Final Report

Revised investigation of *Erwinia chrysanthemi* (*Dickeya dianthicola* and other *Dickeya* spp.) able to infect potatoes

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Project Report 2008/7

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1 Summary

There has been a perceived increase in findings of *Erwinia chrysanthemi* (recently reclassified into 6 species of the genus *Dickeya*) on potatoes in Europe and Mediterranean countries, including a number of findings on potato crops in England and Wales, mostly but not exclusively associated with crops grown from imported seed. This 14 month project mainly aimed to study a collection of over 200 isolates of the pathogen from potato and a wide range of other hosts from diverse locations in order to verify which *Dickeya* species (spp.) present a current risk to potato production, both in Europe and worldwide.

In the first instance, currently available diagnostic methods were evaluated to ensure their suitability for detection, identification and enumeration of the various *Dickeya* species and strains. A modified selective medium (CVPM) allowed growth of all isolates that were also detected using a molecular (real-time PCR) assay but not always by ELISA using currently available antibodies.

A detailed study of the evolutionary relatedness of all of the *Dickeya* isolates involved comparisons of DNA sequence within specific genes. One gene in particular (*recA*) differed sufficiently in DNA sequence to allow identification of the described species and a potential new species isolated from sugar cane in Australia. Four of the six named *Dickeya* species are found in Europe whereas *D. dadantii* appears to be unique to *Pelargonium* from the Comoros Islands near Madagascar and *D. paradisiaca* has been found only on banana in Colombia, Cuba and Jamaica. The other 4 species can all cause disease on potato but in each case their distribution on potato seems to be geographically isolated.

As expected, all isolates from potato in the UK and almost all isolates from potato in Europe were identified as *Dickeya dianthicola*. However, a new strain, distinct from the known *Dickeya* species has also been recently identified in the Netherlands, Israel and possibly elsewhere. *D. dianthicola* appears to be largely confined to Europe and Mediterranean areas and has not been found on potato elsewhere in the world. In contrast to *D. dianthicola*, the other *Dickeya* species appear to have origins in warmer regions of the world and have probably spread to Europe with trade in ornamental plant hosts. *D. dieffenbachiae* has been isolated from potato in Peru and Brazil, whereas potato isolates from Australia and the USA were found to be *D. zeae* and *D. chrysanthemi*, respectively.

Testing of selected seed potato stocks grown in England and Wales revealed a low (around 1%) frequency of infection by *Dickeya* spp. The finding of *Dickeya* in a single sample of home-grown seed in 2007 suggests that pathways exist for the spread of infection from outside of the classification system. As in previous years, several findings of *Dickeya dianthicola*, causing wilting in ware potato crops during the 2007 season, were all associated with seed imports. Continued vigilance is therefore justified to protect home-grown seed from infection with this pathogen.

Dickeya spp. were also isolated from around 35% of watercourses sampled during September in England and Wales at relatively low frequency in comparison with the ubiquitous presence of *Pectobacterium* in the watercourses sampled. No evidence for survival in riparian hosts (such as *Solanum dulcamara*) was found in this study, although infections in this host have been found elsewhere in Northern Europe (Olsson, 1985) and this species was found to be susceptible to artificial infections under glasshouse conditions. *Dickeya* sp. were not consistently detected when watercourses were sampled at the same point on different dates, as is usually the case with the brown rot bacterium (*Ralstonia solanacearum*) following colonisation of *S. dulcamara* along river banks. This study did not determine whether the

species of *Dickeya* isolated from UK and European rivers is the same as that identified in potatoes (*D. dianthicola*). Continued assessment of the risks of waterborne populations are planned to involve identification of rivers where the pathogen is continually detected, investigation of the *Dickeya* spp. involved and potential role of alternative hosts.

Achievement of objectives

- **Determine distribution of *Dickeya dianthicola* and other *Dickeya* spp. (all formerly *Erwinia chrysanthemi*) in England and Wales, Europe and worldwide.**

A collection of 219 *Dickeya* isolates were identified to species and their distribution was mapped by continent and by country in Europe.

- **Determine frequency of occurrence and identification of *Dickeya* spp. on seed and ware potatoes currently entering and grown in England and Wales.**

All isolates of *Dickeya* found on potato entering, or grown in, England and Wales were identified as *D. dianthicola*. The frequency of occurrence on home-grown potatoes was found to be low (around 1%) in 2007. Permission was not obtained to test imported potatoes.

- **Frequency of occurrence and identification of any *Dickeya* spp. in watercourses in England and Wales.**

Dickeya sp. were detected sporadically in 35% of watercourses sampled in England and Wales in September 2007.

- **Determine whether *Solanum dulcamara* or other riparian weed spp. are hosts of *D. dianthicola.***

No evidence was found that *Dickeya* spp. had infected riparian weeds in the rivers in which it was detected. *D. dianthicola* was found to infect *S. dulcamara* under glasshouse conditions and has been found to infect under natural conditions in Sweden.

- **Determine whether current diagnostic methods are suitable for detection, identification and enumeration of *Dickeya* spp.**

A modified selective medium (CVPM) allowed isolation of all *Dickeya* spp. A real time PCR assay allowed detection and identification of all *Dickeya* spp. but insufficient 16S rRNA and *recA* gene sequence variation was found for the development of specific assays for individual detection and identification of the different *Dickeya* spp. Therefore, further development will be required for species-specific PCR detection methods.

2 Experimental Section

Introduction

There has been a perceived increase in findings of *Dickeya* sp. on potatoes in Europe and Mediterranean countries, including a number of findings on potato crops in England and Wales, mostly but not exclusively associated with crops grown from imported seed. The British Potato Council review (Elphinstone and Toth, 2007) of *Dickeya* spp. (*Erwinia chrysanthemi*) indicated some knowledge gaps critical to understanding the current extent of introduction, survival and persistence of the pathogen in GB and the associated risks to our potato industry. This proposed short project, based on previous investigations at CSL, aimed to reanalyse data and isolates obtained over the last 20 years in the light of recent changes to the taxonomy of the pathogen (Samson *et al*., 2005). This involved further investigation of the species found on potatoes and ornamentals, and to further investigate their reported potential for establishment on alternative hosts and transmission in surface water.

Dickeya spp. have not been detected on potato in Scotland, investigations into the frequency of introduction and extent of establishment were therefore restricted to England and Wales, where the pathogen is known to have been introduced since at least 1990, and has since occurred on English seed potatoes. This study aimed to provide baseline information in preparation for a more extensive study on the survival, disease epidemiology and risks posed by this pathogen under GB conditions.

The objectives of this study were to determine:

- Distribution of *Dickeya dianthicola* and other *Dickeya* spp. (all formerly *Erwinia chrysanthemi*) in England and Wales, Europe and worldwide.
- Frequency of occurrence and identification of *Dickeya* spp. on seed and ware potatoes currently entering and grown in England and Wales.
- Frequency of occurrence and identification of any *Dickeya* spp. in watercourses in England and Wales.
- Whether *Solanum dulcamara* or other riparian weed spp. are hosts of *D. dianthicola.*
- Whether current diagnostic methods are suitable for detection, identification and enumeration of *Dickeya* spp.

Material and methods

Identification of *Dickeya* spp. amongst stored *Erwinia chrysanthemi* isolates

Phylogenetic relatedness within a large collection of *E. chrysanthemi* isolates maintained within the NCPPB and research collections at CSL and elsewhere (including isolates from the Netherlands Plant Protection service) was investigated using partial 16S rRNA and *recA* gene sequencing. These isolates were also compared with reference strains representing the 6 *Dickeya* spp. into which *E. chrysanthemi* was recently reclassified (Samson *et al*., 2005). The distribution of the various *Dickeya* spp. on potato and other hosts in GB, Europe and worldwide was then determined. A total of 219 isolates previously characterised as *Erwinia chrysanthemi* according to biochemical, fatty acid and rep-PCR profiles (Table 2) and 18 reference isolates of known *Dickeya*, *Pectobacterium* and *Erwinia* species (Table 3) were used in this study.

Bacterial DNA was prepared by heating (96 ºC for 6 min) aqueous suspensions of each isolate containing approximately 1 x 10^8 cfu per ml. Partial 16S rRNA and *recA* genes were amplified by PCR using primers shown in Table 3. PCR reactions (50 µl each) contained 10 µM primers, 1x PCR buffer (Fermentas), 1.25 U proof reading *Taq* DNA polymerase, 2 mM $MgCl_2$ and O,2 mM dNTP mix. Reaction conditions were 95 ºC for 9 min followed by 35 cycles of 95 ºC for 1 min, 55 ºC for 2 min and 72 ºC for 1 min with a final extension of 72 ºC for 7 min. Amplicons were purified using the Wizard SV gel and PCR clean-up system (Promega A9280) and sequenced at MWG Biotech (www.mwg-biotech.com). Sequence data was aligned using ClustalW software and clipped to 715 and 481 bases for 16S rRNA and *recA* gene analysis respectively. Phylogenetic comparisons were made with Jukes-Cantor algorithms and phylogenetic trees were constructed using the neighbour joining method in Treecon software. Bootstrap values greater than 70% were considered to indicate reliable positioning of each node in the phylogenetic tree.

