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First report of Rhizomonas sp. causing corky root of lettuce in Europe

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Abstract

Typical symptoms of corky root were observed on iceberg lettuce (*Lactuca sativa* L.) in the Netherlands and England, on prickly lettuce (*Lactuca serriola* L.) in Spain, and on sowthistle (*Sonchus oleraceus* L.) in Greece. Slow-growing bacteria with similar colonies as strains of *Rhizomonas suberifaciens* or other *Rhizomonas* species were isolated from soil surrounding plants with corky root symptoms using lettuce seedlings as bait. Crude lysate from all strains was tested for DNA homology with DNA from *R. suberifaciens* strain CA1 (*R. sub.* CA1) and *Rhizomonas* sp. strain WI4 (*R.* sp. WI4). Strains that had homology values higher than that of *R.* sp. WI4 or *R. sub.* CA1 were tested for pathogenicity on 1-wk-old lettuce seedlings, cv. Salinas. Two strains from the Netherlands induced typical symptoms of corky root on lettuce. These strains tested negative with monoclonal antibody MAb-Rs1 specific for *Rhizomonas suberifaciens* in an enzyme-linked immunosorbent assay (ELISA). They had low DNA homology with *R. sub.* CA1 (4-9%) and low to moderate DNA homology with *R.* sp. WI4 (4-17%). Some nonpathogenic strains had moderate to high levels of DNA homology with *R. sub.* CA1 or *R.* sp. WI4 (19-84%). All strains had fatty acid profiles similar to those of *Rhizomonas* species. This is the first report of *Rhizomonas* sp. causing corky root of lettuce in Europe.

Additional keywords: Lactuca sativa, Rhizomonas suberifaciens, pathogenicity, DNA homology, fatty acid profile, ELISA

Introduction

Corky root of lettuce (*Lactuca sativa* L.) has been reported on the American continent in California (Patterson et al., 1986; Van Bruggen et al., 1988), Florida (Guzman, 1982), Wisconsin (Amin and Sequeira, 1966a), New York (Hoff and Newhall, 1960), and Canada (Busch and Barron, 1963). The symptoms include banded yellow lesions on the taproot and main lateral roots, that become dark greenish brown and swollen with numerous longitudinal cracks, adopting a corky appearance. Sometimes, distinct swellings are produced, especially in Florida (Lucas and Guzman, 1980). The only report of similar symptoms in Europe was published in Italy (D'Ercole, 1981).

The etiology of this disease was controversial for many years, and was attributed to toxins liberated from decomposing lettuce debris in Wisconsin (Amin and Sequeira, 1966b), New York (Harnett and Lorbeer, 1971), and Italy (d'Ercole, 1981). Recently, however, corky root was shown to be caused by a gram-negative bacterium in California, Florida, Wisconsin, and New York (Van Bruggen et al., 1989; Datnoff and Nagata, 1990). The causal agent was characterized as a new genus and species, *Rhizo-*

monas suberifaciens (Van Bruggen et al., 1990). So far, *R. suberifaciens* is the only species in the genus *Rhizomonas*. Contrary to strains of *R. suberifaciens*, a corky root inducing strain from Wisconsin, strain WI4, had a brown diffusible pigment, a relatively wide sugar utilization pattern, a relatively high G+C content (63 mol% compared to 59 mol% for *R. suberifaciens*), and a moderate level of DNA homology with DNA of the type strain of *R. suberifaciens* (Van Bruggen et al., 1990). This strain was therefore not classified as *R. suberifaciens* but as an unnamed *Rhizomonas* species (Van Bruggen et al., 1990).

One of the distinguishing characteristics of *Rhizomonas* is the presence of a relatively large proportion of 2-OH-14:0 fatty acid in the whole cell fatty acid profile (Van Bruggen et al., 1990). The only other genus known to have a relatively large proportion of this fatty acid is *Sphingomonas* with *S. paucimobilis* (formerly *Pseudomonas paucimobilis*) as type strain (Dees et al., 1979; Yabuuchi et al., 1990). The ratios of various fatty acids are different enough, however, to enable distinction between these two genera (Van Bruggen et al., 1989).

