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Presence of *Erwinia chrysanthemi* in two major river systems and their alpine sources in Australia

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Erwinia chrysanthemi was isolated over three summers by filtration, with or without enrichment, of water samples from the headwaters of the Murray and Murrumbidgee Rivers and from several locations downstream. The bacterium could not be detected in streams rising adjacent to headwater catchments nor could it be detected in freshly voided faeces of native and domestic animals in the vicinity of the Murrumbidgee headwaters. Although it survived passage through the intestinal tract of a guinea pig and fowl, it did not persist in either animal. The bacterium survived for at least 211 days at 16°C in sterile distilled water. The specific localities from which the bacterium was isolated and the lack of external sources of inoculum indicate that *E. chrysanthemi* is most likely a constituent of the sessile bacterial population on stream weeds and sediments and is not a contaminant of this alpine environment.

In the irrigation areas of southern New South Wales, Australia, whole potato seed tubers sown in summer are susceptible to infection by *Erwinia chrysanthemi* pv. *zeae* (Sabet) Victoria, Arboleda and Munoz (Cother & Powell 1983). Whilst it was initially suggested that the soil population was the main source of inoculum (Cother 1980), subsequent studies elsewhere on the closely related species *E. carotovora* subsp. *carotovora* and *E. carotovora* subsp. *atroseptica* (Gudmestad & Secor 1983; McCarter-Zorner *et al.* 1984; Harrison *et al.* 1987) show that irrigation water may be a primary source of pectolytic bacteria in potato crops.

It is considered in some reports (Perombelon & Hyman 1987; Maddox & Harrison 1988) that *E. carotovora* subsp. *carotovora* and *E. C.* subsp. *atroseptica* are contaminants, deposited in the water by way of aerosols (McCarter-Zorner *et al.* 1984; Franc *et al.* 1985) or by runoff from arable land (Pérombelon & Hyman 1987). Others (Quinn 1985; Graham 1985) suggest that by virtue of their apparently ubiquitous nature in seawater, snow, streams etc., soft-rot *Erwinia* spp. may be aquatic bacteria but this is disputed by Pérombelon & Hyman (1987).

In these studies, which considered the origin of subsp. *carotovora* and *atroseptica*, *E. chrysanthemi* was never detected. Recently Cappaert *et al.* (1988) reported low incidences of *E. chrysanthemi* in surface irrigation water in Oregon but did not speculate on its origin. Other reports on the isolation of *E. chrysanthemi* from water have associated its occurrence with local sources of inoculum. Goto (1979) demonstrated that infected iris plants could serve as a source of inoculum for bacterial foot rot of rice. Olsson *et al.* (1984) isolated *E. chrysanthemi* from par-

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tially purified sewerage water and later recovered the bacterium directly and indirectly from stream water, attributing the source to *Solanum dulcamara* (Olsson 1985).

The major irrigation areas of south eastern Australia are supplied with water from the Murray and Murrumbidgee Rivers. These rivers rise in the Australian Alps and flow inland in a west to south-west direction. The source of both rivers lie within the Kosciusko National Park at 1180 m and 1380 m altitude respectively. There is considerable feral pig and marsupial activity in the vicinity of both sources. The initial stream width of both rivers is $<0{\cdot}5$ m and in summer the slow flow supports prolific aquatic weed growth. The Murray River rises on the boundary of the Pilot Wilderness Area of Kosciusko National Park and flows for approximately 60 km before entering grazing country and a further 30 km to arable land. The Murrumbidgee River rises 140 km north of the Murray source, separated by part of the Great Dividing Range (>2000 m altitude), and flows for approx 70 km before entering grazing country and another 50 km before its flow is influenced by run off from cultivated land.

The occurrence of *Erwinia chrysanthemi* in irrigation water and its alpine source, and in possible niches in this environment was investigated over the summer months (November to March) of 1986 to 1988. This paper reports the isolation of *E. chrysanthemi* from alpine water catchments and discusses the possible autochthonous nature of this bacterium.