TABLE 1: SEQUENCING PCR PRIMERS USED IN THIS STUDY.

Primers	**Sequences**
16S rRNA	ufp1 5’- AGT TTG ATC CTG GCT CAG –3’ (18bp) urp1 5’- GGT TAC CTT GTT ACG ACT T –3’ (19bp)
recA	recAf 5’- GGT AAA GGG TCT ATC ATG CG –3’ (20 bp) recAr 5’- CCT TCA CCA TAC ATA ATT TGG A –3’ (22 bp)

TABLE 2: *DICKEYA* ISOLATES USED IN THIS STUDY

	CSL No.	**NCPPB No.**	**Other Code**	**Host**	**Country**	Year
1	P1	3537	IPO 598	Potato	Peru	1987
2	P2		IPO 648	Potato		
3	P3	3533	IPO 655	Potato	USA	1987
4	P4		IPO 765	Potato		
5	P5		IPO 771	Potato		
6	P6		IPO 502	Potato		
7	P7		IPO 647	Potato		
8	P8		IPO 651	Potato		
9	P9	3536	IPO 754	Potato	Peru	1987
10	P10		IPO 769	Potato		
11	P11		IPO 256	Potato		
12	P12	3532	IPO 646	Potato	Australia	1987
13	P13		IPO 650	Potato		
14	P14	3535	IPO 730	Potato	Finland	1987
15	P15		IPO 767	Potato		
16	P16	3531	IPO 645	Potato	Australia	1987
17	P17		IPO 649	Potato		
18	P18	3534	IPO 713	Potato	Netherlands	1987
19	P19		IPO 766	Potato		
20	P23	3529		Potato	UK (Jersey)	1987
21	P548		A4620/1	*Pelargonium*	UK	1990
22	P762		CFBP 2598	*Kalanchoe blossfeldiana*	Switzerland	1982
23	P1090	3542	PD 483	Potato cv. Ostara	NL	1985
24	P1104		A5299 92/1143B	Potato	UK	1992
25	P1106		A5309/3b	Potato	UK	1992
26	P1116	2030	A63	*Chrysanthemum morifolium*	UK	1967
27	P1221		a	Potato cv. Premiere	UK	1993
28	P1231		PD 1132	*Gymnocalicium* 'Optima Rubra'	NL	1988
29	P1232		PD 552	*Scindapsus pictus*	NL	1985
30	P1233		PD 813	*Phalaenopsis* sp.	NL	1987
31	P1234		PD 554	*Kalanchoe blossfeldiana* 'Pollux'	NL	1985
32	P1235		PD 788	*Cichorium intybus*	NL	1987
33	P1237		PD 593	*Kalanchoe blossfeldiana*	NL	1985
34	P1238		PD 484, IPO 764	Potato cv.Bintje	NL	1984
35	P1239		PD 551	*Kalanchoe blossfeldiana* 'Pollux'	NL	1985
36	P1242		A5873	*Dianthus*	UK	1993
37	P1504	3930	IBSBF 994	*Lycopersicon esculentum*	Brazil	1995
38	P1644		B412 (CSL964816)	Potato	UK	1996
39	P1649		B444 (CSL965208)	Potato cv. Ostara	UK	1996
40	P1650		B444 (CSL965208)	Potato cv. Ostara	UK	1996
41	P2132	518		*Dianthus caryophyllus*	Denmark	1957
42	P2135	427		*Chrysanthemum morifolium*	USA	1957
43	P2138	393		*Dianthus caryophyllus*	UK	1957
44	P2139	377		*Zea mays*	Zimbabwe	1956
45	P2141	708		*Zea mays*	India	1959
46	P2143	1514		*Dieffenbachia picta*	DE	1963
47	P2144	1490		*Dieffenbachia picta*	Puerto Rico	1963
48	P2146	1111		*Dianthus caryophyllus*	UK	1961
49	P2148	911		*Dieffenbachia picta*	USA	1961
50	P2153	2227		*Chrysanthemum morifolium*	UK	1969
51	P2157	2027		*Chrysanthemum morifolium*	UK	1967
52	P2158	1863		*Zea mays* var. rugosa	USA	1966
53	P2159	1861		*Parthenium argentatum*	USA	1966
54	P2160	1849		*Parthenium argentatum*	USA	1966
55	P2161	2513		*Musa paradisiaca* var. dominico	Colombia	1973
56	P2162	2512		*Musa paradisiaca* var. dominico	Colombia	1973
57	P2164	2477		*Musa paradisiaca*	Jamaica	1972
58	P2167	2348	ICPB EC243	*Zea mays*	Italy	1971
59	P2168	2340		*Chrysanthemum morifolium*	UK	1970
60	P2170	2309	ICPB EC240	*Chrysanthemum morifolium*	Italy	1969
61	P2172	2549	ICPB ER108	*Triticum aestivum*	France	1973
62	P2173	2547		*Zea mays*	India	1973

TABLE 2 (CONTINUED): *DICKEYA* ISOLATES USED IN THIS STUDY

	CSL No.	**NCPPB No.**	**Other Code**	**Host**	**Country**	Year
63	P2175	2545		*Zea mays*	India	1973
64	P2176	2542		*Zea mays*	India	1973
65	P2180	2536		*Dianthus caryophyllus*	UK	1973
66	P2181	3144	ICMP 7721	*Zea mays*	Brazil	1981
67	P2182	3138	ICMP 7475	*Dianthus caryophyllus*	Greece	1980
68	P2183	3090		*Oryza sativa*	Japan	1979
69	P2184	3065		Potato	Brazil	1978
70	P2185	2990	A 834	*Dieffenbachia exotica*	UK	1977
71	P2187	2988	A815a	*Hyacinthus* sp.	Netherlands	1977
72	P2188	2924		*Musa* sp.	Panama	1977
73	P2189	2915		*Musa* sp.	Panama	1977
74	P2190	2872		Potato	Netherlands	1976
75	P2191	3478		*Musa* sp	PNG	1986
76	P2193	3477		Potato	PNG	1986
77	P2196	3346		Potato	Australia	1984
78	P2198	3344	CFBP 1888	Potato	France	1984
79	P2199	3211		Orchid sp.	Sri Lanka	1982
80	P2200	3193		*Brassica chinensis*	Malaysia	1982
81	P2201	3538	A3590	Potato	UK (Jersey)	1987
82	P2202	3537	IPO 598	Potato	Peru	1987
83	P2203	3536	IPO 754	Potato	Peru	1987
84	P2204	3535	IPO 730	Potato	Finland	1987
85	P2205	3534	IPO 713	Potato	Netherlands	1987
86	P2207	3532	IPO 646	Potato	Australia	1987
87	P2208	3531	IPO 645	Potato	Australia	1987
88	P2210	3528		Potato	UK (Jersey)	1987
89	P2214	3779	A5098/M2/2	Potato cv. Marfona	UK	1991
90	P2215	3731	CFBP 1596	*Zea mays*	France	1990
91	P2216	3730	CFBP 1805	*Kalanchoe blossfeldiana*	Denmark	1990
92	P2217	3729	CFBP 2982	*Kalanchoe blossfeldiana*	France	1990
93	P2247		PD 1406	Potato cv. Elkana	Netherlands	1989
94	P2248		PD 1022	Potato cv. Astarte	Netherlands	1987
95	P2250		PD 1325	*Kalanchoe* 'Tropicana'	Netherlands	1989
96	P2251		PD 1343	*Kalanchoe* sp.	Netherlands	1989
97	P2269		PD 1405	Potato cv. Bintje	Netherlands	1989
98	P2271		PD 806	*Cichorium intybus*	Netherlands	1986
99	P2272		PD 1077	Potato	Bangladesh	1988
100	P2274		PD 720	*Kalanchoe* sp.	Netherlands	1986
101	P2275		PD 482	Potato cv. Ostara	Netherlands	1984
102	P2277		PD 1619	*Achmea fasciata*	Netherlands	1989
103	P2278		PD 1086	*Ctenanthe lubbersii*	S. Africa	1988
104	P2279		PD 1401	Potato	Netherlands	1989
105	P2280		PD 721	*Kalanchoe* sp.	Netherlands	1986
106	P2284		PD 1750	*Yucca*	Netherlands	1990
107	P2285		PD 1713	*Euphorbia milii*	Netherlands	1990
108	P2286		PD 471	*Philodendron reubescens* 'Emerald King'	Netherlands	1984
109	P2287		PD 916	*Packera* sp.	Taiwan	1987
110	P2455			Potato cv. Carlingford	UK	1997
111	P2629		CRM44C	Cucumber	UK	1997
112	P3166			Potato cv. Lay Rosetta	NL	1998
113	P2455			Potato cv. Carlingford	UK	1997
114	P2629		CRM44C	Cucumber	UK	1997
115	P3166			Potato cv. Lay Rosetta	NL	1998
116			20708100	*Dahlia* stem	UK	2007
117			20708100	*Dahlia* petiole	UK	2007
118			20710970	Potato	Netherlands	2007
119			20711304	Potato	UK	2007
120			20711432	Potato	UK	2007
121			20711537	Potato	UK	2007
122			20711883	Potato	UK	2007
123			20714263	*Phalaenopsis*	Taiwan	2007
124			20714521	*Sedum*	UK	2007
125	P6983	3139	ICMP 7476	*Dianthus caryophyllus*	Greece	1980