Strains of *R. suberifaciens* grow very slowly, and only on relatively low-nutrient media; they are oligotrophic (Van Bruggen et al., 1990). Although a special medium (S-medium) was developed for *R. suberifaciens* (Van Bruggen et al., 1988), this medium is not selective enough to assure a high success rate for isolation of the pathogen from corky lettuce roots. To identify strains of *R. suberifaciens* isolated from lettuce roots, a specific monoclonal antibody, MAb-*Rs*1, was developed (Van Bruggen et al., 1991). However, this antibody may be too specific to screen for other *Rhizomonas* species among colonies isolated from corky lettuce roots, because corky root inducing strains that do not belong to *R. suberifaciens* do not react with MAb-*Rs*1 (Van Bruggen et al., 1991). Polyclonal antibodies produced against *R. sub.* CA1 were equally specific as MAb-*Rs*1 in ELISA tests on microtitre plates (Van Bruggen et al., 1991), but reacted with some unrelated bacterial genera on immunoblots (Van Bruggen et al., 1989). Thus, this polyclonal antibody may not be suitable for screening strains of *Rhizomonas* species either. A better initial screen to select *Rhizomonas* colonies among various colonies isolated from corky lettuce roots may be DNA-DNA hybridization with strains of different *Rhizomonas* species.

In this paper we report isolation of strains of *Rhizomonas* sp. from European soils using lettuce seedlings as baits and DNA-DNA hybridization as initial colony screen. Strains that had DNA homology levels with *R. sub.* CA1 and *R.* sp. WI4 at least as high as that of *R.* sp. WI4 and *R. sub.* CA1, respectively, were tested for pathogenicity on iceberg lettuce, and characterized by fatty acid analysis and reaction with a monoclonal antibody in indirect double antibody sandwich (DAS) ELISA tests.

Materials and methods

Plant and soil samples. In June 1989, roots of sowthistle (*Sonchus oleraceus* L.) and prickly lettuce (*Lactuca serriola* L.) with early corky root symptoms plus surrounding soil were collected in Metsoro, Epirus, Greece, and in La Nava de Ricomalillo, Montes de Toledo, Spain, by Dr Louise Jackson. The soils from Greece and Spain were a grey and red sandy loam, respectively. In August 1989, roots of iceberg lettuce and sowthistle with corky root symptoms and surrounding soil were collected by the first author from Birchington, Kent, England, and from Maasland, South Holland, the

Netherlands, and Hauwert and Nibbixwoud, North Holland, the Netherlands. The soil from England was a brown sandy loam and the soils from Maasland, Hauwert, and Nibbixwoud, The Netherlands, were black silty clay, clay, and silt, respectively.

Isolations. Roots of one lettuce and one sowthistle plant from England and of three lettuce plants from the Netherlands were washed under running tapwater and sonicated for 15 min in 20 ml of sterile distilled water (SDW) as described previously (Van Bruggen et al., 1988). Sonicated suspensions and suspensions of comminuted roots were passed through 0.65 um sterile filters and plated onto S-medium amended with 30 ppm streptomycin sulphate as described before (Van Bruggen et al., 1988 and 1989). After incubating plates at 27 °C for 10 days, strains with colony morphology similar to that of *R. sub.* CA1 or *R.* sp. WI4 were tested for reaction to MAb-*Rs*1 in indirect DAS ELISA tests. Strains with marginally positive reactions were tested for pathogenicity on lettuce seedlings (see below). Strains isolated from field-grown roots were not subjected to DNA-DNA hybridization tests (see below) because this technique was used only after repeated faillure to screen strains of *R. suberifaciens* from roots and soil collected from Europe using MAb-*Rs*1 in ELISA tests.

To isolate bacteria causing corky root from soil, soil suspensions were poured at the stem bases of one-wk-old lettuce seedlings, cv. Salinas, as described previously (Van Bruggen et al., 1989). Water and a suspension of a five-day-old culture of *R. sub.* CA1 were included as negative and positive controls. There were four to six replications. Plants inoculated with suspensions from different sources were kept in separate insect-proof cages to prevent cross contamination by fungus gnats (midges, belonging to the family Chironomidae). The pots received 25 ml of water, half-strength Hoagland's solution, or a solution of $Ca(NO_3)_2$ + KNO_3 (each at 0.005 M) on alternate days. Supplemental lighting was provided by 400 W multivapor lamps for 14 h per day. Daily temperatures fluctuated between 24 °C and 28 °C during the day and 20 °C and 22 °C at night. Three to four weeks after inoculation, isolations were made from roots with corky root symptoms (including the positive controls) and attempted from the negative controls as described before (Van Bruggen et al., 1988 and 1989), except that roots were not washed before sonication, a 0.8 μm sterile filter was used, roots were not surface disinfected with bleach, and 100 ppm cycloheximide was added to the medium in addition to 30 ppm streptomycin sulphate. Bacterial strains to be tested for pathogenicity were initially selected based on similar colony growth and morphology as strains of *Rhizomonas* and reaction to MAb-*Rs*1 in indirect DAS ELISA tests. Later, the selection criteria were colony growth and morphology and percent DNA homology with DNA of *R. sub.* CA1 and *R.* sp. WI4 (see below).

