SOME CHARACTERISTICS OF PECTOLYTIC BACTERIA ASSOCIATED WITH POTATO IN TASMANIA

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Abstract

Gram-negative pectolytic bacteria, predominantly green fluorescent pseudomonads, were obtained from approximately 13% of apparently sound selections of seed tubers in the Tasmanian Certified Seed Potato Scheme. Pseudomonads were also associated with tuber soft rot and were isolated from wilted plants in the field. *Flavobacterium*-like organisms were isolated from tubers with soft and dry rots and from potato field soil.

On the basis of a limited number of biochemical tests, green fluorescent pseudomonads were tentatively assigned to biotypes A, B or F, and G of *Pseudomonas fluorescens* and to biotype B of *P. putida* in the classification described by Stanier, Palleroni, and Doudoroff (1966). Isolates of Enterobacteriaceae were identified as strains of *Erwinia carotovora* (Jones) Holland, and as species of *Aerobacter* (*Enterobacter*).

I. INTRODUCTION

A certified seed potato scheme is conducted in Tasmania based on the multiplication of single plant selections which are checked as being disease-free by regular observation and testing. A method for the detection of tuber-borne *Erwinia carotovora* var. *atroseptica* (van Hall) Holland, the cause of the root rot, plant wilt, and tuber soft rot disease known as potato blackleg, has been used as a practical technique, utilizing the ability of this and related organisms to lyse a thin layer of calcium pectate gel (Dowson 1957). During this program a number of isolates of pectolytic bacteria were obtained from apparently healthy tubers. Single-colony isolates were also obtained from plants with wilt symptoms of the blackleg type, from tubers and seed pieces with soft and hard rots, from stems of plants that were outwardly healthy, and from krasnozem soils used for potato production. Genera in addition to *Erwinia* were obtained.

In addition to *E. carotovora* and its varieties, fluorescent pseudomonads have also been implicated as a cause of soft rot and other disorders (Folsom and Friedman 1959; Rudd Jones 1959; Vorenkovich 1960; Huether and McIntyre 1969). Pectolytic isolates of *Aerobacter aerogenes* (Kruse) Beijerinck have been obtained by enrichment culture from activated sludge and sewage (Dias 1967), of *Flavobacterium* sp. (or spp.) from soil (Dorey 1959; Rudd Jones 1959), and a *Flavobacterium*-like organism from healthy cauliflower tissue (Lund 1969).

A limited amount of descriptive work has been carried out with a number of local isolates which possess pectolytic ability.

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II. Media and Methods

Seed stocks were sampled by removing a 1-cm cube of tissue containing the stolon attachment from a tuber of each single plant selection used in the Tasmanian Certified Seed Potato Scheme. Diseased material obtained largely from commercial crops was sampled by removing tissue immediately adjacent to obviously diseased tissue. Samples were surface-sterilized for 2 min in 0·1% $HgCl_2$ containing 0·1% Teepol and then rinsed in three changes of sterile tap water. Samples from single plant selections were divided through the stolon attachment. Other tissues were sliced to expose unsterilized surfaces and the pieces of each sample were incubated at 27°C for 48 hr in Oxoid MacConkey broth. Soil samples were similarly incubated, using approximately 0·1 g of soil per millilitre. Suspensions were streaked onto Wieringa pectate plates (Dowson 1957), and where depressions resulted after 24 hr incubation at 27°C a representative isolate was obtained by streaking out a single colony. Source, appearance of colony, and an accession number for representative isolates are listed in Table 1.