TABLE 2 (CONTINUED): *DICKEYA* ISOLATES USED IN THIS STUDY

	CSL No.	**NCPPB No.**	**Other Code**	**Host**	**Country**	Year
126	P6962	1120	ICPB EC215	*Ananas comosus*	Malaysia	1962
127	P6963	1121	ICPB EC216	*Ananas comosus*	Malaysia	1962
128	P6964	1125	ICPB EC217 ATCC 43763	*Ananas comosus*	Malaysia	1962
129	P6961	1066		*Zea mays*	Egypt	1961
130	P6965	1157		*Dieffenbachia picta*	USA	1961
131	P6966	1515		*Dieffenbachia picta*	DE	1963
132	P6967	1516		*Dieffenbachia picta*	DE	1963
133	P6968	1609		*Dahlia* sp.	Netherlands	1964
134	P6969	1956		*Dahlia* sp.	Netherlands	1964
135	P6970	2149		*Euphorbia pulcherrima*	UK	1968
136	P6971	2347		*Zea mays*	Italy	1971
137	P6972	2351		*Syngonium podophyllum*	USA	1971
138	P6973	2476		*Zea mays*	Malaysia	1972
139	P6974	2540		*Zea mays*	USA	1973
140	P6975	2543		*Zea mays*	India	1973
141	P6976	2544		*Zea mays*	India	1973
142	P6977	2881		*Dieffenbachia picta*	UK	1976
143	P6978	2899		*Daucus carota*	USA	1976
144	P6979	2929		*Colocasia esculenta*	Solomon Is.	1977
145	P6980	2948		*Philodendron* sp.	UK	1977
146	P6981	2957		*Ipomoea batatas* cv. Georgia jet	USA	1977
147	P6982	2958		*Ipomoea batatas* cv. Red Jewel.	USA	1977
148	P6984	3273		*Philodendron* sp.	UK	1983
149	P6985	3274		*Aglaonema* sp	St Lucia	1983
150	P6986	3306		*Polyscias filicifolia*	UK	1984
151	P6987	3459		*Dieffenbachia picta*	Hungary	1986
152	P6988	3476		*Ipomoea batatas*	PNG	1986
153	P6989	3882		*Dianthus* sp. - Calnet Joy	UK	1993
154	P6990	4097		*Euphorbia pulcherrima* (Poinsettia).	Denmark	2000
155	P6991	4305		?	UK	2002
156	P6952	426		*Dianthus caryophyllus*	UK	1957
157	P6953	429		*Dianthus caryophyllus*	UK	1957
158	P6954	430		*Dianthus caryophyllus*	UK	1958
159	P6955	452		*Dianthus caryophyllus*	UK	1957
160	P6956	551		*Ananas comosus*	Malaysia	1958
161	P6957	912		*Dieffenbachia picta.*	USA	1961
162	P6958	913		*Dieffenbachia picta*	USA	1961
163	P6959	914		*Dieffenbachia picta*	USA	1961
164	P6960	915		*Dieffenbachia picta*	USA	1961
165	P2131	569	PD 870	*Saccharum officinarum*	Australia	1958
166	P2133	516 (T)	CFBP 1270 ICMP 1547	*Parthenium*	DK	1957
167	P2134	453 (T)	PD 718 ICMP 6427 CFBP 1200 LMG 2485	*Dianthus caryophyllus*	UK	1956
168	P2136	402 (T)	PD 587 ATCC11663 ICMP 5703	*Chrysanthemum morifolium*	USA	1956
169	P2137	394		*Chrysanthemum morifolium*	USA	1957
170	P2145	1385	PD 858	*Dahlia*	Romania	1962
171	P2149	898 (T)	PD 859	*Pelargonium capitatum*	Comoro Is.	1961
172	P2152	2302	PD 857	Pelargonium	UK	1969
173	P2156	2028		*Chrysanthemum morifolium*	UK	1967
174	P2163	2511	ATCC 33242 ICMP 2349 CFBP 2811 LMG 2545 PD 865	*Musa paradisiaca* var. dominico	Colombia	1973
175	P2165	2454		*Dieffenbachia* sp.	UK	1972
176	P2166	2421	PD 823	*Begonia bertinii*	NL	1971
177	P2177	2541	CFBP 1528	*Zea mays*	USA	1973
178	P2178	2539		*Zea mays*	USA	1973
179	P2179	2538 (T)	PD 688, ICMP 5704	*Zea mays*	USA	1973
180	P2206	3533	IPO 655	Potato		1987
181	P2209	3530		Potato	Jersey	1987
182	P2211	3881	A4713/14	Potato cv. Sante	UK	1993
183	P2220	3710	A4674	Potato cv. Sante	UK	1990

TABLE 1 (CONTINUED): *DICKEYA* ISOLATES USED IN THIS STUDY

	CSL No.	**NCPPB No.**	**Other Code**	**Host**	**Country**	Year
184	P2244		PD 552	*Scindapsus pictus*	NL	1985
185	P2252		PD 787	*Cichorium intybus*	NL	1986
186	P2268		PD 753	*Eryngium alpinum*	NL	1986
187	P2270		PD 382	*Aechmea fasciata*	NL	1982
188	P2282		PD 1461	*Freesia* 'Oberon'	NL	1989
189	P2283		PD 1084	*Ctenanthe lubbersii*	SA	1988
190	P2288		PD 168	*Gymnocalicium mihanovichii* 'Red'	NL	1979
191		454		*Philodendron*	USA	1957
192		1851		*Zea mays*	USA	1966
193			10504/1	Potato cv. Markies	UK	2007
194			10504/2	Potato cv. Markies	UK	2007
195			Ech 1991	Potato	Israel	2007
196			G-87	Potato	Israel	2007
197			G-115	Potato	Israel	2007
198			G-118	Potato	Israel	2007
199			G-120	Potato	Israel	2007
200			G-121	Potato	Israel	2007
201			G-122	Potato	Israel	2007
202			G-169 9	Potato	Israel	2007
203			Imp.1	Potato	Israel	2007
204			Imp.23.1	Potato	Israel	2007
205			Imp.24	Potato	Israel	2007
206			Pc 3	Potato	Israel	2007
207			Pc 26.4	Potato	Israel	2007
208			Pc 36.1	Potato	Israel	2007
209			Pc 41.1	Potato	Israel	2007
210	P2169	2339		*Chrysanthemum morifolium*	UK	1970
211			20708100	*Dahlia*	UK	2007
212			20711307	Potato	UK	2007
213			20712027*	Potato	UK	2007
214			20712027	Potato	UK	2007
215			20713326	Potato	UK	2007
216			20714261	*Phalaenopsis*	Taiwan	2007
217	P2147	1065		*Zea mays*	Egypt	1961
218	P2140	179		*Gypsophila*	USA	1946
219	P2197	3345		Potato	France	1984