Strains were named after the country they were isolated from, i.e. GB strains from Great Britain, GR strains from Greece, NL strains from the Netherlands, and SP strains from Spain.

Immunoassays. Indirect DAS ELISA tests were performed essentially as in Clark and Adams (1977). Duplicate samples were suspended in ELISA sample buffer to a concentration of 2-8 $\times$ 10^9 cells/ml. The sample buffer consisted of PBS tween (0.01 M phosphate, 0.14 M NaCl, and 0.1% tween 20) plus 2 g/l bovine serum albumin and 20 g/l polyvinylpyrrolidone (MW 10000). In a replicate set of tests, bacterial colonies were scraped from solid S-medium, dispersed, and sonicated (Van Bruggen et al.,

1990) in sample buffer to the same approximate concentration. Polystyrene 96-well microtiter plates (Immulon 1 plates, Dynatech Laboratories, Chantilly, VA) were coated with MAb-*Rs*1 (Van Bruggen et al., 1991), followed by antigen, polyclonal antibody CA1-Ab1 (Van Bruggen et al., 1989), and anti-rabbit-alkaline-phosphatase (Sigma Chemical Co., St. Louis, MO) as conjugate. Concentrations of antibodies and antigen were described previously (Van Bruggen et al., 1991). Plates were read after 20 min and one h on a Vmax Kinetic microplate reader (Molecular Devices Corp., Palo Alto, CA) at 405 nm. There were six replicates per strain on three ELISA plates, arranged in a randomized complete block design with two replicates per block. Reactions were considered positive if the absorbance at 405 nm exceeded those of the negative controls by at least 2 standard deviations (Van Bruggen et al., 1991).

DNA-DNA hybridization. Colonies with growth rates and morphologies similar to those of *R. sub.* CA1 or *R.* sp. WI4 were subjected to a crude-lysate quick-screen test for DNA homology to these two strains. Cultures were streaked on S medium and incubated for five days at 28 °C. One or two colonies were transferred to 5 ml TEN buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, and 10 mM NaCl) and sonicated for 30 to 45 sec with a Microson ultrasonic cell disruptor model MS-50 equipped with a CM-1 convertor (Heat Systems Electronics, Inc. Farmingdale, N.Y.) at 80% power to break up colonies. The concentration of bacteria in suspension was assessed with a spectrophotometer at 650 nm (Spec 20, Bausch and Lomb, Inc., Rochester, N.Y.), and adjusted to 10^6 to 10^7 cells per ml. Two hundred μl of suspension was transferred to an eppendorf tube. Cells were lysed and DNA was denatured by adding 200 μl of 0.8 M NaOH and 20 mM EDTA and heating at 90-100 °C for 10 min. The suspension (100 μl) was transferred onto Zeta-probe membranes (BioRad Laboratories, Richmond, CA) with a slot-blot vacuum minifold (Minifold II, Schleicher and Schuell, Keene, NH). Blots were washed with 0.4 M NaOH and rinsed in 2 × SSC, air dried and fixed at 80 °C in a vacuum oven for 1 h. DNA from *R. sub.* CA1 was denatured on a boiling water bath for 5 min, and labeled with P^{32} using an oligolabeling kit (Random Primed DNA Labeling Kit; Boehringer Mannheim). Unincorporated nucleotides were removed by passing the mixture through Sephadex G-50. Slot blots were prehybridized for 4-6 h. Denatured, labeled probe was added to the blots, and hybridized for 16 h at 60 °C (stringent conditions, Van Bruggen et al., 1990). Blots were washed and rinsed as described before (Van Bruggen et al., 1990) and autoradiographed on Kodak XAR 5 film (Kodak, Rochester, NY). The extent of hybridization was determined by measuring counts per min on the blots using the Ambis Radioanalytic Imaging System (Ambis Systems, Inc., San Diego, CA). Counts per min were compared to those of the homologous control and expressed as percent homology. Strains with crude-lysate homology values higher than that of *R.* sp. WI4 or *R. sub.* CA1 with purified DNA of *R. sub.* CA1 and *R.* sp. WI4, respectively, were tested for pathogenicity on 1-wk-old lettuce seedlings, cv. Salinas.