Materials and Methods

SAMPLING PROCEDURE

Water and/or stream sediment samples were collected from both rivers on several occasions between November 1985 and March 1988 (Figs. 1 and 2, Table 1). Sites 1 to 12 which represent the source of the Murrumbidgee are in terrain classified as perennial swamp although over the sampling period this was very restricted as a result of below average rainfall. Sites 36 to 38 were sampled only in 1988 because of restricted access to a designated wilderness area. Two streams, Yarrangobilly River and Jounama Creek, which rise 4 km and 15 km west respectively of site 1, separated from it by Fiery Range, were also sampled. Both streams empty eventually into Blowering Reservoir (site 21) on the Tumut River (site 27), which joins the Murrumbidgee at Gundagai (site 29). Eucumbene River (site 15) flows into Lake Eucumbene where it is diverted underground to the Murray River above Khancoban (site 44).

Water samples (2 to 4 l) were collected each year in sterile plastic bottles by immersing the bottle midstream where appropriate or approx. 60 cm from the riverbank. Water pH and temperature were recorded at the time of sampling. Where a previous sample from a particular site was negative for pectolytic *Erwinia* spp. in 1986 and 1987, an additional sample was collected after disturbance of stream sediment or submerged weeds. This procedure was adopted for all sites in 1988. Bottles were transported in polystyrene foam boxes to minimize temperature fluctuations and the time lapsed between sampling and processing varied from 2 to 40 h depending on distance from the laboratory.

In 1985 and 1986 samples from sites 25 to 35 were filtered through sterile Whatman No. 1 filter paper with diatomaceous earth as a filtering aid (Hammarstrom & Ljutov 1954). Filters were shaken in 50 ml of sterile distilled water on a Vortex mixer and 20 μl were spread with a bent glass rod on the surface of a semi-selective agar medium (see below), two plates per dilution.

For subsequent sampling in 1986 and 1987, samples were divided into two equal subsamples and filtered through sterile 1·0 μm glass fibre filters, then through 0·45 μm cellulose acetate membranes. Sample volume filtered varied from 2 l for irrigation canal samples containing large quantities of suspended mineral and organic matter to 4 l for relatively clear stream water. Filters were shaken in 50 ml of sterile distilled water for 30 s on a Vortex mixer followed by addition of 50 ml of double strength PT enrichment medium (Burr & Schroth 1977). Filters were incubated for 48 h at room temperature (21–30°C), those from one subsample aerobically and the other filters anaerobically in BBL GasPak or Oxoid Anaerobic jars. The filter suspensions were shaken again on a Vortex mixer prior to preparation of a dilution series in sterile distilled water and plating of aliquots as above.