TABLE 3: REFERENCE ISOLATES USED IN THIS STUDY

	Pathogen	**CSL No.**	**Other Codes**	**Host**	**Country**	**Year**
220	*Dickeya chrysanthemi* bv. *chrysanthemi* (T)	P2136	NCPPB 402 CFBP 2048 LMG 2804 Z96093	*Chrysanthemum morifolium*	USA	1958
221	*Dickeya chrysanthemi* bv. *parthenii* (T)	P2133	NCPPB 516 CFBP 1270, ICMP 1547	*Parthenium argentatum*	Denmark	1957
222	*Dickeya dadantii* (T)	P2149	NCPPB 898 CFBP 1269 AF520707	*Pelargonium capitatum,*	Comoros Is	1960
223	*Dickeya dianthicola* (T)	P2134	NCPPB 453 CFBP 1200 AF520708 PD 718 ICMP 6427 LMG 2485	*Dianthus caryophyllus*	UK	1956
224	*Dickeya dieffenbachiae* (T)		NCPPB 2976 CFBP 2051 AF520712	*Dieffenbachia* sp.,	USA	1957
225	*Dickeya paradisiaca* (T)		LMG 2542 Z96096 CFBP 4178 (NCPPB 2511)	*Musa paradisiaca*	Colombia	1970
226	*Dickeya paradisiaca*	P2162	NCPPB 2512 CFBP 3477 AF520710 ICMP 2349 LMG 2545 LMG 6250	*Musa paradisiaca*	Colombia	1973
227	*Dickeya paradisiaca*	P2163	NCPPB 2511 CFBP 2811	*Musa paradisiaca*	Colombia	1973
228	*Dickeya zeae* (T)	P2179	NCPPB 2538 PD 688 ICMP 5704 CFBP 2052 LMG 2505 AF520711.2	*Zea mays*	USA	1973
229	*Enterobacter cancerogenus* (T)		LMG 2693 Z96078	*Populus canadensis* var. regenerata		1969
230	*Pectobacterium atrosepticum*		BBA 9201 AF373180	Potato	Germany	
231	*Pectobacterium carotovorum subsp. carotovotum*		E161 AF373189	Potato	Canada	
232	*Pectobacterium carotovorum* subsp. *brasiliensis*		8 AY207086	Potato	Brazil	2001
233	*Pectobacterium carotovorum subsp. wasabiae* (T)		ICMP 9121 AJ223408	Horseradish	Japan	1987
234	*Pectobacterium carotovorum* subsp. *odoriferum* (T)	P2213	NCPPB 3839 CFBP 1878, ICMP 11533	*Cichorium intybus*	France	1992
235	*Pectobacterium rhapontici* (T)	P2142	NCPPB 1578 ICPB ER102 ATCC 29283 ICMP 1582	*Rheum rhaponticum*	UK	1963
236	*Pectobacterium carotovorum* subsp. *betavasculorum* (T)	P2171	NCPPB 2795 ATCC 43762 CFBP 1539 ICMP 4226	*Beta vulgaris*	USA	1975
237	*Erwinia amylovora*	P2150	NCPPB 683 ATCC 15580 CCM 1114 ICMP 1540 CFBP 1232 LMG 2024	*Pyrus communis*	UK	1959

Assessment of current diagnostic methods for detection, identification and enumeration of *Dickeya* spp.

Reference isolates, representative of the 6 *Dickeya* spp. (Table 3) were used to assess the efficiency of isolation and cavity formation on CVP medium compared with a modified double layer CVPM medium. The CVPM medium was prepared as follows:

CVPM basal layer

Peptone from Casein (Roth Co. No. 89862)	8.00g
Yeast Extract (DIFCO No. 0127-01)	2.40g
Sodium Chloride (Fisher No. 7647-14-5)	4.00g
Sodium Dodecyl Sulphate (BDH 1086735)	0.20g
L Asparagine (MERCK No. 1.00126)	2.00g
Tryptone (DIFCO No. 0123-17-3)	6.00g
Agar (Bacto)	12.00g
Distilled water	1 Litre

CVPM overlayer

Crystal Violet Solution 0.075% (w/v) SIGMA C3886	2.00 ml
Calcium chloride $2H_2O$ 10% solution freshly prepared	13 ml
Sodium Nitrate	2.00g
Tri sodium citrate dehydrated	5.00g
Agar (Bacto)	4.00g
Sodium polypectate (x 914-02 CP Kelco)	15.00g
Distilled water	1 Litre

Ingredients for the basal layer were mixed and dissolved at 80 ºC. The pH was adjusted to 7.2 by addition of 1M NaOH prior to autoclaving for 15 min at 121 ºC. Plates were poured when the autoclaved medium had cooled to 50 ºC. For the overlayer all ingredients were dissolved at 80 ºC except for the sodium polypectate which was then added gradually while whisking. The pH was adjusted to 7.2 by addition of 1M NaOH prior to autoclaving for 15 minutes at 121 ºC. The overlayer was poured, before cooling below 50 ºC, over the set basal layer. The media was stored at 4 ºC for up to 1 month before use. The plates were dried before use by ventilating in a laminar flow cabinet.

All isolates in Tables 1 and 2 were also tested by real-time PCR assays developed at CSL (recently validated through Potato Council-funded project R253 "Improving decision-making for the management of potato diseases using real-time predictive diagnostics") to ensure specificity for detection of *Dickeya* spp.

Efforts were also made to identify variation in 16S rRNA and *recA* gene sequences between the 6 *Dickeya* spp. for exploitation in development of species-specific PCR assays. Sequence data was aligned using ClustalW software and examined for exploitable variation using Primer Express software.

Identification and frequency of occurrence of *Dickeya* spp. on seed and ware potatoes entering and grown in England and Wales

Real time PCR assays were used to screen for presence of the pathogen in seed potato samples submitted to CSL as part of the annual ring rot and brown rot surveys. Only those samples from which permission had been received from the person registering the crop were used in this study. Of 1342 potato samples received at CSL between 1st April 2007 and 31st March 2008, permission was received to test 258 samples (19%) for the presence or absence of *Dickeya* spp. Although 71% of samples were submitted anonymously, 76% of those which were identified were produced from UK grown seed, 6% were grown from imported seed entering the seed potato classification scheme and the remaining 3% were grown from imported seed.

Testing was performed on randomly selected samples, each containing 200 tubers. A core containing vascular tissue was removed from the stolon end of each tuber and shaken in phosphate buffer for at least one hour at room temperature or overnight with refrigeration. Bacteria in the supernatant were then concentrated by centrifugation and resuspended in 1 ml of PB. DNA was then extracted from 100 µl aliquots using automated magnetic separation with a Wizard® Magnetic DNA purification system for food kit (Promega).

Real time PCR was performed using *Dickeya*-specific primers and probe (Table 4). The same extracts were also tested for total pectolytic bacteria (*Pectobacterium* and *Dickeya* spp.) as well as for potato cytochrome oxidase sequence (as positive control for the DNA extraction procedure and each PCR reaction). For each reaction, the PCR buffer contained 0.1% $MgCl_2$, 200 µM d-nTP mix, 300 nM primers, 100nM probe, and 0.63U DNA polymerase (AmpliTaq® Gold). Reaction conditions were a denaturation phase of 10 min at 95 ºC and 40 PCR cycles of 15 sec at 95 ºC followed by 60 sec at 60 ºC. PCR reactions were performed in an ABI 7900 sequence detector TaqMan system.

TABLE 4: PRIMERS AND PROBES FOR REAL TIME PCR USED IN THIS STUDY.

	Sequences
Primers	
ECH-1F	5' GAG TCA AAA GCG TCT TGC GAA 3'
ECH-1R	5' CCC TGT TAC CGC CGT GAA 3'
PEC-1F	5' GTG CAA GCG TTA ATC GGA ATG 3'
PEC-1R	5' CTC TAC AAG ACT CTA GCC TGT CAG TTT T 3'
COX-F	5'- CGT CGC ATT CCA GAT TAT CCA -3'
COX-R	5'- CAA CTA CGG ATA TAT AAG AGC CAA AAC TG -3'
Probes	
ECH	5'-[JOE]- CTG ACA AGT GAT GTC CCC TTC GTC TAG AGG -[TAMRA]-3'
PEC	5'-[FAM]- CTG GGC GTA AAG CGC ACG CA -[TAMRA]-3'
COX-P	5'-[VIC]- TGC TTA CGC TGG ATG GAA TGC CCT -[TAMRA]-3'

Identification and frequency of occurrence of *Dickeya* spp. in watercourses in England and Wales

Water samples submitted to CSL for official brown rot surveys in June and September were tested for the presence of *Dickeya* spp. using selective isolation on CVPM medium. Bacterial extracts from pectolytic colonies were then screened using real-time PCR assays.

Water samples (500 ml each) were collected on 4 occasions from 36 sampling points on 7 different watercourses in June 2007. In September, samples were collected on up to 4 occasions from a further 127 sampling points on 48 watercourses. The watercourses sampled were either (a) designated as contaminated with *Ralstonia solanacearum* (Fossdyke Navigation, Pig Water, Stanground Lode, River Teifi, River Culm and River Avon), (b) a recently de-designated watercourse (River Witham) or (c) watercourses sampled as part of a national survey in Central Southern and South East England.

Results

Phylogeny of *Erwinia chrysanthemi* isolates

Phylogenetic analysis, based on the limited sequence variation found within the 715 nucleotide portion of the 16S rRNA gene, discriminated clades containing *D. chrysanthemi* and *D. paradisiaca* type strains, but not the other species type strains (Fig. 1). Furthermore, some *Dickeya* isolates were not clearly differentiated from those of *Pectobacterium atrosepticum* or *carotovorum* on the basis of partial 16S rRNA sequence variation. There was therefore insufficient sequence variation in this portion of the 16S rRNA gene for reliable identification of unknown isolates to species or even genus.