Table 1

ORIGIN OF ISOLATES AND SOME GENERAL PROPERTIES

Accession No.	Origin	Metabolism of Glucose*	Appearance on Medium B of King, Ward, and Raney (1954)†
382, 383, 384, 385, 386, 389	Sound Kennebec tubers	O	GDF
390	Sound Brownell tuber	O	GDF
391, 392	Soft rot of Brownell seed pieces	O	GDF
393, 394	Stem of Kennebec plants with wilt	O	GDF
395	Stem of mature healthy Kennebec plant	O	GDF
372, 374, 397	Stem of Sebago plants with wilt	F	C
373, 376, 396	Stem of Kennebec plants with wilt	F	C
375	Soft rot of Kennebec plant	F	C
377, 378	Stem of Brownell plants with wilt	F	C
379	Tuber from Kennebec plant with wilt	F	C
380	Necrotic lesion on washed Kennebec tuber	F	C
381	Stem of healthy Kennebec plant	F	C
398	*Erwinia carotovora* culture	F	C
399, 400	*E. carotovora* var. *atroseptica* cultures	F	C
401	Stem of healthy Kennebec plant	F	C
387	Soil from potato field	O	Y
PS4	Soft rot of swede turnip "root"	O	Y
PS22, PS28	Necrotic lesions on Kennebec tubers	O	Y
PS27	Soft rot of Kennebec seed pieces	O	Y
NCIB9059	*Flavobacterium pectinovorum* (Dorey 1959)	O	Y

* F, fermentative; O, oxidative.

† GDF, green diffusible fluorescent pigment; C, cream colony; Y, yellow colony.

Cultures were maintained in nutrient broth and propagated on Difco peptone yeast extract (PYE) agar incubated for 48 hr at 27°C. Most tests were performed with a water suspension of at least 10^8 bacteria per millilitre, care being taken to avoid including agar from the plates. Catalase, Kovacs' oxidase, and plant tests other than the injection of tobacco leaves were performed with bacteria on the PYE plate or removed with a loop.

Tests with potato stems were performed by forcing small cotton plugs coated with bacteria into the cavity of stems removed from plants 9–12 in. tall. The condition of the stem at the point of inoculation was observed after incubation for 24 hr in polythene bags held at room temperature.

Methods used for the pseudomonads were those used by Allen *et al.* (1970). Those of particular relevance to the Enterobacteriaceae were performed as described by Ewing (1960). Fermentative or oxidative metabolism of glucose and other carbohydrates was determined by the method of Hugh and Leifson (1953), as modified by Hayward (1964).

III. Results

Some general properties of the isolates are listed in Table 1. These include type of metabolism of glucose and the appearance of the colony on medium B of King, Ward, and Raney (1954).

The nature of the test and results obtained for the 14 oxidative organisms are shown in Table 2. The tabulated results of tests with 16 fermentative and six yellow, oxidative organisms are available as Accessory Publications.*

TABLE 2

PHYSIOLOGICAL PROPERTIES OF FLUORESCENT PSEUDOMONADS

All cultures positive for: motility; catalase; Kovacs' oxidase test; arginine dihydrolase; malonate oxidation; Simmons' citrate utilization; Christensen's citrate utilization; growth at 5°C, growth in 2 and 3% NaCl; conversion of calcium lactate to calcium carbonate; oxidation of fructose, glucose, galactose, arabinose, glycerol; growth on lactate, malate, succinate, and *p*-hydroxybenzoate; rotting of potato slice and stems. All cultures negative for: Gram stain, blue pigment on medium A of King, Ward, and Raney (1954); hydrolysis of starch, chitin, and deep pectate gel; lipase activity (except 390); deamination of phenylalanine; growth at 40°C, growth in 10% NaCl; oxidation of maltose, salicin, lactose, adonitol, and dulcitol; growth on maleate; hypersensitive reaction on tobacco.

+, positive reaction; −, negative reaction; ±, weak or doubtful positive reaction; *, colonies larger than on PYE agar, with wrinkled surface (not domed)

Nature of Test	Accession No.											
	382	383	384	385	386	389	395	390	391	392	393	394
Nitrate reduction	+	+	+	+	+	+	+	−	+	−	−	−
Gas from nitrate	+	+	+	+	+	+	+	−	−	−	−	−
Gelatin liquefaction	+	+	±	+	±	−	±	+	+	±	+	±
Levan production	+	+	+	+	*	+	+	+	+	−	−	−
Oxidation of sucrose	+	+	+	+	+	+	+	+	+	−	−	−
Oxidation of inositol	+	+	+	+	+	+	+	−	+	−	−	−
Oxidation of mannitol	+	+	+	+	+	+	+	+	+	−	−	−
Oxidation of sorbitol	+	+	+	+	+	+	+	−	+	−	−	−
Oxidation of trehalose	+	+	+	+	+	+	+	+	+	−	−	−
Oxidation of gluconate	+	+	+	+	+	+	−	+	+	−	−	−
Aesculin hydrolysis	+	+	+	+	+	+	+	−	+	−	−	−