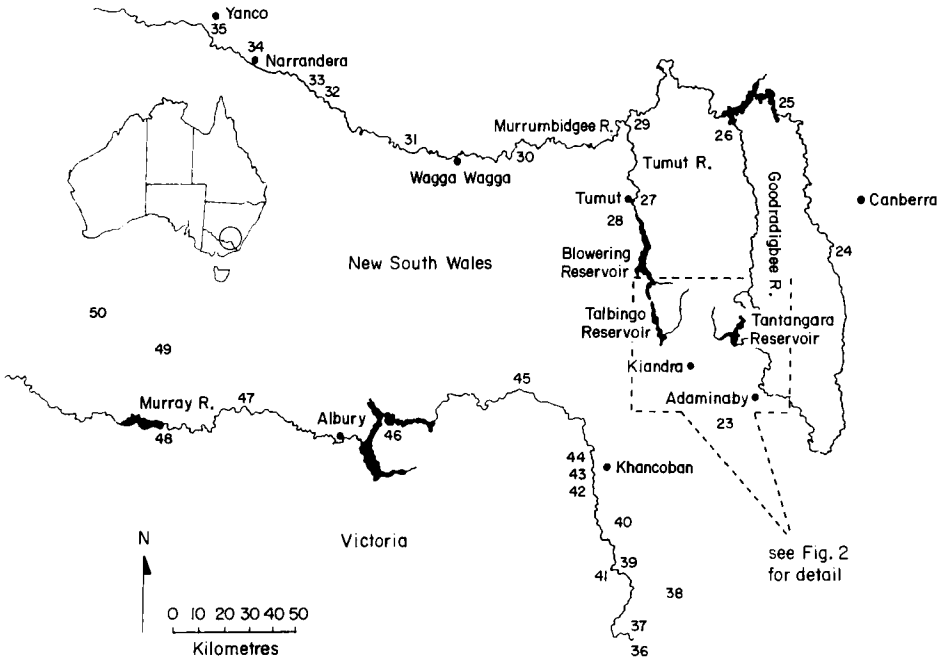

Fig. 1. Location of water sampling sites on the Murray and Murrumbidgee River systems.

Media

The crystal violet pectate (CVP) medium of Cuppels & Kelman (1974) proved unsuitable for *E. chrysanthemi* because of the strong pectolytic activity of the organism. The two layer VX medium of Pérombelon & Lumb (pers. commun.) was used for all attempted isolations. The medium contained (g/l): agar, 15;

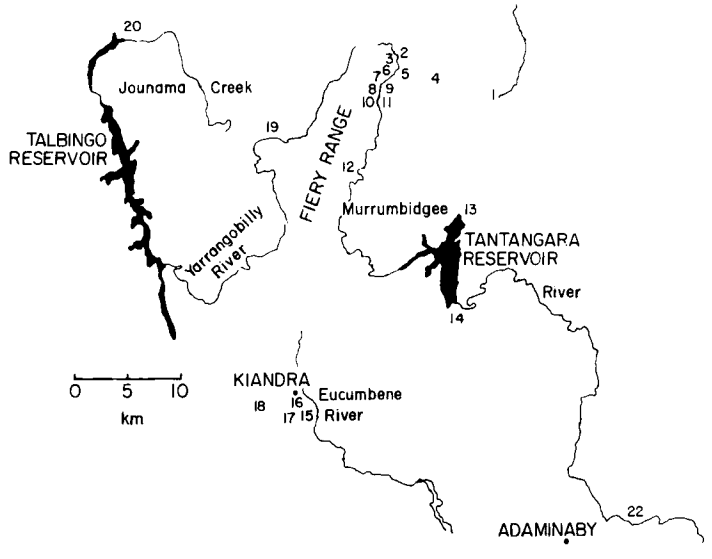

Fig. 2. Location of water sampling sites in the vicinity of the headwaters of the Murrumbidgee River.

Table 1. Location of, and isolation of *Erwinia chrysanthemi* from, water sampling sites on the Murrumbidgee and Murray Rivers