Additional DNA-DNA hybridization tests were conducted with purified DNA from strains that were pathogenic to lettuce or had relatively high levels of DNA homology with *R. sub.* CA1 or *R.* sp. WI4 in the crude- lysate quick-screen test. DNA from *R. sub.* CA1 and *R.* sp. WI4 and from the type strain of *S. paucimobilis* (ATCC # 29837) were used as probes. The techniques for DNA isolation were described earlier (Van Bruggen et al., 1990), and those for random labeling with ^{32}P and DNA-DNA

hybridization on filter membranes were the same as for the quick-screen test. Hybridization conditions were less stringent than those for the quick-screen test (50 °C rather than 60 °C). The extent of hybridization was determined as described above. There were three replicates per strain, completely randomized over the blots. The percentages hybridization were compared with Duncan's multiple range test.

Pathogenicity on lettuce. Lettuce seed cv. Salinas was surface sterilized in 0.5% sodium hypochlorite for 5 min, rinsed, and planted in 5-cm-wide pots with autoclaved UC mix (Matkin and Chandler, 1957). The plants were thinned to one per pot. One week after planting, three seedlings were inoculated by dispensing 2.0 ml of a cell suspension (approximately 10^8 CFU/ml scraped from S-agar into sterile distilled water) of each strain at the stem base of each plant. Three plants inoculated with *R. sub.* CA1 or sterile distilled water served as positive and negative controls, respectively. Growing conditions of the plants were the same as described under Isolation. The plants were uprooted 3-4 weeks after inoculation, and rated for corky root severity based on a 0-9 scale (Brown and Michelmore, 1988). Inoculated strains were reisolated from roots with corky root symptoms as described under Isolations. Reisolated strains were compared to the original strains with respect to colony morphology. All strains were tested for pathogenicity in three to five experiments. The average disease scores per experiment were subjected to Duncan's multiple range test, while experiments were considered as blocks.

Fatty acid analyses. Fatty acid analyses were performed on 11 unidentified strains from Europe by Microcheck, Inc, Northfield, Vermont. Strains CA1 and FL11 of *R. suberifaciens* from California and Florida (van Bruggen et al., 1991), strains WI4, NY3, CA15 and CA16 of unnamed *Rhizomonas* species from Wisconsin, New York, and California (van Bruggen et al., 1991), and the type strain of *S. paucimobilis* (ATCC # 29837) were included for comparison. All strains were grown on S-medium, and fatty acids were extracted and methylated using the procedure of Moore et al. (1987). Fatty acid methyl ester fractions were separated in a 5% methyl phenyl silicone capillary column of a gas chromatograph (Hewlett-Packard Co., Palo Alto, California) equipped with a flame ionization detector. Standards containing known fatty acids and fatty acid extracts from *Xanthomonas maltophilia* were run simultaneously. The peaks were identified with the Hewlett-Packard MIS software package. Fatty acid profiles from 11 unidentified strains, two strains of *R. suberifaciens*, four strains of *Rhizomonas* sp., and *S. paucimobilis* were subjected to cluster analysis (Microcheck, Inc., Northfield, Vermont).

Results

Typical symptoms of corky root were observed on iceberg lettuce in all three locations in the Netherlands, and on iceberg lettuce and sowthistle in Birchington, England. Disease severity was mild, however, compared to that commonly found in the Salinas Valley in California or in Florida. Early symptoms of corky root (yellow bands) were observed on prickly lettuce in Montes de Toledo, Spain, and on sowthistle in Metsoro, Epirus, Greece. No pathogenic strains were isolated directly from field-grown lettuce or sowthistle roots with symptoms of corky root from England and the Netherlands,

Table 1. Percentages homology of purified DNA with DNA from strain CA1 of *R. suberifaciens*, strain WI4 of *Rhizomonas* sp., and the type strain of *Sphingomonas paucimobilis*, corky root severity (0-9 scoring scale), and absorbance values (at 405nm) in an ELISA test with MAb-*Rs*1 for bacterial strains isolated from soil from the Netherlands (NL), Great Britain (GB), Greece (GR), and Spain (SP).