Tests for the presence of pectolytic bacteria in tubers of single plant selections revealed that 53 of 401 tubers (13·2%) contained pectolytic bacteria in 1969 and 42 of 375 tubers (11·2%) in 1970. Percentages for particular varieties are shown in

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Table 3 for the two seasons. In the 1970 season, 94 of 691 (13·6%) selections of local, Australian, and imported named varieties and hybrids maintained for potato breeding and selection work contained pectolytic bacteria.

TABLE 3

PERCENTAGES OF SELECTIONS OF VARIOUS POTATO VARIETIES FROM WHICH PECTOLYTIC PSEUDOMONADS WERE ISOLATED

Variety	Percentages of Selections		Percentage of 1970 Selections which were Carriers in both Seasons
	1969	1970	
Kennebec	13·3	7·7	0
Sebago	20·0	7·7	0
Pinkeye	9·8	7·7	2·2
Bismark	19·7	15·2	3·1
Up-to-date	8·5	5·7	1·9
Brownell	18·7	22·2	2·1

IV. DISCUSSION

The organisms listed in Table 2 have the properties generally associated with the green fluorescent pseudomonads; however, they are heterogeneous in their biochemical properties and may be assigned to several taxa. All would fall into groups IVa and IVb of the classification proposed by Lelliott, Billing, and Hayward (1966). These authors regard *Pseudomonas marginalis* (Brown) Stevens as the species most representative of their group IVa. Comparison of the results presented here with those of Stanier, Palleroni, and Doudoroff (1966) indicates that none of the isolates conforms to their biotypes C, D, or E of *P. fluorescens* Migula, or to biotype A of *P. putida* (Trevisan) Migula. However, our results are consistent with the classification of isolate 390 into biotype A of *P. fluorescens*; isolates 382, 383, 385, and 391 into biotype B or F, and isolate 393 into biotype G. If a limited ability to liquefy gelatin is accepted as a positive result, then isolates 384, 392, 394, and 395 would also be assigned to *P. fluorescens*: 384 as biotype B or F, and 392, 394, and 395 as biotype G. However, these conclusions are tentative because they are not based on the full range of tests carried out by Stanier, Palleroni, and Doudoroff (1966). Isolate 386 did not produce the domed, mucoid colonies typical of levan-producing cultures of *Pseudomonas* on 5% sucrose peptone agar. This isolate is therefore difficult to classify to biotype using the scheme of Stanier, Palleroni, and Doudoroff (1966).

P. putida includes those fluorescent pseudomonads which do not liquefy gelatin. Isolate 389 thus resembles biotype B of Stanier, Palleroni, and Doudoroff (1966).

The relationship between *P. fluorescens*, *P. putida*, and *P. marginalis* is obscure and further work is required to clarify the interrelationships of these species. It is possible that some isolates which have been classified in the past as *P. marginalis* resemble *P. fluorescens* while others resemble *P. putida*. Misaghi and Grogan (1969) found that the overall similarity between *P. fluorescens* and *P. putida* was 78% whereas that between *P. marginalis* and *P. fluorescens* was only slightly less, being 74%. Sands, Schroth, and Hildebrand (1970) also found a high overall percentage similarity between *P. marginalis* and the saprophytic pseudomonads, exemplified by

P. fluorescens and *P. putida*, in nutritional and biochemical properties; however, they examined insufficient isolates of *P. marginalis* to be certain about the variability of this species.