Site No.	Locality	Sampling date(s)	Isolation of *E. chrysanthemi*
Murrumbidgee catchment			
1	Source of Goodradigbee R	2/88	−
2	Peppercorn Hill, source of Murrumbidgee R	2/88	−
3	Soil, adjacent site 2	2/88	−
4	Soil in creek bed	2/88	−
5	Sphagnum peat bog draining to site 6	2/88	−
6	Murrumbidgee R, Long Plain	2/88	+
7	Murrumbidgee R, Long Plain	4/88	+
8	Murrumbidgee R, Long Plain	4/88	+
9	Murrumbidgee R, Long Plain	4/88	+
10	Murrumbidgee R, Long Plain	4/88	−
11	Designated headwaters of Murrumbidgee R	2/87, 1/88	+, +
		2/88, 4/88	+, +
12	Swampy watercourse draining into Murrumbidgee, 2 km below site 11	2/88	−
13a	Causeway above Tantangara Reservoir	2/88	+
b	Sediment and water weed	2/88	+
14a	Water discharge from Tantangara Dam	2/88	+
b	Stream sediment below Tantangara Dam	2/88	+
15	Eucumbene River	2/88	−
16	Billabong adjoining Eucumbene River	2/87	−
17	Bullocks Head Creek	2/87	−
18	Stream sediment Bullocks Head Creek	3/87	−
19	Yarrangobilly River	2/87, 2/88	−, −
20	Jounama Creek	2/87, 2/88	−, −
21	Sediment, edge of Blowering Dam	2/87, 2/88	−, −
22	Murrumbidgee River, Rosedale	3/86, 1/87	−, +
		2/88, 3/88	+, +
23	Lake Jindabyne	1/87	+
24	Murrumbidgee River, Angle Crossing	3/86, 1/87	+, +
25	Murrumbidgee R. confluence with Burrinjunck Reservoir	2/86	+
26	Goodradigbee R. confluence with Burrinjuck Reservoir	2/86, 1/87	+, +
27	Tumut River	2/86	−
28	Farm dam Batlow	2/86	+
29	Murrumbidgee R, Gundagai	2/86, 2/87	−, −
30	Murrumbidgee R, Wantabadgery	2/86	−
31	Murrumbidgee R, Mundoway Bridge	2/86	−
32	Murrumbidgee Irrigation Area main canal below Berembed Weir	2/86	−
33	M.I.A. canal, Thelhaven	2/86, 3/87	+, +
34	M.I.A. canal, Narrandera	2/86, 3/87	+, +
35	M.I.A. canal, Yanco	12/85, 2/86	+, +
		3/87, 1/88	+, +
Murray catchment			
36	Cowombat Flat, source of Murray R	3/88	+
37	Pilot Creek	3/88	−
38	Swampy watercourse Pilot Wilderness Area	3/88	−
39	Leather Barrel Creek	1/87	−
		3/88	−
40	Swampy Plain Creek	1/87	−
41	Murray R, Tom Groggin	3/88	+
42	Murray R, Damms Bridge	3/88	+
43	Murray R, Indi Bridge	3/88	+
44	Murray R, Khancoban	3/86, 1/87	+, +
		3/88	+
45	Murray R, Jingellic	2/87, 3/88	−, +
46	Murray R, Bethangra Bridge	3/86, 2/87	−, −
47	Murray R, Corowa	3/86, 2/87	−, +
48	Lake Mulwala	3/86, 2/87	−, −
49	Irrigation canal, Savanche	3/86, 2/87	−, −
50	Irrigation canal, Berrigan	3/86, 2/87	−, +

$CaCl_2.6H_2O$, 5·5; phenol red, 0·15; $MnSO_4$, 0·12; tryptone, 1; 1 N NaOH, 5·5 ml in the base layer and: Na polypectate (Wisconsin Alumni Research Foundation), 12·5; $NaNO_3$, 1; 1 N NaOH, 7·5 ml; disodium salt of EDTA, 0·1; blended and autoclaved 10 min, as the overlayer. The quantity of alkali used is dependent on the source of polypectate used. Inoculated plates were incubated at 30°C for 24 h and examined for the presence of vertical sided cavities. Bacterial colonies at the base of the cavity in the pectate overlayer were removed and plated on Nutrient Agar (Oxoid) to obtain single pure colonies. Each colony type was then retested for pectolytic activity by spot inoculation on VX agar. Cavities were sampled at random to a maximum of 25 per water sample incubation method (i.e. 50 per sample). Each pectolytic strain was tested for growth at 37°C, glucose fermentation, oxidase activity, lecithinase and phosphatase production (Cother & Sivasithamparam 1983) and for indole production by the method of Cother & Blakeney (1987). Oxidase-negative strains, positive for the other reactions were presumptively identified as *E. chrysanthemi*.