Species	Strain	Source	DNA homology (%)[a]			Corky root severity[c]	Absorb. (405 nm)[d]
			R. sub. CA1	*R. sp.* WI4	*S. pauc.*[b] #29837		
S. paucimobilis	ATCC #29837	England[e]	5.2 c	6.8 ef	100.0 a	–[f]	0.04
R. suberifaciens	CA1	Salinas	100.0 a	7.5 ef	4.5 h	4.2 a	3.00 (+)
R. sp.	WI4	Madison	9.6 c	99.9 a	8.2 defgh	3.8 a	0.02
	NL1	Maasland	8.7 c	33.0 d	8.6 cdefg	1.0 b	0.10
	NL2	Maasland	3.8 c	3.9 f	4.4 h	2.8 a	0.08
	NL3	Maasland	4.4 c	6.0 ef	10.2 bcde	0.8 b	0.01
	NL4	Maasland	8.5 c	17.4 e	7.9 defgh	3.6 a	0.04
	NL5	Maasland	7.9 c	60.7 c	6.7 efgh	0.7 b	0.04
	NL6	Maasland	20.5 b	8.0 ef	5.6 gh	0.3 b	1.07 (+)
	NL7	Maasland	10.2 c	83.9 b	7.9 defgh	0.5 b	0.02
	NL8	Maasland	19.3 b	6.3 ef	4.6 h	0.3 b	1.01 (+)
	GB1	Birchington	3.5 c	4.8 ef	10.1 bcdef	0.3 b	0.00
	GR1	Metsoro	9.4 c	15.4 ef	11.9 bc	1.0 b	0.06
	GR2	Metsoro	4.9 c	5.7 ef	12.6 b	0.6 b	0.02
	GR3	Metsoro	8.2 c	14.2 ef	10.9 bcd	0.5 b	0.12
	SP1	Montes de Toledo	6.4 c	6.2 ef	8.6 cdefg	1.0 b	0.01
	SP3	Montes de Toledo	4.7 c	5.3 ef	6.3 fgh	0.5 b	0.04

[a] Average of three replicates per test. Means followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

[b] *Sphingomonas paucimobilis* type strain (American Type Culture Collection, ATCC # 29837)

[c] 0-9 scoring scale. Average of five tests with two or three replicates per test. Means followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

[d] Average of six replicates per test. Absorbance values larger than two standard deviations above the mean of the negative control are considered (+).

[e] From a hospital respirator.

[f] Not tested; *S. paucimobilis* was not pathogenic to lettuce in previous tests (Van Bruggen et al., 1990)

when MAb-*Rs*1 was used to select strains similar to *R. suberifaciens*. Nonpathogenic strains were discarded.

Lettuce seedlings that were inoculated with extracts of the different soil samples displayed typical corky root symptoms 3-4 weeks after inoculation even after soil had been stored in the refrigerator for one year. Attempts to isolate the pathogen failed, however, for three consecutive inoculation and isolation cycles when indirect ELISA tests with MAb-*Rs*1 were used as initial screen to identify potential colonies of *Rhizomonas* species. Use of DNA-DNA hybridization with *R. sub.* CA1 and *R.* sp. WI4 of *Rhizomonas* sp. as initial colony screen resulted in successful isolation of two strains from the Netherlands (NL2 and NL4) that induced corky root on lettuce cv. Salinas (Table 1). Disease severity was lower with these strains than with *R. sub.* CA1 and *R.* sp. WI4, however. Several plants inoculated with the other strains isolated from soil had slight yellow lesions on their taproots, but these strains were not pathogenic in all tests, and the mean disease severity scores did not differ significantly from those for noninoculated control plants (4 out of 14 plants with score 1). Bacteria with colonies similar to those of inoculated strains were reisolated from 67%, 78%, 50% and 67% of the plants inoculated with strains CA1, WI4, NL2, and NL4, respectively. Colonies isolated from noninoculated control plants did not resemble any of these strains.

DNA isolated from strains that caused corky root had only low homology with DNA of *R. sub.* CA1 (4-9%) and low to moderate homology with DNA of *R.* sp. WI4 (4-17%) (Table 1). These strains had no affinity to MAb-*Rs*1 (Table 1), but had fatty acid profiles similar to those of *Rhizomonas* and *Sphingomonas* species (Table 2).

Nonpathogenic strains had low to moderate DNA homology with DNA from *R. sub.* CA1 (Table 1). The highest levels of hybridization were obtained with strains NL6 and NL8 (21% and 19%, respectively). These strains were also the only strains that tested positive with MAb-*Rs*1 (but to a lesser extent than *R. sub.* CA1). These strains were not pathogenic, however. The fatty acid profiles of strains NL6 and NL8 were similar to that of *R. sub.* CA1 but differed slightly in the contents of 16 : 1, 17 : 1, 18 : 1, and 19 : 0-cyclo fatty acids (Table 2).