In comparison, greater sequence variation (82-94%) observed, within the 481-nucleotide *recA* locus, between *Dickeya* species type strains, provided a much stronger phylogenetic signal to indicate relatedness between the 219 isolates studied (Fig. 2). Isolates belonging to the genus *Dickeya* were found to be distinct from the *Erwinia amylovora* type strain used to root the phylogeny, as well as isolates of *Enterobacter* and *Pectobacterium* species. New information on the degree of phylogenetic relatedness between the type species of the genus *Dickeya* and the 219 isolates formerly classified as *E. chrysanthemi* could then be obtained.

The type strains of *D. paradisiaca*, *D. zeae* and *D. chrysanthemi* grouped into distinct clades, clearly differentiated with long branches. Bootstrap analysis clearly supported the separation of these clades as distinct species. None of the other strains sequenced grouped with the *D. paradisiaca* type strain from banana. The *D. zeae* clade contained all the maize strains and split into two well-defined sub-clades that have not been previously identified. Both clades had a high proportion of sequence variants from strains isolated from a variety of hosts. Of the six *Chrysanthemum* strains five grouped in a clade with the *D. chrysanthemi* type strain, and shared close sequence similarity with each other, as well as to strains from other ornamentals. Potato, tomato and *Parthenium* strains made up the remainder of the clade.

The other type strains of *D. dianthicola, D. dieffenbachiae* and *D. dadantii,* although clearly distinct from the other three species, were found to be more closely related to each other. Although these species type strains each grouped into well-defined clades they were distinguished only by relatively small branch lengths, of similar size and supported by a range of bootstrap values. These grouped into a well-defined single group supported with a bootstrap value of 100%. Within this large clade, a significant sub-clade structure was recognized to comprise six major groups, three of which contained the species type strains.

The clade containing the *D. dianthicola* type strain was well defined with minimal or no sequence diversity and supported with a bootstrap value of 100%. This clade comprised isolates from potato, chicory, *Dianthus, Kalanchoe* and some other ornamentals (including *Dahlia* and *Begonia*).

The *D. dieffenbachiae* containing clade was also well defined with a supporting bootstrap value of 88%. The type strain grouped in a sub-clade exclusively comprising *Dieffenbachia* isolates. Another sub–clade comprises mainly *Dieffenbachia* and *Philodendron* isolates. A third sub-clade has greater sequence diversity and comprises strains from a wide variety of hosts, including strains previously classified as *D. dadantii* (Samson *et al*., 2005).

A distinct sub-clade (with a bootstrap value of only 58%), identified within the *D. dieffenbachiae* clade, comprises isolates from potato grown in Israel all with identical *recA* sequences. This same level of relatedness was also determined using a further two algorithms for distance estimation. Since the level of bootstrap support is relatively low there is a possibility that its assignment to the closely related species within the complex may not be stable. To account for this possibility this clade has been identified as *D. dieffenbachiae* species complex intermediate clade 1 (DSIC-1).

A second well-defined clade was identified which grouped apart from the existing species in the complex with a low bootstrap value. Five of the six constituent strains were from monocot hosts. To indicate the intermediate status of this clade within the species complex, it is referred to as DSCI-2. The low bootstrap values that distinguish these clades, prevent their elevation to species level.

The type strain of *D. dadantii*, was found to be the sole representative of a branch, which was distinguished from other species within the complex with only weak bootstrap support. Its *recA* sequence differed from the other type strains by at least 6%.

The recA sequence for one strain (P2131), from sugar cane, was sufficiently distinct to be considered as a new species-level clade within the *Dickeya* genus.

FIG. 1: PHYLOGENY OF *DICKEYA* ISOLATES BASED ON PARTIAL 16S rRNA GENE SEQUENCE

FIG. 2: PHYLOGENY OF *DICKEYA* ISOLATES BASED ON PARTIAL *RECA* GENE SEQUENCE

Distribution and host range of the *Dickeya* isolates studied

Phylogenetic characterization of the isolates formerly identified as *Erwinia chrysanthemi* has resulted in a clearer indication of the distribution and host range of the various *Dickeya* species around Europe and worldwide. Figs. 3 and 4 show the continents and countries of Europe where each *Dickeya* spp. has been isolated. Table 5 shows the host range and countries of isolation for the *Dickeya* isolates studied here and by Samson *et al*. (2005).

Four of the six named species are found in Europe whereas the *D. dadantii* strain appears to be unique to *Pelargonium* from the Comoros Islands near Madagascar and *D. paradisiaca* has been found only on banana in Colombia, Cuba and Jamaica.

With the exception of single isolates of *Dickeya dianthicola* from *Chrysanthemum* in the USA and potato in Bangladesh, all other *D. dianthicola* isolates studied were found in Europe. The isolate from Bangladesh likely originated on seed potato produced in Europe. All other potato isolates of *D. dianthicola* studied were found in Europe. Furthermore, amongst the isolates studied, none of the other known *Dickeya* spp. were found on potato in Europe, although a unique *Dickeya* strain (designated DSIC-1 in Fig. 2) has also been isolated from potato in Israel and the Netherlands. All UK potato isolates tested were identified as *D. dianthicola*. Other diverse hosts of *D. dianthicola* were confirmed as *Dianthus*, *Dahlia*, *Kalanchoe*, *Begonia,* tomato and artichoke.

In contrast to *D. dianthicola*, the other *Dickeya* species appear to have origins in warmer regions of the world and have probably spread to Europe with trade in ornamental plant hosts. *D. dieffenbachiae* has been isolated from potato in Peru and Brazil, whereas potato isolates from Australia were found to be *D. zeae* and potato isolates from the USA were *D. chrysanthemi*.