In 1988 a resuscitation phase was introduced before the membrane filters were added to the enrichment broth. Whatman filter papers were saturated with either Enterobacteriaceae Enrichment Broth (Merck) or a resuscitation medium modified from Hall (1985) containing (g/l): tryptone, 10; DL-tryptophan, 1; mannitol, 1: yeast extract (Oxoid), 1; Na pyruvate, 0·1; Na glycerophosphate, 0·2; Na acetate, 1; $MgSO_4.7H_2O$, 0·2; pH 7·6. After sample filtering, membrane and glass fibre filters were placed face-up on the saturated paper and incubated at 30°C for 2 h after which the filters were carefully removed and added to the enrichment medium as above.

PRESENCE OF *E. chrysanthemi* IN, OR SURVIVAL THROUGH, ANIMAL AND BIRD INTESTINES

Although *E. chrysanthemi* is the only species in the Enterobacteriaceae that does not possess the enterobacterial common antigen it has nonetheless DNA–DNA relatedness to other members of this family (Jones 1988). The likelihood that native or domestic animals in the river catchments may act as reservoirs of pectolytic *Erwinia* spp. was examined by attempting to isolate them from freshly voided faeces and from inoculated animals.

Detection of Erwinia *in faeces*

Five to 10 g samples of freshly voided faeces were collected in the field in January 1987 from sheep (2 samples), cattle (4) near site 24, kangaroo (8), wombat (6), broad toothed rat (*Mastacomys* sp.; 1), native bush rat (*Rattus fuscipes*; 1), near site 23 and wood duck (*Chenonetta jubata*, 6) near sites 20 and 35. The faecal samples from rats came from trapped animals and could have been up to 12 h old. Sheep, cattle and duck faeces were collected immediately after voiding and the kangaroo and wombat faeces were less than 2 h old. Samples were collected, avoiding contact with soil, and placed in sterile polycarbamate jars containing 30 ml of enrichment broth. Samples were taken 2 to 3 h later and incubated with an equal quantity of double strength enrichment broth at room temperature (20–25°C) in anaerobic jars for 24 and 48 h.

Samples of stomach and large intestine contents of 12 feral pigs (*Sus scrofa*) trapped in the vicinity of sites 1 to 11 in March 1988 were also tested. Samples were received in the laboratory 24 to 30 h after collection and two 20 g wet weight sub samples were taken. One sample was spread on filter paper saturated with resuscitation medium, incubated at 30°C for 2 h, then transferred to PT broth. The other sample was placed directly into single strength PT broth and both samples were incubated at 30°C for 24 and 48 h before serial dilution and plating on VX agar.

Survival of Erwinia through animal and bird intestines

For ease of experimental use, a domestic hen approx. 6 months old and a male guinea pig approx. 5 months old were chosen as representative of avian and mammal fauna.

Hen. The bird was caged for 3 weeks after receival and fed commercial poultry pellets. The presence of resident pectolytic organisms was determined by incubating 3 g wet weight of freshly voided faeces in 75 ml of PT broth, aerobically and anaerobically for 48 h and plating

20 μl volumes on VX agar. After a 24 h fast, 5 ml of a suspension of *E. chrysanthemi* (DAR 35633; $1{\cdot}34 \times 10^{10}$ cfu/ml in physiological saline) was administered orally to the hen and normal feeding resumed. Further bacterial drenches were given 23 and 48 h later with suspensions of $1{\cdot}09 \times 10^{10}$ and $3{\cdot}25 \times 10^{10}$ cfu/ml respectively. Faeces were collected at varying times after drenching (Table 2) and incubated for 24–36 h in PT broth prior to streaking on VX agar. Representative pit-forming colonies were tested for glucose fermentation, lecithinase and phosphatase.

Guinea pig. The resident pectolytic microflora was determined by incubating 0·8 g of faeces as above in PT broth. After a 24 h fast, 2 ml of the above suspension was administered orally and the animal fed dried apple (high pectin content) for 24 h, and then commercial rodent pellets. The subsequent drench programme followed that of the hen.