The cream-coloured isolates (Table 1) conformed to the family Enterobacteriaceae defined by the subcommittee on Enterobacteriaceae of the ICNB (Skerman 1967). With knowledge of the origin of these isolates and using the determinative scheme of Ewing and Edwards (1960) and the results of Dias (1967) isolates 380 and 381 are tentatively assigned to the genus *Aerobacter* (*Enterobacter*—cf. Skerman 1967, p. 152), 380 as *A. aerogenes* (Kruse) Beijerinck, and 381 as *A. cloacae* (Jordan) Bergey, but the correspondence is not close. Tests with stems and tubers show the remaining isolates to have the ability to rot potato tissue but again only tentative identification as to species is possible. Consistently positive methyl red and negative Voges–Proskauer reactions were obtained only for *E. carotovora* var. *atroseptica* by Graham and Dowson (1961) and this species may be represented by isolates 375, 376, 377, and 399. Isolates 374, 378, and 396 did not produce gas in any test and may be representative of *E. carotovora* var. *aroideae*. There was no clear correspondence between these tentative identifications and colony sizes on the defined medium used by Logan (1966) to distinguish *E. carotovora* var. *atroseptica* from other varieties of *E. carotovora* in tests with isolates from Northern Ireland.

The remaining results do not indicate that two or more varieties of *E. carotovora* were included in the tests. It is inferred that tests of this kind are of no additional value for the routine identification of varieties of *E. carotovora*. The results are in general agreement with those of a number of workers (Mushin, Naylor, and Lahovary 1959; Graham and Dowson 1961), who often obtained variable reactions with isolates considered to be *E. carotovora* and *E. carotovora* var. *aroideae*.

The yellow bacteria isolated from soil and potato tuber tissue appear similar to, but distinct from, *Flavobacterium pectinovorum*. Lund (1969) reached a similar conclusion with regard to isolates obtained from fresh cauliflower. The properties of the isolates from cauliflower described by Lund and those of the isolates obtained from soil, potato, and swede turnip are in close agreement and it is probable that they represent the same organism. The local isolates migrated through semisolid agar quite promptly, although this property was erratically displayed, but no motility was observed in hanging-drop preparations, nor were flagella seen in a preliminary examination with the electron microscope. This suggests that motility could be due to gliding movement. Further clarification of the taxonomy of the flavobacteria appears necessary before these organisms can be assigned to a particular subgroup of those suggested by Hendrie, Mitchell, and Shewan (1968).

The limited data summarized in Table 3 indicates that pectolytic pseudomonads associated with local seed stocks were not efficiently transmitted via seed tubers from one season to the next. Of the limited number of isolates which were obtained in pure culture from 1970 selections, eight were fermentative, of which one was strongly pectolytic, while 57 were pseudomonads producing a green diffusible fluorescent pigment on medium B of King, Ward, and Raney (1954).

The significance of the occurrence of pseudomonads in apparently healthy plants and tubers has yet to be determined and is the subject of further study. *Verticillium albo-atrum* was not obtained from such plants as listed in Table 1, and pink-eye tuber symptom on Kennebec described by Huether and McIntyre (1969) as

associated with *Verticillium* wilt and infection with *P. fluorescens* has not been recognized in Tasmania.

The local occurrence of *E. carotovora* associated with soft rot and plant wilt symptoms is confirmed. Its presence in apparently sound tubers has considerable quarantine significance, as exemplified by the origin of isolate 401 from Canada. Contamination of seed potato stocks with *E. carotovora* without any disease expression has been recognized in further field studies.

The technique used in screening seed stock was convenient to carry out on the scale required, but the occurrence of pectolytic pseudomonads in particular reduced its value as a practical and rapid means of detecting *E. carotovora*. *Flavobacterium*-like organisms and *Aerobacter* (*Enterobacter*) species are only of passing interest as inhabitants of potato tissue. Distinction between the main genera that were represented was conveniently achieved by colony appearance on medium B of King, Ward, and Raney (1954), reaction in the medium of Hugh and Leifson (1953), using glucose and maltose as substrates, and Thornley's arginine test (Allen *et al.* 1970). However, the identification of pectolytic bacteria required considerable extra work to obtain representative single colony isolates.

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