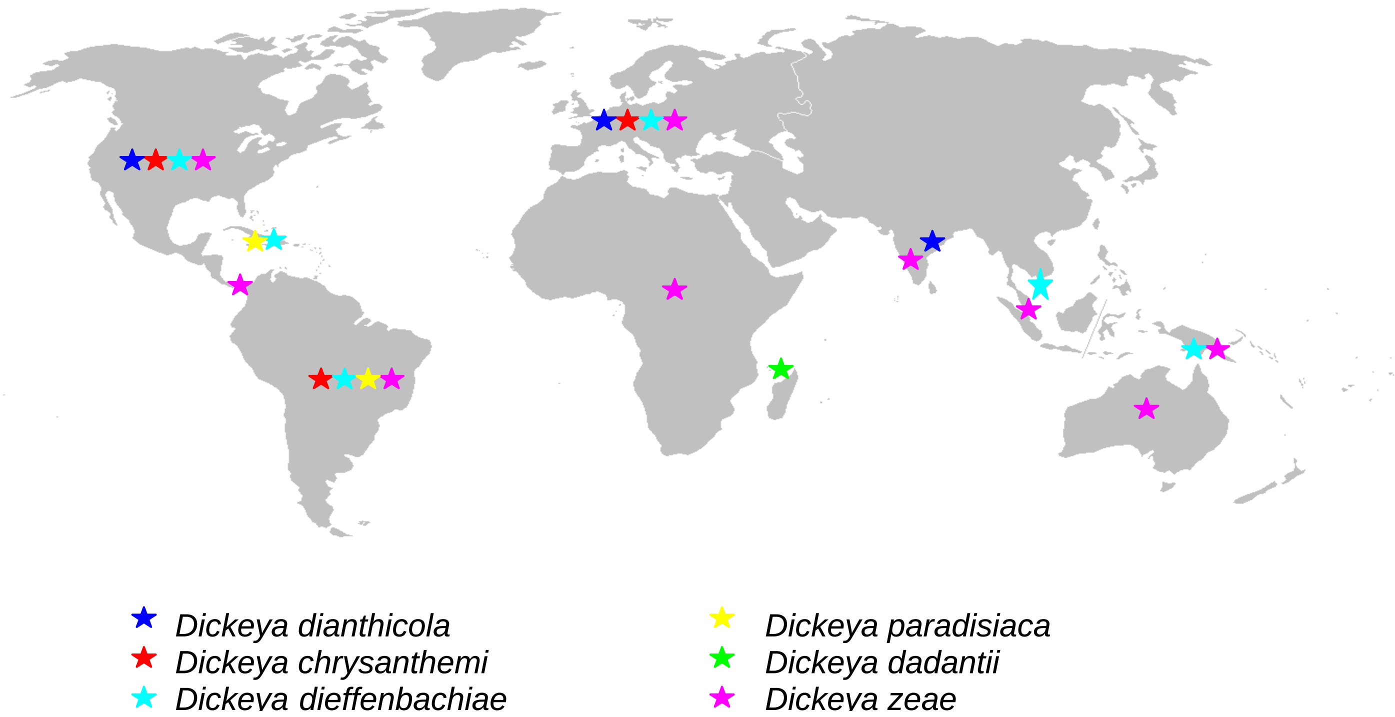

FIG. 3: WORLDWIDE DISTRIBUTION OF *DICKEYA* ISOLATES STUDIED BY CONTINENT

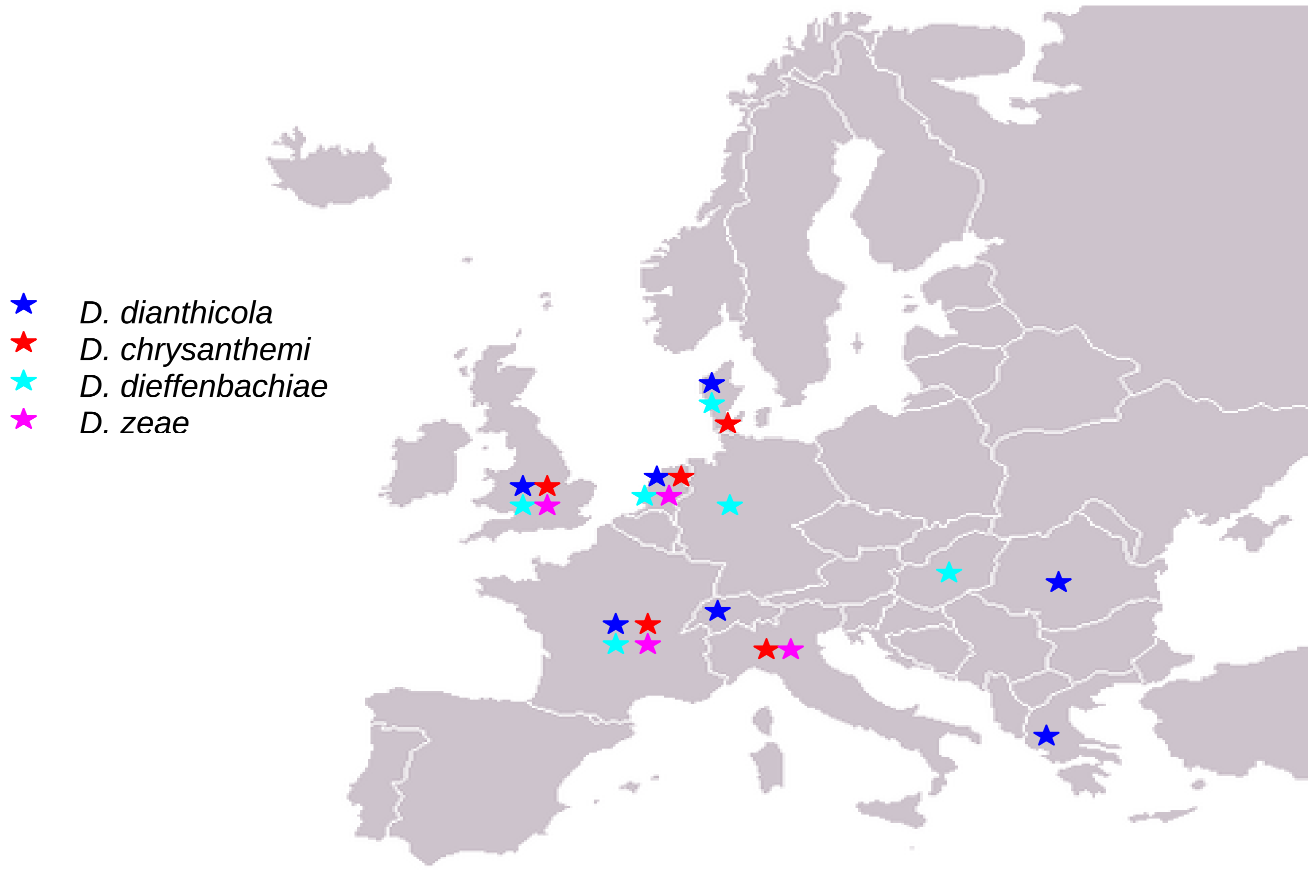

FIG 4: DISTRIBUTION IN EUROPE OF *DICKEYA* ISOLATES STUDIED

TABLE 5: HOST RANGE OF *DICKEYA* SPP. STUDIED

***Dickeya* species**	**Host**	**Country of isolation**
Dickeya dianthicola	**Potato**	UK, Netherlands, France, Switzerland, Bangladesh
	Tomato	France
	Dianthus	UK, Netherlands
	Dahlia	UK, Netherlands, France, Romania
	Kalanchoe	UK, Netherlands, Denmark, France, Switzerland
		Netherlands, France, Switzerland
	Chicory	Netherlands
	Begonia	France
	Artichoke	USA
	Chrysanthemum	
Dickeya dieffenbachiae	*Dieffenbachiae*	UK, USA, France, Germany, Netherlands, Hungary
	Philodendron	UK, USA, Netherlands
	Ipomoea	Papua New Guniea, USA,
	Potato	Brazil, Peru
	Tomato	Cuba
	Eryngium	Netherlands
	Gymnocalicium	Netherlands
	Banana	Jamaica, Cuba
	Euphorbia	Netherlands, Denmark
	Packera	Taiwan
Intermediate clade 1	**Potato**	Israel, Netherlands
Intermediate clade 2	*Yucca*	Netherlands
	Banana	Panama
	Polyscias	UK
	Orchid	Sri Lanka
	Colocasi	Solomon Islands
	Phalaenopsis	Netherlands
Dickeya dadantii	*Pelargonium*	Comoros Islands
Dickeya chrysanthemi	*Chrysanthemum*	UK, USA, Italy
	Potato	USA
	Tomato	Brazil
	Carrot	USA
	Parthenium	Denmark, USA
	Kalanchoe	Netherlands
	Chicory	Netherlands
	Euphorbia	UK
	Helianthus	France
Dickeya paradisiaca	Banana	Columbia, Cuba
	Maize	Cuba
Dickeya zeae	Maize	Italy, France, USA, Panama, Egypt, India, Zimbabwe
	Wheat	France
	Potato	Australia, Papua New Guinea
	Brassica	Malaysia
	Pineapple	Malaysia
	Banana	Panama, Papua New Guinea
	Ctenanthe	South Africa
	Chrysanthemum	UK
	Achmea	Netherlands
	Dieffenbachiae	Puerto Rico
Dickeya sp.	Sugar cane	Australia

Detection and frequency of occurrence of *Dickeya* spp. on seed and ware potatoes entering and grown in England and Wales

Only 3 (1.2%) of the 258 tuber samples tested positive for *Dickeya* spp. Two of these samples, were grown from imported seed and came from the same place of production in Pembrokeshire. The third sample was grown from homegrown seed in Suffolk. In contrast, *Pectobacterium* sp. was detected in 176 (68%) of the 258 samples. This high frequency of *Pectobacterium* contamination was expected given the known ubiquitous nature of *Pectobacterium carotovorum* pv. *carotovorum*.

Detection and frequency of occurrence of *Dickeya* spp. in watercourses in England and Wales

None of the 136 water samples from the 8 watercourses sampled in June 2007 tested positive for *Dickeya* spp. although *Pectobacterium* spp. were detected in 98.4% of the samples (Table 6).

TABLE 6: DETECTION OF *DICKEYA* SPP. IN RIVER WATER SAMPLES IN JUNE 2007

Watercourse	No samples tested	% samples with pectolytic colonies on CVP	% samples containing *Dickeya* sp.
River Witham	4	100	0
Pig Water	24	100	0
Stanground Lode	12	100	0
Fossdyke Navigation	4	100	0
River Culm	4	100	0
River Teifi	12	67	0
River Avon	4	0	-
River Idle	72	100	0
Total:	136	98.4	0

In September 2007, 27 (6.5%) of 417 river water samples tested positive for *Dickeya* spp. (Table 7). Positive samples came from 17 (35.4%) of the 48 watercourses sampled but were rarely found at the same sampling point on more than one occasion, indicating transient rather than established populations. *Pectobacterium* sp. were detected in 394 (94.5%) of the samples. *Solanum dulcamara* was not found at the sampling sites on watercourses where *Dickeya* spp. were detected in river water. Artificial inoculation of *S. dulcamara* seedlings with *D. dianthicola* isolates under glasshouse conditions resulted in infection of the plants with slight wilting and necrosis of the vascular tissues in the base of the stem.