DNA homologies of nonpathogenic strains with DNA of *R.* sp. WI4 varied substantially (Table 1). Strains NL1, NL5, and NL7 had 33%, 61%, and 84% homology, respectively, with *R.* sp. WI4. These strains did not cause typical corky root symptoms on cv. Salinas, and, like *R.* sp. WI4, did not have significant affinity to MAb-*Rs*1. Fatty acid profiles of strains NL1 and NL7 were very similar to that of WI4, except for a higher percentage of 16:0 fatty acid (Table 2). Strain NL5 was not subjected to fatty acid analysis. Three other strains had moderate levels of DNA homology (14-17%) with *R.* sp. WI4, namely NL4, GR1, and GR3. However, these homology levels were not statistically significantly different from most lower levels of DNA homology.

Cluster analysis of the fatty acid profiles (Fig. 1) grouped strains NL6 and NL8 together with strains CA1 and FL11 of *R. suberifaciens* at a Euclidian distance smaller than 10. Strains GB1 and GR2 were very closely related to the *R. suberifaciens* cluster, at a Euclidian distance of 11. Fatty acid profiles of strains NL1 and NL7 clustered in the same group with *R.* sp. WI4 and strain CA16, a non-pathogenic strain of *Rhizomonas* sp. (Van Bruggen et al. 1991), at a Euclidian distance of 9. Strain NY3, a nonpathogenic strain from New York (Van Bruggen et al., 1991), was closely related to this group at a Euclidian distance of 11. Strains NL4, NL2, and SP1 clustered together with a pathogenic strain from California, strain CA15 (Van Bruggen et al., 1991), at a Eucli-

Table 2. Fatty acid composition of whole cells of 11 unidentified bacterial strains causing corky root of lettuce as determined by gas chromatography. Data represent percentages of fatty acids for each strain[a]. Fatty acid profiles of *Rhizomonas suberifaciens* strain CA1, *Rhizomonas* sp. strain WI4, and the type strain of *Sphingomonas paucimobilis* are included for comparison.

Fatty acid	*R. sub.* CA1[b]	*R. sp.* WI4	*S. p.* type[c]	NL1	NL2	NL4	NL6	NL7	NL8	GB1	GR1	GR2	GR3	SP1
14 : 0	0.7	2.9	0.9	–	2.9	1.9	–	–	–	–	–	–	–	2.1
14 : 0-2-OH	8.9	10.5	6.0	7.4	15.0	8.3	8.5	10.3	8.7	15.8	7.6	12.0	7.8	17.3
15 : 0	–	2.7	–	–	–	–	–	–	–	–	–	–	–	–
16 : 0	11.1	19.3	8.9	24.6	10.9	18.0	10.3	16.9	9.9	12.4	8.8	16.2	9.1	9.9
16 : 0-2-OH	0.2	–	–	–	–	–	–	–	–	1.7	2.1	–	1.8	–
16 : 1	9.4	8.4	4.2	9.2	18.9	20.7	16.1	7.3	14.8	3.4	35.1	2.2	36.0	11.9
17 : 1	2.3	–	3.6	–	–	–	–	1.3	0.9	–	–	1.7	–	–
18 : 0	0.9	–	–	–	–	–	–	–	–	–	–	–	–	–
18 : 1	56.7	52.2	75.4	54.1	47.5	43.6	61.5	60.3	63.5	63.1	43.3	61.9	41.4	50.4
18 : 1-2-OH	–	–	–	–	–	–	–	–	–	1.1	–	–	–	–
18 : 1-10-CH3	4.8	4.2	–	4.7	3.2	7.5	2.2	4.0	2.1	2.6	3.1	4.5	3.9	2.8
19 : 0-cyclo	4.7	–	0.2	–	1.7	–	–	–	–	–	–	1.5	–	5.4
20 : 0 iso	–	–	0.8	–	–	–	–	–	–	–	–	–	–	–
20 : 4 cis	–	–	–	–	–	–	1.3	–	–	–	–	–	–	–

[a] Fatty acid analyses were performed mainly by Microcheck, Inc. Additional fatty acid analysis for strain CA1 was performed by Microbial ID, Newark, Delaware.

[b] Means of three replicates.

[c] *Sphingomonas paucimobilis* type strain (American Type Culture Collection, ATCC # 29837).