SURVIVAL OF SELECTED STRAINS IN WATER

Two type cultures DAR 35625, pv. *chrysanthemi*, and DAR 35630, pv. *zeae*; and four strains from water DAR 64759 from site 7, 64781 from site 26, 64780 from site 36 and 64793 from site 45 were each grown for 24 h on TGE agar (Fahy & Hayward 1983). Cells were suspended in sterile distilled water, pH 5·7, in duplicate 30 ml screw-capped bottles and incubated at 16°C. Survival was determined periodically by dilution plating of 1 ml amounts on TGEA and incubation at 25°C for 48 h.

Results

Erwinia chrysanthemi IN RIVER WATER

Erwinia chrysanthemi was isolated from streams forming the headwaters of the Murray and Murrumbidgee Rivers and at several sites downstream (Table 1). Sites from which the bacterium was isolated at the first sampling date generally remained so when sampled in subsequent summers. Sites 22 and 45 where the bacterium was not detected in the first sample, were positive at later sampling dates when either stream/weed sediment was included in the sample or a resuscitation phase was used in the isolation procedure.

Restricted access to many sites within the National Park precluded winter sampling. The regularity of isolation at site 11 suggests a continuity in its presence at least during summer.

The bacterium was not detected in peat bog or soil samples from the upper most reaches of the Murrumbidgee River immediately adjacent to water sites from which it was isolated. Nor was it detected in the Murrumbidgee between the Burrinjuck Reservoir and the main Murrumbidgee Irrigation Area supply canal several hundred kilometres downstream. It was however, isolated from a farm dam at Batlow (site 28) which although adjacent (500 m) to a

Table 2. Isolation of *Erwinia chrysanthemi* from faeces of hen and guinea pig at various time intervals after oral drenching of the animal with bacterial suspension

	Guina pig		Hen	
Animal drenched	Faeces void time (h)	*Echr* isolation	Faeces void time (h)	*Echr* iolation
Monday 1330				
Tuesday 1100	1600–2020	+	1200–1400	+
			1400–2040	+
	2030–0650	+	2100–0715	+
Wednesday 1100	1200–1600	+	1200–1600	+
	1600–0700	–	1630–0700	+
	1200–1600	–	1200–1600	+
			0920	–

seed potato growing field, received runoff from native eucalypt forest only.

Erwinia chrysanthemi was not detected in streams rising in close proximity to the source of the Murrumbidgee (sites 1, 15, 17, 19 and 20) nor in tributaries flowing into the Murray River (sites 37, 39 and 40).

Failure to detect the bacterium between sites 26 and 33 and 45 to 49 may be due to its absence or because it was masked by competition as the nutritional base for the resident microflora of the river water increases in both quantity and complexity. This does not explain, however, the consistency of isolation without enrichment from the Murrumbidgee Irrigation canal.

At sites 6 to 11 and 36 *E. chrysanthemi* was more readily isolated when stream sediment or sediment on submerged weeds was included in the water sample. At site 6 in February 1988 it was isolated only from material retained on a Whatman No. 1 filter paper used to remove particulate matter from the sample; it was not isolated from the resulting filtrate with a 0·45 μm membrane filter. There was little advantage in anaerobic incubation of filters in PT broth except at site 30 where *E. chrysanthemi* was not isolated from aerobic cultures. At site 26, however, the sample taken in February 1986 was positive for the bacterium only after anaerobic incubation but the following year *E. chrysanthemi* could be isolated only from aerobic cultures.

Water temperatures at sampling ranged from 11°C in alpine areas to 24°C downstream; pH varied from 5·1 to 6·0. The temperature of water samples positive for *E. chrysanthemi* was 16·2°C or warmer but there was no relationship to water pH.

No attempt was made to quantify bacterial populations especially where enrichment procedures were used because it is believed that absolute numbers *per se* are less important than establishing the presence or otherwise of the bacterium. When samples produced many pits on VX agar the large majority of bacteria isolated from such pits were *E. chrysanthemi*. Sites negative for this bacterium were also negative for other pectolytic bacteria capable of growth on VX agar except for sites 15, 16, 20, 30 and 40 where bacteria resembling *E. carotovora* were isolated.