TABLE 7: DETECTION OF *DICKEYA* SPP. IN RIVER WATER SAMPLES IN SEPTEMBER 2007

Watercourse and sampling point	OS grid ref.	Week 1	Week 2	Week 3	Week 4
Pig Water					
Yaxley	TL 192923	-	-	-	-
Horsey Toll	TL 222963	+	+	-	NT
Horsey Toll (under bridge)	TL 222964	-	-	+	-
River Witham					
Works outfall, Lincoln	SK 990710	-	-	-	-
Downstream of Canwick sewage works	TF 020709	-	-	-	-
Five Mile Bridge	TF 059715	+	-	-	-
Bardney Lock	TF 104700	-	-	NT	-
Kirkstead Bridge at Martin Dales	TF 176621	-	-	NT	-
Tattershall Bridge	TF 196563	+	-	NT	-
Fossdyke Navigation					
Landing Stage east of Torksey Lock	SK 839780	+	-	-	-
Saxilby	SK 894752	+	+	-	NT
Bridge on B1273, Lincoln	SK 969714	+	-	-	-
Stanground Lode					
Crown Lakes, at outfall to drain feeding into Lode	TL 196945	-	NT	NT	-
At A1139	TL 198959	-	-	-	-
At junction with Back River	TL 201977	-	-	-	-
River Teifi					
Between Cellan and _lanfair Clydogau	SN 613498	-	-	-	NT
Stream (Dulas) by Co-op in Lampeter – Bridge	SN 581477	-	-	-	NT
River Teifi at Co-op in Lampeter – Bridge	SN 580478	-	-	-	NT
Dulas brook off A485 (past Organic Farm Foods) – Bridge	SN 579494	-	-	-	NT
South side of river off A485 by old railway line (near fishermans car park) – wall	SN549459	-	-	-	NT
Measycrugiau – bridge	SN473413	-	-	-	NT
Llanybydder – Bridge	SN 519344	+	-	-	NT
Cefn Bryn on A485 between Llanybydder and Lampeter – Shingle Bank	SN 559463	-	-	-	NT
Sewage outfall at Lampeter – Wall	SN 576473	-	-	-	NT
Pipe footbridge – Bridge	SN 569471	-	-	-	NT
River Avon					
Barford Bridge	SP 268609	-	+	-	-
Confluence of Dene and Avon	SP 259563	-	-	-	-
Bomfords abstraction point	SP 248562	-	-	-	NT
Alveston Village	SP 239562	-	-	-	-
Tiddington Village	SP 221561	-	-	-	-
Stratford Bridge	SP 201540	-	-	-	-
Sopley	SZ 150977	-	-	NT	-
Bicton	SU 148126	-	-	NT	-

TABLE 7 (CONTINUED): DETECTION OF *DICKEYA* SPP. IN RIVER WATER SAMPLES IN SEPTEMBER 2007

Watercourse and sampling point	OS grid ref.	Week 1	Week 2	Week 3	Week 4
Lymington River					
Boldre	SZ 307940	-	-	NT	-
Brokenhurst	SU 302029	-	NT	NT	-
Avon Water					
Lymington	SZ 307940	-	-	-	-
Sway	SZ 277974	-	NT	-	-
River Test					
Kimbridge	SU 329255	-	-	-	-
Longparish	SU 429440	-	-	-	-
River Anton					
Cottenworth	SU 378394	-	-	-	-
Monxton	SU 312444	-	-	-	-
River Itchen					
Otterbourne	SU 467224	-	-	-	NT
New Alresford	SU 574325	-	-	-	-
River Meon					
Wickham	SU 574115	-	-	-	-
Exton	SU 612208	-	-	-	-
Hogwell Sewer					
Near Brook Farm	TR220680	-	-	-	NT
Reculver Seweage Works	TR229693	-	NT	NT	NT
North Stream					
Opposite Little Grays Farm	TQ228675	-	-	NT	NT
River Great Stour					
Grove Ferry	TR238632	-	-	-	NT
Shalmsford St Bridge	TR089549	-	-	-	NT
Manhood Peninsula					
River Ems Emsworth	SU749066	-	-	-	-
Ham Brook Nutbourne	SU775058	-	-	-	-
Bosham Stream Broadbridge	SU809058	-	-	-	-
Stream Fishbourne	SU836049	-	-	-	-
Canal Whophams Lane	SU840017	NT	-	+	-
Aldingbourne Rife Bersted Park	SU927402	-	-	-	-
Lydsey Rife College	SU977003	-	-	-	-
Stream East Wittering	SZ801973	-	-	-	-
Keynor Rife Norton	SZ848967	-	NT	NT	-
Broad Rife Norton	SZ848954	NT	-	NT	-
Bremere Rife Pagham Harbour	SZ881299	NT	-	NT	-
Pagham Rife Pagham Road	SZ881992	-	+	+	-
Ford Drain Marina	TQ000439	-	-	NT	-
Ryebank Rife Acton	TQ016402	NT	-	-	-
Black Ditch Lyminster	TQ024488	NT	-	-	-
Ferring Rife Kingston Gorse	TQ090020	-	-	-	-

TABLE 7 (CONTINUED): DETECTION OF *DICKEYA* SPP. IN RIVER WATER SAMPLES IN SEPTEMBER 2007

Watercourse and sampling point	OS grid ref.	Week 1	Week 2	Week 3	Week 4
River Lavant					
Appledram	SU843042	-	-	-	-
Tilmore Brook Heath	SU759240	-	-	-	-
Ashford stream Mill	SU759252	-	-	-	-
Rother River Durleigh Marsh	SU781237	-	-	-	-
Hammer River Chithurst	SU845240	+	NT	-	-
Costers Brook West Lavington	SU898215	-	-	-	-
Rother river Amersham Bridge	SU914216	-	-	-	-
Lodsworth Drain Halfway Bridge	SU930225	NT	-	-	-
Rother river Coultershaw Bridge	SU970198	-	-	-	-
Rother river Shopham Bridge	SU983189	-	-	-	-
Rother river Fittleworth Bridge	TQ008187	-	-	-	-
Chichester River Adur					
Bay Bridge	TQ163211	-	-	-	-
River Arun					
Pallingham Bridge	TQ358212	-	-	-	-
Loxwood	TQ040315	-	-	-	-
Boxal Brook Green Bridge	TQ041261	-	-	-	NT
Chilt River Wickford Bridge	TQ064180	-	-	-	-
New Bridge	TQ081331	+	-	-	-
Brockhurst Brook Brockhurst bridge	TQ071248	-	-	-	-
Stor River Hurston Place	TQ072160	-	-	-	-
Wanford Bridge	TQ081331	+	-	-	-
North River Slaughter Bridge	TQ134334	-	-	-	-
Broadbridge	TQ150305	NT	-	-	-
Black Bridge	TQ164305	-	-	-	NT
Boldings Brook Warnham Station	TQ166344	-	-	-	-
Channels Brook Holbrook	TQ188338	-	-	-	-
Wey & Arun Navigation					
Waterside, base of bridge in Tannery Lane	TQ005457	-	-	-	NT
Windle Brook					
Bridge over Windle Brook on A319 at Chobham, next to Charlotte interiors	SU972614	-	-	-	NT
Basingstoke Canal					
South towpath, under Guildford Road Bridge on A3012	SU892564	NT	NT	-	NT
The Bourne					
Dunford Bridge on A320	TQ018619	-	-	-	NT
River Wey					
Main channel at Bowers Lock where flowing	TQ012529	+	NT	+	NT
Smarts Heath Lane off A380,	SU984554	-	-	-	NT
Adur – Lewes					
Wineham Bridge.	TQ236196	-	+	-	-
Ouse – East Sussex					
Sheffield Park Bridge.	TQ 398324	+	-	-	-
Sharps Bridge.	TQ 439208	-	-	-	NT
Anchor Inn.	TQ 443160	-	-	-	-
River Uck					
Isfield Mill Bridge.	TQ508294	-	-	-	-
River Cuckmere					
Chilvers Bridge.	TQ537068	+	-	-	NT
River Brede					
Under Ferry Bridge.	TQ904179	+	-	+	NT

TABLE 7 (CONTINUED): DETECTION OF *DICKEYA* SPP. IN RIVER WATER SAMPLES IN SEPTEMBER 2007

Watercourse and sampling point	OS grid ref.	Week 1	Week 2	Week 3	Week 4
River Rother-East Sussex					
Under Bodiam Bridge	TQ783253	-	-	-	NT
River Eden					
Eden Bridge	TQ444195	-	-	-	NT
Salmans weir	TQ524875.	-	-	+	-
River Medway					
Ensfield Bridge, Penshurst.	TQ544628.	+	-	-	-
Hartlake Bridge, Hadlow	TQ624325.	-	-	NT	-
Royal Military Canal					
Appledore	TQ957291	-	+	NT	NT
Ruckinge.	TR026333	-	-	-	-
New Sewer					
Brenzett	TR008271	NT	-	NT	-
Paternoster Bridge	TR060263	NT	NT	-	NT
White Kemp Sewer					
Wheelsgate	TR024242	-	-	-	-
Sconce Bridge, Brookland	TR970252	NT	NT	NT	-
River Emmbrook					
Black Swan Sailing Club	SU785727	-	-	NT	NT
The Rye					
The Rye	TQ152576	-	-	-	NT
The Mole					
The Mole Water	TQ165552	-	NT	-	NT
Tanners Brook					
Tanners Brook Water	TQ196495	-	-	-	NT
Tillingbourne					
Tillingbourne Water	TQ084478	NT	-	-	NT
Salford stream					
Salford Stream Water	TQ267466	-	-	-	NT
Deanoak Brook					
Deanoak Brook Water	TQ244457	NT	-	-	NT
River Loddon					
Bridge on downstream side	SU780730	-	+	NT	NT
Whistley Mill (Tributary of R Emmbrook)					
Bridge on downstream side	SU793748	NT	-	NT	NT
The Cut, Paley Street					
Bridge on downstream side.	SU870762	-	-	NT	NT
The Channel, Bisham					
Bridge on upstream side	SU861857	-	-	NT	NT
White Brook, Cookham					
Bridge on upstream side	SU898842	-	-	NT	NT

Assessment of current diagnostic methods for detection, identification and enumeration of *Dickeya* spp.