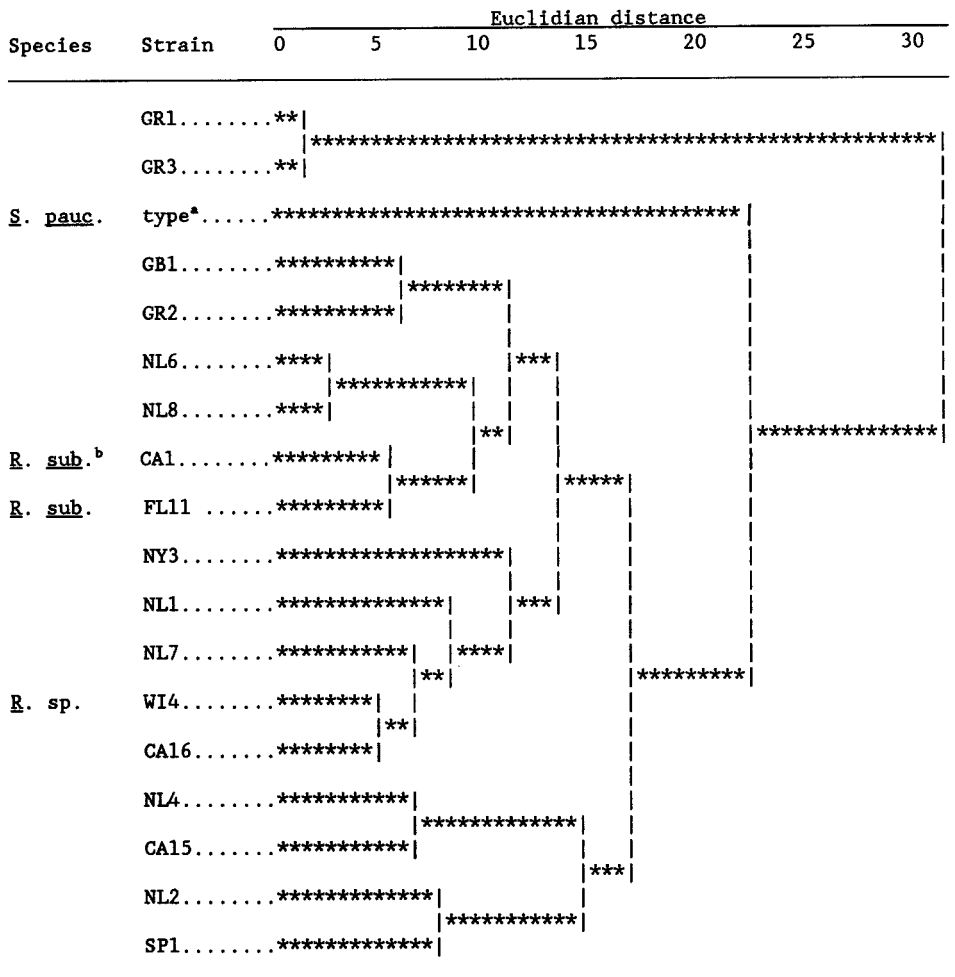

Fig. 1. Cluster analysis of whole-cell fatty acid profiles of strains isolated from soils from Greece (GR-strains), Great Britain (GB-strain), the Netherlands (NL-strains), Spain (SP-strain), California (CA-strains), Florida (FL-strain), and New York (NY-strain). The type strain of *Sphingomonas paucimobilis*, strains CA1 and FL11 of *Rhizomonas suberifaciens*, and strain WI4 of *Rhizomonas* sp. were included for reference.

[a] *Sphingomonas paucimobilis* type strain (ATCC # 29837)

[b] *Rhizomonas suberifaciens*

[c] Unidentified *Rhizomonas* species

dian distance of 15. This cluster was connected to the clusters with *R. sub.* CA1 or *R.* sp. WI4 at a Euclidian distance of 18. *Sphingomonas paucimobilis* was connected to the *Rhizomonas* cluster at a Euclidian distance of 22. Strains GR1 and GR3 were connected with the *Rhizomonas* and *Sphingomonas* clusters at a Euclidian distance of 32.

Discussion

Initial attempts to isolate strains of bacteria causing corky root of lettuce from lettuce roots with corky root symptoms and from soil were unsuccessful. This was probably due to the fact that the search was limited to strains that would react with monoclonal antibody MAb-*Rs*1 specific for *R. suberifaciens* (Van Bruggen et al., 1991). Moreover, it is extremely difficult to isolate *R. suberifaciens* from field-grown roots, because it is easily overgrown by secondary bacteria. When the search was broadened to include strains that had some DNA homology with *R. sub.* CA1 or *R.* sp. WI4 in a crude-lysate quick-screen test, two strains were obtained from Dutch soils (strains NL2 and NL4) that induced typical symptoms of corky root on lettuce. Fatty acid analysis indicated that these strains belong to the genus *Rhizomonas.* However, they do not belong to *R. suberifaciens*, considering the low level of DNA homology with *R. sub.* CA1 and a Euclidian distance of 18 from the cluster containing *R. sub.* CA1 in a cluster analysis of fatty acid profiles. Clusters at a Euclidian distance larger than 10 but less than 25 are generally considered to be different species of the same genus (M. Sinclair of Microcheck, Inc., personal communication). Strains NL2 and NL4 tested negative with monoclonal antibody MAb-Rs1 specific for *R. suberifaciens* in an indirect DAS ELISA test, and would not have been obtained if reaction with MAB-*Rs*1 had been used as selection criterion.