Erwinia chrysanthemi IN FAECES

No pectolytic bacteria were detected in any faecal samples regardless of method or length of incubation. After incubation for 48 h samples were added to fresh PT broth and incubated anaerobically or aerobically at various temperatures from 30 to 42°C for up to 7 d but no pectolytic bacteria were isolated on VX agar.

ERWINIA PASSAGE THROUGH HEN AND GUINEA PIG

No faecal pectolytic flora was detected by direct plating of faeces on VX agar prior to drenching. After drenching with *E. chrysanthemi*, incubation of faeces in PT broth for longer than 24 h was necessary to isolate the bacterium. *Erwinia chrysanthemi* could be isolated from faeces up to and including the third sampling of guinea pig faeces (Table 2) and the 6th sample from the hen. Even though sampling continued for a further 6 d at 12 h intervals the bacterium was not detected after this time even when enrichment of the original suspension, or of samples in fresh PT broth, exceeded 56 h.

Passage through the intestinal tract may stress the cells to that viable but non-culturable cells are voided. A resuscitation phase, however, was not introduced as preliminary experiments with mixed suspensions of *E. chrysanthemi* with *Escherichia coli* or with faeces indicated that whilst *Escherichia coli* multiplied only slowly in PT broth relative to *E. chrysanthemi*, resuscitation medium accelerated growth of *Escherichia coli* so obscuring pit development on VX agar.

SURVIVAL IN WATER

Population levels of all strains remained relatively high throughout the sampling period (Table 3). The number of detectable cells of DAR 64781 fell markedly at day 156 and this strain was not culturable by day 211. The cultures were transported to another laboratory on day 157 and were out of the incubator for 18 h. Temperature fluctuations in transit may have affected this bacterium.

Table 3. Survival of various strains of *Erwinia chrysanthemi* in sterile distilled water pH 5·7 at 16°C expressed as cfu/ml

Isolate	Sampling Time (days)						
	0	7	14	40	100	156	211
Type cultures							
DAR 35625	$1{\cdot}7 \times 10^{7}$	$1{\cdot}9 \times 10^{8}$	$1{\cdot}5 \times 10^{8}$	$7{\cdot}8 \times 10^{6}$	$3{\cdot}5 \times 10^{6}$	$1{\cdot}1 \times 10^{6}$	$4{\cdot}0 \times 10^{4}$
35630	$1{\cdot}8 \times 10^{7}$	$2{\cdot}6 \times 10^{8}$	$1{\cdot}8 \times 10^{8}$	$3{\cdot}0 \times 10^{7}$	$7{\cdot}5 \times 10^{6}$	$2{\cdot}0 \times 10^{6}$	$2{\cdot}8 \times 10^{5}$
Water isolates							
64759	$4{\cdot}1 \times 10^{7}$	$5{\cdot}2 \times 10^{7}$	$4{\cdot}2 \times 10^{7}$	$7{\cdot}7 \times 10^{6}$	$2{\cdot}8 \times 10^{6}$	$2{\cdot}6 \times 10^{6}$	$7{\cdot}1 \times 10^{3}$
64780	$1{\cdot}4 \times 10^{8}$	$1{\cdot}5 \times 10^{8}$	$1{\cdot}3 \times 10^{8}$	$2{\cdot}1 \times 10^{7}$	$2{\cdot}4 \times 10^{6}$	$5{\cdot}8 \times 10^{5}$	$4{\cdot}5 \times 10^{4}$
64781	$5{\cdot}5 \times 10^{7}$	$6{\cdot}7 \times 10^{7}$	$5{\cdot}5 \times 10^{7}$	$9{\cdot}3 \times 10^{6}$	$3{\cdot}5 \times 10^{6}$	$1{\cdot}0 \times 10^{5}$	not detected
64793	$1{\cdot}4 \times 10^{8}$	$1{\cdot}7 \times 10^{8}$	$9{\cdot}9 \times 10^{7}$	$1{\cdot}6 \times 10^{7}$	$4{\cdot}2 \times 10^{6}$	$6{\cdot}7 \times 10^{5}$	$1{\cdot}1 \times 10^{5}$

Discussion

This is the first record of the isolation of *E. chrysanthemi* from natural water sources in the absence of known diseased hosts. The presence of this pectolytic enterobacterium in an alpine environment raises questions as to its origin.