Whereas some isolates of different *Dickeya* spp. failed to grow on CVP medium normally used to culture *Pectobacterium*, all isolates tested from each species grew on the modified CVPM medium and produced characteristic deep cavities within 48 hours.

Similarly, not all isolates of each *Dickeya* spp. were detected in ELISA using currently available antisera to *Erwinia chrysanthemi*. However, all of the *Dickeya* isolates studied gave a positive reaction with the Ech real-time PCR assay. None of the other isolates of *Erwinia*, *Enterobacter* or *Pectobacterium* reacted positively with this assay. Similarly, healthy extracts from potato, tomato, *Dianthus*, *Kalanchoe* and *Begonia* reacted negatively.

Alignment of 16S rRNA and recA gene sequences indicated that all sequence variation between *Dickeya* species occurred as single nucleotide mutations. No suitable sequence for species-specific PCR assay development was therefore identified.

Discussion

Identification methods for *Dickeya* (*Erwinia chrysanthemi*) strains based on natural host range (pathovars) or biochemical tests (biovars) have historically been irreproducible. Detection methods usually employed for *Pectobacterium* (*Erwinia carotovora*) have also been shown to be unreliable for all strains of *Dickeya*. New methodology, based on a modified selective CVPM medium and real time PCR, has been used in this study and found to be robust and reliable for detection of *Dickeya*. Further development will be needed to produce specific assays for individual detection and identification of the various *Dickeya* species.

The description of *Dickeya* (Samson *et al*., 2005) was constituted by transfer of *Erwinia* (*Pectobacterium*) *chrysanthemi* and *Brenneria paradisiaca*, and the description of four newly recognised species *D. dadantii*, *D. dianthicola*, *D. dieffenbachiae* and *D. zeae*. Surprisingly, no inter-species DNA/DNA homology data or, open reading frame phylogenetic analysis was provided to support the identification of these four species. Their differentiation relied heavily on phenotypic characteristics, which often do not reflect relatedness and 16S sequence data. The 16S phylogeny of species type strains was based on minimal sequence variation, (between 96% and 99.5% nucleotide differences), which is too low to reliably indicate species-level distinctiveness or relatedness. The recent phylogenetic analysis of the plant pathogenic *Enterobacteriacae* using concatenated *atpD*, *carA* and *recA* loci, (Young and Park, 2007) reported sequences only for *D. chrysanthemi* and *D. paradisiaca*. This study analysed the *recA* locus of more than 200 strains held in culture collections and is the largest and most discriminating phylogenetic analysis that has been applied to the *Dickeya* genus.

Sequence analysis of the partial *recA* gene has provided new insights into the evolutionary diversity and relatedness of species and strains previously identified as *Erwinia chrysanthemi.* This has proved a useful tool for the re-identification of archived and newly isolated strains from European and worldwide origins in the light of the reclassification of *E. chrysanthemi* into 6 distinct species of *Dickeya.* Phylogenetic analysis has shown that *D. paradisiaca*, *D. chrysanthemi*, and *D. zeae* were well discriminated, thus justifying their separation into distinct *Dickeya* species. However, previously unrecognised close relatedness, identified between *D. dadantii*, *D. dianthicola* and *D. dieffenbachiae,* places in doubt their previous classification as individual species.

Nevertheless, *D. dianthicola* was recognised as a well defined clade within this species complex with little or no *recA* sequence variation. *D. dianthicola* appears to be almost unique to Europe and the only recognised *Dickeya* species affecting potato in Europe. All potato isolates obtained in England and Wales to date have been identified as *D. dianthicola*. However, a newly recognised strain (DSIC-1), discriminated within the same species complex, was also found to be present on potato in Israel and the Netherlands. This strain has only been isolated from potato crops in Israel grown from seed imported from the Netherlands (Tsror *et al*., 2006). This strain may also be present in other countries, including Finland (J. van der Wolf and M. Pirhonen, personal communication). None of the other recognised *Dickeya* species have been isolated from potato in Europe. Elsewhere in the world, *Dickeya* species found on potato appear to vary with geographic location (*D. zeae* in Australia, *D. dieffenbachia* in South America and *D. chrysanthemi* in the USA). Further investigation is justified to determine whether *D. dianthicola* and the newly identified strain DSIC-1 are more adapted to European climates than the other species, and therefore pose a greater risk to European potato production. In particular, the critical conditions required for each species to cause disease in relation to those required by *Pectobacterium* spp. require determination to allow assessment of risks associated with expected climate change and dispersal of the various species on seed potatoes and other hosts.

Testing of selected seed potato stocks grown in England and Wales revealed a low frequency of infection by *Dickeya* spp. Stocks tested appeared to be mostly produced from home-grown seed. As in previous years, several findings of *Dickeya dianthicola* in ware potatoes during the 2007 season were again all associated with seed imports. The finding of *Dickeya* in a single sample of home-grown seed in 2007 suggests that pathways exist for the spread of infection from outside of the classification system. Continued vigilance is therefore justified to protect home-grown seed from infection with this pathogen.

There are several reports of findings of *Erwinia chrysanthemi* in temperate watercourses and alternative weed hosts (including *Solanum dulcamara*) from several countries, including Sweden (Olsson, 1985), the Netherlands (Van Vuurde and de Vries, 1992), Finland (Laurila *et al*., 2006) and Australia (Cother 1992). The results presented here have confirmed that *Dickeya* sp. can also be isolated from watercourses in England and Wales at relatively low frequency in comparison with the ubiquitous presence of *Pectobacterium* in the watercourses sampled. No evidence for survival in riparian hosts (such as *Solanum dulcamara*) was found in this study, although infections in this host have been found elsewhere in Northern Europe (Olsson, 1985). *Dickeya* spp. were not consistently detected when watercourses were sampled at the same point on different dates, as is usually the case with the brown rot bacterium (*Ralstonia solanacearum*) following colonisation of *S. dulcamara* along river banks. This study did not determine whether the species of *Dickeya* isolated from UK and European rivers is the same as that identified in potatoes (*D. dianthicola*). Continued assessment of the risks of waterborne populations should involve identification of rivers where the pathogen is continually detected, investigation of the *Dickeya* spp. involved and potential role of alternative hosts.

Conclusions

- Analysis of partial *recA* gene sequences identified a wide diversity amongst 219 *E. chrysanthemi* isolates and allowed their association with the 6 *Dickeya* species recently described by Samson *et al*., 2005.

- *Dickeya dianthicola* was shown to be a homogeneous and readily identifiable taxon within a species complex which also includes *D. dadantii*, *D. dieffenbachia* and two other intermediate taxa (named DSIC-1 and DSIC-2).

- *D. dianthicola* is the only currently recognised species affecting potato in Europe although DSIC-1 has also been isolated from potato in the Netherlands and Israel and may also be present in Finland.

- All *Dickeya* isolates from potato in the UK were identified as *D. dianthicola*.

- Other diverse hosts of *D. dianthicola* were confirmed as *Dianthus*, *Dahlia*, *Kalanchoe*, *Begonia,* tomato and artichoke.

- Other *Dickeya* species affecting potatoes in other regions of the world are *D. zeae* in Australia, *D. dieffenbachiae* in Peru and *D. chrysanthemi* in the USA.

- A potentially new *Dickeya* species was identified on sugar cane in Australia.

- A modified selective medium (CVPM) allowed isolation of all *Dickeya* strains.

- A real time PCR assay allowed detection and identification of all *Dickeya* strains.

- Insufficient 16S rRNA and *recA* gene sequence variation was found for further development of PCR assays for *Dickeya* species identification.

- *Dickeya* sp. was detected in 3 (1.2%) of 258 seed potato samples, mostly taken from selected stocks grown in England and Wales in 2007.

- *Dickeya* sp. was also detected in 27 (6.5%) of 417 river water samples collected in September 2007 from 17 (35.4%) of 48 selected watercourses in England and Wales.

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