Two strains from the Netherlands (NL6 and NL8) tested positive with MAb-*Rs*1, had a moderate level of DNA homology with *R. sub.* CA1 , but did not induce typical corky root symptoms. Cluster analyses of whole- cell fatty acid profiles grouped strains NL6 and NL8 into the same cluster as *R. sub.* CA1 and *R. suberifaciens* strain FL11 at a Euclidian distance of 10, indicating that these strains belong to the same species (M. Sinclair of Microcheck, Inc., personal communication).

Most strains isolated from soil and selected according to DNA homology with *R. sub.* CA1 or *R.* sp. WI4 had fatty acid profiles similar to those of *Rhizomonas* and *Sphingomonas* species. These strains were grouped together with strains of *Rhizomonas* in a cluster analysis of fatty acid profiles at a Euclidian distance of 17, indicating that they belong to the genus *Rhizomonas.* The grouping of strains within the *Rhizomonas* cluster based on fatty acid analysis corresponded very well with the results from DNA-DNA hybridization. However, strains GR1 and GR3 which had relatively high DNA homology with strain WI4 of *Rhizomonas* sp. were grouped in a separate cluster at a fairly large distance from the *Rhizomonas* cluster. *S. paucimobilis* was placed in a separate cluster by itself, connected with the *Rhizomonas* cluster at a Euclidian distance of 22. According to the criterion of separate genera at a Euclidian distance larger than 25, *S. paucimobilis* would be in the same genus as strains of *Rhizomonas.* However, the low DNA homology between *R. suberifaciens*, the type species of *Rhizomonas*, and *S. paucimobilis* argue against placing strains of *Rhizomonas* and of *Sphingomonas* in the same genus. In addition, the guanine-cytosine content of the DNA of strains of *R. suberifaciens* is 7-8 mol% lower than that of *S. paucimobilis* (Van Bruggen et al., 1990), and the melting temperature of DNA-rRNA hybrids of *S. paucimobilis* and *R. sub.* CA1 is 70 °C compared to 79 °C for hybrids of strains of *R. suberifaciens* with *R. sub.* CA1 (Jochimsen and Van Bruggen, 1991). These differences in guanine-cytosine content and melting temperature of DNA-rRNA hybrids are sufficiently large to warrant placement in different genera (De Vos

and De Ley, 1983; Owen and Pitcher, 1985).

This is the first report of strains of *Rhizomonas* spp. isolated from Europe. Most of these strains were not or only slightly pathogenic to lettuce seedlings in the greenhouse. Corky root symptoms on lettuce in fields in England and the Netherlands were also less severe than those observed in California or Florida. Pathogenic strains isolated from Europe belonged to different species of *Rhizomonas* than *R. suberifaciens* but not to different genera.

In this study we showed that there is a large variability among pathogenic *Rhizomonas* strains, both in their genetic make-up and the extent to which they cause corky root on lettuce cv Salinas. Most of the strains isolated from the U.S. belong to *R. suberifaciens*. However, strains of *R. suberifaciens* from Florida are distinct from those from California with respect to DNA homology and reaction to monoclonal antibody MAb-*Rs*2 (Van Bruggen et al., 1991). So far, two pathogenic strains isolated from the U.S. (*R.* sp. WI4 and CA15) belong to different species of *Rhizomonas* (Van Bruggen et al., 1991). Based on clustering of the fatty acid profiles, one strain from the Netherlands (NL4) belongs to the same species of *Rhizomonas* as strain CA15, and the other strain (NL2) may belong to a different species.

The large variability among strains that induce corky root on lettuce may be of concern to lettuce producers and breeders since all cultivars with resistance to corky root were derived from cultivars Green Lake or Montello with the same source of resistance (Guzman, 1984; Sequeira, 1970), conferred by a single recessive gene (Brown and Michelmore, 1988) which may not be effective against all pathogenic strains of *Rhizomonas*.

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