Although Jorge & Harrison (1986) suggested that pectolytic *Erwinia* spp. may multiply in stream bed niches and release cells into the water more or less constantly, they and other workers (McCarter-Zorner *et al.* 1984; Maddox & Harrison 1988) have placed greater emphasis on considering external sources of inoculum (aerosols, precipitation, agricultural runoff or sewerage). Pérombelon & Hyman (1987) regarded these bacteria as contaminants, suggesting that they were not a natural part of the existing microflora of the sites from which they were isolated.

In contrast to this viewpoint, the results of this study suggest that *E. chrysanthemi* may possibly be indigenous in this environment. The existence of the bacterium at the source of each river and the apparent absence of *E. chrysanthemi* from neighbouring streams suggest that the bacterium inhabits specific (as yet unknown) microsites on these two rivers and is not an introduced 'contaminant' from external sources. Should *E. chrysanthemi* be introduced into alpine areas by rain or aerosols one would expect it to be more widely distributed in the catchment areas.

There are no known sources of *E. chrysanthemi* in diseased plants in situations suitable for aerosol generation in Eastern Australia. The bacterium is a potato tuber pathogen in the semi-arid irrigation areas west of the mountains and has not been recorded there as a foliage pathogen. At the time of greatest disease occurrence there is no foliage present in the potato crops, temperatures are high (>30°C), relative humidity is low (30%) and storms are infrequent. Moreover, climate in the alpine areas is influenced as much from the eastern seaboard, where there are no known hosts of the bacterium, as from the west where conditions in summer do not favour aerosol generation.

There is no evidence from this study that a background population of *E. chrysanthemi* inhibits the intestinal tract of indigenous or introduced fauna and which could act as a constant source of transient inoculum to the streams. The locality of isolations argue against the faecal contamination theory as the range of animal movement is far wider than the specific sites of positive isolation.

In all natural ecosystems studied there is a universal tendency for bacteria to adhere to surfaces and to form biofilms (Costerton *et al.* 1986). The improved isolation, or in some cases the only isolation, of *E. chrysanthemi* from water sampled after stream and/or weed sediment disturbance attests to the presence of the bacterium more as a sessile rather than as a planktonic population. It would be this planktonic population only that was sampled at those sites (e.g. 22) where the bacterium was isolated from clear stream water, free of sediment. The release from a larger sessile population must be a more-or-less continuous process to account for the bacterium's regular presence.

If *E. chrysanthemi* has not successfully colonized specific niches in these alpine streams, its

introduction into water must be constant or the population would fall, by dilution or by competition, to below detectable levels. Moreover the length of survival of culturable cells in sterile water contrasts the conclusion of Pérombelon & Hyman (1989) that erwinias, having adapted to grow in nutrient-rich plant tissue will die out more or less rapidly in a low nutrient environment.

Escherichia coli has recently been isolated from water within bromeliads and from dry leaf surfaces in tropical areas (Bermudez & Hazen 1988; Rivera *et al.* 1988) indicating that bacteria long since regarded as 'contaminants' may indeed cycle naturally in certain ecosystems.

Whilst it is unclear how *E. chrysanthemi* was introduced into this environment it is likely to have been in association with human activity. The source of the Murrumbidgee River and to a much lesser extent that of the Murray are accessible during the summer months to bushwalkers and fishermen. Before inclusion in the National Park in the 1950s both areas supported isolated settlements of tin miners or cattlemen, and the area immediately adjacent to the source of the Murray River reportedly supported a vegetable farm in the 1930s. It is possible that *E. chrysanthemi* was introduced to these areas during that time and has occupied a specific niche ever since.

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