacteria*, Budapest

Samson R, Poutier F, Sailly M, Jouan B (1987) Caractérisation des *Erwinia chrysanthemi* isolées de *Solanum tuberosum* et d'autres plantes-hôtes selon les biovars et sérogroupes. *Bull OEPP, EPPO Bull* 17, 11-16

Suslow TV, Schroth MN, Isaka M (1982) Application of a rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without staining. *Phytopathology* 72, 917-918

Thomson SV, Hildebrand DC, Schroth MN (1981) Identification and nutritional differentiation of the *Erwinia* sugar beet pathogen from members of *Erwinia carotovora* and *Erwinia chrysanthemi*. *Phytopathology* 71, 1037-1042

Thornley MJ (1960) The differentiation of *Pseudomonas* from other Gram-negative bacteria on the basis of arginin metabolism. *J Appl Bacteriol* 23, 37-52

Young JM, Dye DW, Bradbury JF, Panagopoulos CG, Robbs CF (1978) A proposed nomenclature and classification for plant pathogenic bacteria. *NZJ Agric Res* 21, 153-177

Zherebilo OE, Gvozdjak RI (1976) On detecting aminoacid decarboxylases in bacteria of the genus Erwinia. *Mikrobiol Zh* 38, 293-298